inal mucosal cells results in the formation of lysophosphatidic acid (LPA). These results indicate that LPA is an intermediate in the formation of phosphoglycerides in the intestinal mucosa during fat absorption.

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Effect of Inhibitors of Protein Synthesis on Renal Pressor Factors* (33879)

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The influence of vasoactive proteins on hemodynamics was approached from a fresh viewpoint with the recent demonstration in this laboratory that several inhibitors of proteins synthesis, actinomycin D, acetoxycycloheximide, and chloramphenicol promptly and markedly reduce blood pressure of normotensive and hypertensive rats (1, 2). From the available evidence it appears that this depressor effect may be mediated through specific derangements of, as yet unknown, proteins or related compounds acting on vascular tissues rather than through nonspecific actions such as pharmacological toxicity, malnutrition, or cachexia following suppression of adrenal cortex function. In a further effort to clarify the mechanism of the depressor effect, the integrity of arterial smooth muscle during the hypotensive state was studied to determine whether interference with phasic contractile protein function might be responsible. It was shown, however, that while blood pressure was appreciably reduced within several hours after initiation of antimetabolite administration, no decrease in phasic contractility was observed until after 14 days of such treatment (3). It appears therefore that more labile vasoactive proteins than those responsible for smooth muscle contraction are involved in the depressor effect induced by inhibitors of protein synthesis.

In this regard, it was considered likely that the protein metabolic inhibitors might deter the synthesis of renin in the kidney, thus removing support for the maintenance of vascular resistance, resulting in a decline in blood pressure. A similar hemodynamic effect might also stem from a situation in which the inhibitors do not interfere with renin synthesis but diminish its vasoconstricting effectiveness. The present experiments were designed to study these two questions.

The effect of antimetabolites on renin formation was tested physiologically by comparing the pressor effect of kidney extracts from normal rats to similar extracts from rats treated with antinomycin D, an inhibitor of DNA-dependent protein synthesis. The second experiment consisted of an examination of the pressor effects of graded doses of angiotensin on rats during the hypotensive state induced by actinomycin D and by acetoxycycloheximide, an inhibitor of RNA-dependent protein synthesis, to determine

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whether the pressor response to this derivative of renin was suppressed.

Methods. The kidneys of normal rats were removed promptly after death, sliced into four pieces, weighed, and immersed into 6 vol of cold acetone (4°). After 6 hr the acetone was discarded and replaced with an additional amount to be left overnight at 4°. The acetone was removed and replaced with 6 vol of cold ether. After several hours, the ether was discarded and the dehydrated and defatted kidneys were ground to powder.

The kidney powder was then extracted for partially purified renin by modification of the method described by Helmer and Page (4) as follows: To the powder was added a volume of 2% NaCl in water equivalent to twice the weight of fresh kidney and kept overnight at 4°. The mixture was filtered and adjusted to pH 4.5 with acetic acid. This mixture was centrifuged and to the supernatant was added 1 *M* KH_2PO_4. This was centrifuged and the solution was adjusted to 2 *M* KH_2PO_4 and again centrifuged. The precipitate was dissolved in 0.9% saline so that 1 ml was equivalent to 4 g of fresh kidney. The solution remained overnight at 4° and was centrifuged. The clear solution was used for pressor activity assay.

Extracts of kidneys from actinomycin D-treated rats were prepared by the same method. Animals received 40 μg of actinomycin D intraperitoneally twice on day 1, at a 6-hr interval, 20 μg on days 2 and 3 and they were killed on day 4 and their kidneys were removed and extracted as described above. Indirect blood pressure determinations were obtained daily and significant hypotension was found on the second and subsequent days, thus confirming the depressor effectiveness of the antimetabolite.

The pressor responses to the kidney extracts were determined as follows: Test animals were anesthetized with ether, the abdomen was opened and a cannula was inserted into the abdominal aorta at the femoral bifurcation. This cannula was attached to a pressure transducer (Statham P 23) which was connected to an apparatus which continuously photographed pulse and blood pressure, (Electronics for Medicine DR8). After the blood pressure was shown as stabilized on the oscilloscope, 0.5 ml of kidney extract was injected intravenously and the pressure changes were recorded. Measurements of blood pressure response were obtained from the photographs.

The influence of inhibitors on the pressor responses to the renal factor was studied by measuring the blood pressure elevations following intravenous injections of graded doses of angiotensin[1] into rats treated with these agents as follows: Four groups of rats were subjected to two injections of 40 μg of actinomycin D[2] on day 1, 6 hr apart; 20 μg on days 2 and 3, and were tested for pressor response with intravenous injections of 0.10, 0.25, 0.50, and 1.0 μg of angiotensin on day 4. Each dose was injected three times at 3-min intervals to detect the possible development of tachyphylaxis. Four other groups of rats treated similarly with 50 μg acetoxycycloheximide[3] twice on day 1 and 30 μg on day 2, were tested with these amounts of angiotensin as well as four groups of untreated rats.

