7. Sutherland, E. W., Wosilait, W. D., *ibid.*, 1956, v218, 459.
8. Fiske, C. H., Subbarow, Y., *ibid.*, 1925, v66, 375.
9. Lowry, O. H., Rosebrough, N. J., Farr, A. L., Randall, R. J., *ibid.*, 1951, v193, 265.
10. Glock, G., McClean, P., *Biochem. J.*, 1953, v55, 400.
11. Boulouard, R., *Fed. Proc.*, 1963, v22, 750.
12. Kelly, T. L., Nelson, E. D., Johnson, R. B., Vestling, C. S., *ibid.*, 1955, v212, 545.
13. Kobayashi, S., Yago, N., Morisaki, M., Ichii, S., Matsuba, M., *Steroids*, 1963, v2, 167.
14. Yago, N., Kobayashi, S., Morisaki, M., Ichii, S., Matsuba, M., *ibid.*, 1963, v2, 175.
15. Eisenstein, A. B., PROC. SOC. EXP. BIOL. AND MED., 1959, v100, 111.
16. Greenberg, L. J., Glick, D., *J. Biol. Chem.*, 1962, v237, 3552.

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Actinomycin D Inhibits Parathyroid Hormone and Vitamin D Activity.* (29501)

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Administration of excessive doses of either Vit. D or parathyroid hormone to animals results in increased absorption of calcium from the gastrointestinal tract, hypercalcemia and dissolution of bone, among other effects (1-3). The hormone, at least, causes increased reabsorption of phosphate by the kidney(4). It has recently been proposed that a number of hormones act by inducing the synthesis of specific DNA directed RNA which directs the synthesis of enzymes which, in turn, cause the observed effects of hormone action (5-7). There is suggestive evidence in this regard in a recent publication in which the action of parathyroid extract on organ cultures was described(8). In these experiments it was demonstrated that addition of parathyroid extract to the culture medium resulted in destruction of bone with release of moieties of collagen into the culture medium. The size of the fragments was such as to indicate that the destruction of the collagen, which forms the bulk of the organic matrix of bone, was due to enzymatic digestion rather than simple solubilization. Such enzymatic digestion could occur either by release of enzymes from sequestered pockets within the cell such as lysozomes, activation of enzymes, or synthesis of new enzymes. If the last were true, the action of the hormone on bone should be inhibited by the action of Actinomycin D, an antibiotic which is considered to inhibit specifically the synthesis of DNA directed or messenger RNA(9-11).

Materials, methods and results. Six-week-old male Swiss-Webster mice were divided into 4 equal groups. One group was injected intraperitoneally with 150 units of parathyroid extract (Eli Lilly & Co.), a second with 0.01 mg of Actinomycin D, a third with 0.01 mg of Actinomycin D and 150 units of parathyroid extract and a control fourth group received an equal volume of 0.9% NaCl. After 18 hours, the animals were anesthetized and blood obtained by decapitation. Hormone activity was assessed by measuring the level of serum calcium using an atomic absorption spectrometer and the production of specific bone lesions, which have been well described(12). Similar experiments were designed to test the ability of Actinomycin D to inhibit the ability of excessive dosages of Vit. D to induce hypercalcemia except that 10,000 units of Vit. D were injected instead of parathyroid extract. Analysis of the data for serum calcium is presented in Table I. Actinomycin completely suppressed the hypercalcemic effects of both parathyroid extract and Vit. D. Histologic studies of the proximal tibial epiphysis of mice injected

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TABLE I. 95% Confidence Limits of Mean Serum Calcium Levels* (mg %).

Animal	Hypercalcemic agent and control solutions	Actinomycin D		Total No. of animals in exp
		Present	Absent	
Mouse	Vit. D	7.52–10.66	11.55–13.41	
	Control (corn oil)	8.79–10.27	9.44–11.14	44
Mouse	Parathyroid extract	8.74–10.32	13.26–17.19	
	Control (.9% NaCl)	9.86–11.71	9.79–10.70	37
Rat	Vit. D	12.13–15.18	16.12–17.43	
	Control (corn oil)	10.01–11.55	10.92–11.91	23

* 95% $CL_{\bar{y}} = Y \pm (t_{.05} \times S_{\bar{y}})$.

These data also were analyzed by analysis of variance to determine the significance of effects of the agents used and their interactions. This analysis provided evaluations similar to those reflected in the Table.

with parathyroid extract and Actinomycin D showed complete suppression or alteration of the histologic response to parathyroid extract in every instance.

Statistical analyses also indicated possible effects of Actinomycin D alone on levels of serum calcium, and that Actinomycin D in the presence of parathyroid extract lowered serum calcium levels below those of control mice. These data were too variable in the different groups of animals to be convincing.

An additional experiment was done using male Wistar rats to test the ability of Actinomycin D to depress the level of vitamin D-induced hypercalcemia after the serum calcium had been raised. The rats were divided into 4 equal groups similar to those described above and daily injections of 100,000 units of Vit. D were given for 12 days. During the last 5 days 0.02 mg of Actinomycin D was given to some rats which were receiving Vit. D and some which received control fluids. From the results presented in Table I it is apparent that serum calcium levels in rats receiving both Actinomycin D and Vit. D were intermediate between control rats and those receiving Vit. D alone. Bone lesions in rats receiving both Vit. D and Actinomycin were different from those receiving Vit. D alone, largely in that osteoid production was diminished.