Results A. Pressor activity of kidney extracts. Blood pressure elevations were measured following intravenous injection of kidney extracts from normal and actinomycin D-treated rats.

A 0.50-ml portion of each extract was tested on nine rats, this dose being equivalent to 2 g of fresh kidney. There was little difference in response between extracts of each type. Thus the average response to injections of extracts of normal kidneys was 22 ± 1.9 mm Hg and to extracts of kidneys of actinomycin D-treated rats, 26 ± 2.7 mm Hg. It was observed, however, that the sys-

[1] Hypertensin - Ciba - Lyophilized (angiotensin amide) supplied by Ciba Pharmaceutical Products, Inc., through the courtesy of Dr. William E. Wagner, Summit, New Jersey.

[2] Actinomycin D supplied by Merck, Sharp and Dohme Research Laboratories, Rahway, New Jersey, through the courtesy of Dr. Walter B. Gall.

[3] Acetoxycycloheximide supplied by the John L. Smith Memorial for Cancer Research, Chas. Pfizer and Co., Inc., Maywood, New Jersey, where the material was produced under support of NIH contract no. PH43-68-45 with the National Cancer Institute through the courtesy of Dr. T. J. McBride.

TABLE I. Response to Repeated Injections of Angiotensin at 3-min Intervals.

Treatment	No. of rats	Angiotensin dose (μg)	Av systolic BP (mmHg)	Av systolic pressor response (mmHg) to each injection		
				1st	2nd	3rd
Control	14	0.10	113 ± 2.9	42 ± 2.8[a]	41 ± 3.2	38 ± 3.0
Actinomycin D	10	0.10	81 ± 4.6	23 ± 3.6 (p <0.01)	23 ± 3.7	21 ± 2.4
Acetoxycyclohexi-mide	12	0.10	73 ± 4.4	38 ± 2.1 NS	39 ± 2.4	38 ± 2.7
Control	12	0.25	114 ± 2.7	66 ± 4.1	67 ± 4.3	64 ± 4.4
Actinomycin D	12	0.25	86 ± 3.6	36 ± 4.0 (p <0.01)	33 ± 2.2	30 ± 3.2
Acetoxycyclohexi-mide	14	0.25	76 ± 4.9	48 ± 2.8 (p <0.05)	44 ± 2.3	43 ± 2.0
Control	12	0.50	118 ± 2.9	84 ± 2.8	91 ± 3.1	83 ± 4.0
Actinomycin D	11	0.50	85 ± 2.9	43 ± 3.6 (p <0.01)	39 ± 2.9	40 ± 3.3
Acetoxycyclohexi-mide	14	0.50	84 ± 2.2	67 ± 2.8 (p <0.05)	62 ± 2.7	57 ± 3.0
Control	8	1.0	117 ± 2.4	94 ± 3.8	90 ± 3.0	92 ± 3.7
Actinomycin D	10	1.0	80 ± 2.6	56 ± 2.0 (p <0.01)	50 ± 2.8	44 ± 2.6
Acetoxycyclohexi-mide	8	1.0	83 ± 3.4	84 ± 4.1 NS	74 ± 4.4	62 ± 3.7

[a] SE of mean.

tolic blood pressure of treated rats at the time of kidney removal to obtain extracts was substantially depressed below normal, 81 ± 3.4, compared to 114 ± 4.5 mm Hg of untreated rats.

B. Pressor response to graded doses of angiotensin. The average pressor responses to multiple injections of graded doses of angiotensin in normal rats and in those treated with actinomycin D and with acetoxycycloheximide are shown in Table I. These results show clearly that the average pressor response in actinomycin D-treated rats was about one-half that of normal at all four dose levels of angiotensin.

On the other hand, the pressor responses to angiotensin injections in rats treated with acetoxycycloheximide were relatively inconsistent and at no time as effectively suppressed as in rats treated with actinomycin D. For example, the average response to the injection of 0.10 μg of angiotensin could not be distinguished from normal. A relatively normal response was also observed after the first injection of 1.0 μg but subsequently there was a decline of approximately 10% with the second and third injections, apparently due to tachyphylaxis. The responses to 0.25 and 0.50 μg were approximately 70 and 80% respectively, of normal. It may also be noted that in spite of the greater suppression in pressor responses between the groups treated with actinomycin D and acetoxycycloheximide, the average systolic blood pressures of both groups were equally depressed at the start of the tests.

The responses to the repeated injections of angiotensin of both groups and the controls were averaged and are presented in Fig. 1.

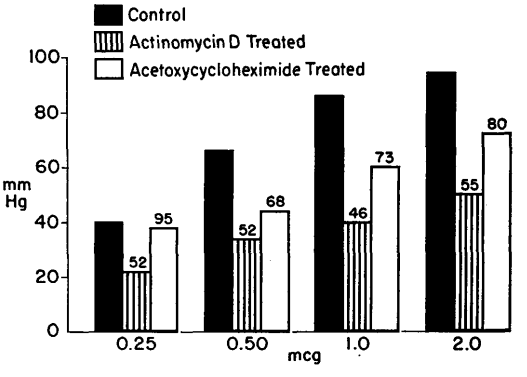

FIG. 1. Average blood pressure elevation after repeated injections of angiotensin in control and treated rats: nos. refer to percentage of normal response.

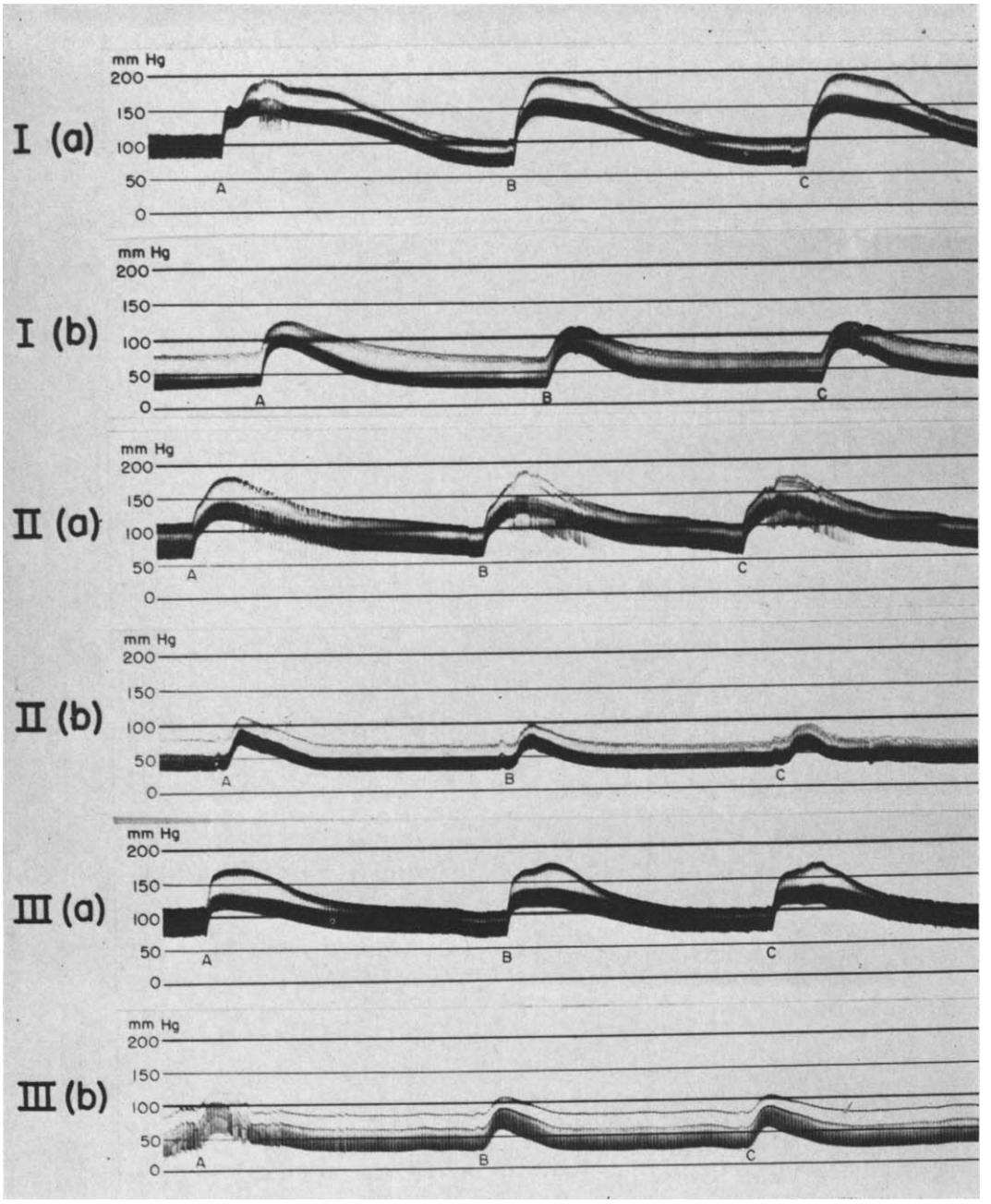

FIG. 2. Pressor responses to angiotensin at three dosage levels (μg): I = 0.50; II = 0.25; and III = 0.10 in (a), control; and (b) actinomycin D-treated rats.