Discussion. Actinomycin D completely suppressed the hypercalcemia induced in mice by injections of either Vit. D or parathyroid extract. It also inhibited the bone lesions induced by excess of these agents, and thus presumably inhibited the chemical changes responsible for them which are apparently cellular and enzymatic in nature(8,12). The data, therefore, indicate that both the hormone and vitamin may act, at least in part, by inducing formation of DNA directed or messenger RNA which, in turn, directs the synthesis of enzymes responsible for the observed effects of these agents. Actinomycin D does not destroy RNA already formed. The observation that in rats with chronic hypervitaminosis D, the antibiotic lowered serum calcium toward but not to normal may indicate that the Actinomycin inhibited further synthesis of messenger RNA, but that the RNA formation induced by the previous Vit. D injections was still operating. Other interpretations are obviously possible. The use of rats also shows that the Actinomycin D-vitamin D interaction is not species specific.

It should be pointed out, however, that both Vit. D and parathyroid extract have been shown to cause release of calcium from isolated mitochondria(13), an effect which would not appear to involve RNA synthesis. In addition, with lower more physiological doses many of the changes in serum calcium levels which occur in mammals can be explained on a mitochondrial basis(14). Thus, in light of present knowledge these agents would seem to have different effects in bone than in soft tissues.

Since this manuscript was submitted for publication, Rasmussen *et al*(15) using parathyroid hormone but not Vit. D, have published data leading to conclusions similar to those proposed here. They have, in addition, shown an inability of Actinomycin D to in-

hibit the renal effect of parathyroid hormone.

Summary. Actinomycin D inhibits the hypercalcemia induced by large doses of parathyroid extract or Vit. D. It also alters the bone changes induced by these agents. The data thus indicate that these agents may act by inducing synthesis of new enzymes in bone induced by DNA directed RNA which in turn enzymatically degrade bone.

1. Harris, L. J., Innes, J. R. M., *Biochem. J.,* 1931, v25, 367.

2. Nicolaysen, R., Eeg-Larsen, N., *Vitamins and Hormones,* 1953, v11, 29.

3. Cramer, C. F., Suiker, A. P., Copp, D. H., *The Parathyroids,* ed., R. P. Creep, R. V. Talmage, Springfield, C. C Thomas, 1951, 158.

4. Talmage, R. V., *Ann. N. Y. Acad. Sci.,* 1956, v64, 326.

5. Mueller, G. C., Gorski, J., Aizawa, Y., *Proc. Nat. Acad. Sci.,* 1961, v47, 1964.

6. Greengard, O., Weber, G., Singhal, R. L., *Science,* 1963, v14, 160.

7. Tata, J. R., *Nature,* 1963, v197, 1167.

8. Stern, B. D., Mechanic, G. L., Glimcher, J. M., Goldhaber, D., *Biochem. and Biophys. Res. Comm.,* 1963, v13, 137.

9. Hurwitz, J., Furth, J. J., Malany, M., Alexander, M., *Proc. Nat. Acad. Sci.,* 1962, v48, 1222.

10. Reich, E., Franklin, R. M., Shatkin, A. J., Tatum, E. L., *ibid.,* 1962, v48, 1238.

11. Davidson, E. H., Allfrey, V. G., Mirsky, A. E., *ibid.,* 1962, v49, 53.

12. Heller, M., McClean, F. C., Bloom, W., *A. M. J. Anat.,* 1950, v87, 315.

13. DeLuca, H. F., Engstrom, G., Rasmussen, H., *Proc. Nat. Acad. Sci.,* 1962, v48, 1604.

14. Rasmussen, H., DeLuca, H., Arnaud, C., Hawker, C., von Stedingk, M., *J. Clin. Invest.,* 1963, v42, 1940.

15. Rasmussen, H., Arnaud, C., Hawker, C., *Science,* 1964, v144, 1019.

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Inhibition of Autoregulation of Renal Blood Flow by SKF 525-A and Fluoroacetate.* (29502)

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The ability of the kidney to maintain renal blood flow and glomerular filtration at relatively constant rates over a wide range of systemic arterial pressures has been termed autoregulation(1,2). To explain this phenomenon various mechanisms, both passive and active, have been proposed. Of the passive mechanisms, a cell separation theory has been advanced by Pappenheimer(3) and an intrarenal pressure theory by Winton(4) and Hinshaw(5). An active mechanism is suggested by the myogenic theory of Miles *et al* (6) and Waugh and Shanks(7) which implies that autoregulation occurs as a result of constriction of renal vascular smooth muscle in response to the stimulus of an increase in distending pressure. Support for this active mechanism has in part been obtained using chemical inhibitors(6,7). The inhibitory action of β-diethylaminoethyl-diphenylpropylacetate hydrochloride (SKF 525-A) and sodium fluoroacetate described here provides further evidence for an active myogenic mechanism of autoregulation.

Methods. Twenty-five male or female mongrel dogs, 18 to 25 kg, were used. Pentobarbital sodium, 30 mg/kg, was administered intravenously to anesthetize the animals and supplemental doses were given as necessary. The trachea was cannulated to insure an adequate airway. Systemic blood pressure was monitored from a carotid artery using a Statham arterial pressure transducer. The left kidney was exposed retroperitoneally by

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