Figure 1 shows that there was a marked loss of reactivity to angiotensin in rats treated with actinomycin D and an overall moderate decrease in rats treated with acetoxycycloheximide. Tracings of the pressor responses to injections of angiotensin in actinomycin D treated and control animals is shown in Fig. 2.

Discussion. In spite of considerable evidence that renin, secreted by the juxtaglo-

merular cells, is responsbile for hypertension of renal origin, it is controversial whether renin content of kidneys can be correlated to blood pressure levels. Thus Tobian reported a positive relation between extractable renin of ischemic kidneys and blood pressure level (5); and Haas and Goldblatt found an increased renin content in the ischemic kidneys of dogs and hypertensive patients (6). On the other hand, Regoli *et al.* (7) found no consistent relationship between blood pressure levels and renin content. It is even less clear whether blood pressure levels of normotensive rats are a function of renin production. Evidence is unavailable which would indicate that hypotension results from a failure of renin secretion to maintain peripheral resistance. It would appear that suppression of renin synthesis is not involved in the induction of the depressor effect in rats treated with protein inhibitors. It might be pointed out that inhibitors are capable, under certain conditions of suppressing renin synthesis. Meyer and associates (8) demonstrated a reduction in renin content of kidneys in rats treated intraperitoneally with doses of actinomycin D about three times as great as were used in the present experiments. Likewise, puromycin injected into kidneys via the renal artery resulting in relatively high tissue concentrations was also effective in this respect according to MacDonald and Blacket (9).

An examination was made of the alternative question whether antimetabolites might lower blood pressure through interference with vasoconstriction from endogenous renin thus reducing peripheral resistance. The results of this study showed that injections of angiotensin in doses ranging from physiologic to pharmacologic (0.10–1.0 μg) into actinomycin D-treated rats, consistently produced about one-half the pressor effect obtained in normal rats. This may be interpreted as evidence that endogenous renin might be unable to maintain adequate peripheral resistance resulting in hypotension. On the other hand, equally hypotensive rats treated with another inhibitor, acetoxycycloheximide, failed to demonstrate a comparable suppressed pressor response to similar doses of angiotensin. At two dosage levels, there was a mild decrease in pressor activity and at two levels, practically none, indicating a weak correlation between declines in blood pressure and pressor response in acetoxycycloheximide-treated rats. It seems possible from this data that interference with renin (angiotensin) activity in the periphery may be, at least partially involved in the depression of blood pressure following the administration of protein synthesis inhibitors. However, there may be some question as to the validity of using the angiotensin to test renin function since the latter requires enzymatic activity for conversion into angiotensin and these enzymes may be subjected to the action of the inhibitors. Such doubts would be considered more serious were claims made that reduction in pressor response was a major factor in the depression of blood pressure.

It is of considerable interest that angiotensin is relatively ineffective in rats treated with actinomycin D while *l*-norepinephrine induces a normal response in similarly treated animals (5). While the hemodynamic response of these two pressor agents have apparently different receptor sites in vascular tissues, angiotensin acting on the precapillary vessels and *l*-norepinephrine directly on the vascular muscle (10), there appears to be some common physiologic mechanism in the response of the two compounds, in part due to release of catecholamines from adrenal medulla and sympathetic nerve endings by angiotensin (11–13). It is therefore difficult to resolve the marked disparity in pressor responses to the two compounds. It is suggested, however, that since a drug which suppresses protein synthesis impairs the response to angiotensin but not to *l*-norepinephrine, the former might utilize an intermediate protein system not required for the action of catecholamines on smooth muscle contraction, thus revealing a fundamental difference between angiotensin and catecholamine action which has not yet been suspected.

The depressor mechanism may involve interference with other humoral factors with hemodynamic action such as the inotropic proteins in plasma reported by Hajdu *et al.*

(14), "kinekard," a protein in plasma shown by Nayler and associates (15) to stimulate myocardial contraction and vasoconstriction; and a substance in plasma which, according to Bohr and Johansson (16), influences arterial tone through shortening vascular smooth muscle. It is conceivable that suppression of synthesis of one or more of these factors may be involved in the depression in blood pressure.

Summary. Actinomycin D administration in amounts capable of markedly lowering blood pressure of rats did not reduce the renin content of kidneys. This treatment induced significant reductions in pressor responses to injections of graded doses of angiotensin but rats made equally hypotensive by another inhibitor, acetoxycycloheximide showed only a mild suppression of blood pressure elevation to such injections. Diminished pressor response to renal pressor factor may be, at least, partially involved in the depressor effect induced by protein synthesis inhibitors. However, interference with synthesis of other vasoactive proteins may possibly participate in the hypotensive response.

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