5. E. Dodt and K. Echte, *J. Neurophysiol.* **24**, 427 (1961).
6. R. A. Cone, *J. Gen. Physiol.* **46**, 1267 (1963).
7. J. E. Dowling and G. Wald, *Proc. Natl. Acad. Sci. U.S.* **46**, 587 (1960); J. E. Dowling, *J. Gen. Physiol.* **46**, 1287 (1963).
8. The data in Fig. 2 have been corrected for the loss of pigment after bleaching, by using the method of reference (*7*), so that the intensity scale refers uniformly to the number of quanta absorbed by each rod per flash. This correction, though relatively small on the logarithmic scale, (ΔI = —0.5 log unit at 27 minutes), is important in principle, because it makes possible a direct comparison between bleaching and other kinds of adaptation, with all the data being presented in terms of the actual initial photochemical stimulus in the rods.
9. It is interesting to compare these results on the rat ERG with those found by Hartline for spikes from a single optic nerve fiber in *Limulus*. The latency of the first spike is little changed during slow dark-adaptation, but the number and frequency of spikes is greatly altered, like the ERG amplitude here. [H. K. Hartline, *J. Opt. Soc. Am.* **30**, 239 (1940), Figs. 5 and 6.]
10. T. Tomita, *J. Opt. Soc. Am.* **53**, 49 (1963).
11. R. Granit, *J. Physiol.* **77**, 207 (1933); T. Tomita, M. Murakami, Y. Hashimota, Y. Sasaki, *The Visual System: Neurophysiology and Psychophysics*, R. Jung and H. Kornhuber, Eds. (Springer, Berlin, 1961), pp. 24–30.
12. R. A. Cone, *J. Gen. Physiol.*, in press.
13. This research was supported by an AEC contract with the University of Chicago.

18 February 1964

Actinomycin D and the Response to Parathyroid Hormone

Abstract. *Actinomycin D inhibits the effect of parathyroid hormone upon bone, as measured by calcium mobilization, without altering its effects upon the renal excretion of phosphate and calcium.*

The administration of parathyroid hormone to a parathyroidectomized animal produces a characteristic sequence of events. An immediate increase (within 10 to 20 minutes) in the renal excretion of phosphate, a decrease in calcium excretion, and a concomitant fall in the concentration of phosphate in the plasma are followed by a gradual rise in the concentration of calcium in the plasma (*1*). The rise in plasma calcium may not appear for an hour or more and it is eventually accompanied by a progressive increase in the excretion of calcium in the urine. Once established, hypercalcemia and hypercalciuria persist and their magnitude are proportional to the dose of administered hormone. This change in plasma calcium is thought to result from an increased absorption of calcium from the gastrointestinal tract, an increased reabsorption of calcium by the renal tubule, and the mobilization of calcium from bone (*2, 3*). The last is by far the most important in quantitative terms. The response of the gastrointestinal tract appears to be least important. Thus the two major organs which respond to this hormone are kidney and bone. There is good evidence that the hormone acts directly on both organs (*3*). The renal response is characterized by a rapid onset which is limited in magnitude, and a high sensitivity to small changes in hormone concentration. The response of bone on the other hand is sluggish in onset, relatively insensitive, but of a nearly unlimited capacity. The basis for this difference in the time course and sensitivity of their responses is not known. We suggested previously that the difference may be due to the striking difference in the rate of blood flow to these two organs (*2*). Further consideration has led to an alternative or perhaps complementary explanation for these differences.

Parathyroid hormone exerts a direct stimulatory effect upon the process of bone resorption (*4*); the process is highly complex and poorly understood, but current opinion favors the view that resorption of bone is brought about by specialized cells, osteoclasts (*3–7*). Furthermore, all available histological evidence indicates that these cells have arisen from other types of bone cells, and that they have an average lifetime of 36 to 48 hours (*6*). In view of these facts, a fundamental action of parathyroid hormone upon bone cells may be that of initiating the development or differentiation of bone-destroying osteoclasts. In such a case, the time course of hormone response in this organ might well be a reflection of the rate of cellular differentiation.

It could be predicted that underlying this hormonally induced change in cell structure and function would be a change in the rate of synthesis of either the type or the amount, or both, of ribonucleic acids and proteins within these cells. If this were the case, then the administration of agents known to block these synthetic activities should lead to an inhibition of hormone action upon bone.

Of the many agents which have been reported to interfere with either RNA or protein synthesis, actinomycin D appears to be one of the most specific and best characterized (*8*). It appears to block the DNA-directed RNA synthesis catalyzed by RNA polymerase by binding at specific sites on the DNA primer.

In order to test our hypothesis, we followed the time course of the change in the concentrations of plasma calcium and phosphate after the administration of purified bovine hormone (*9*) to either parathyroidectomized animals or parathyroidectomized animals which had received 1 μg of actinomycin D per gram of body weight 2 hours before administering the hormone. Parathyroidectomy was performed several

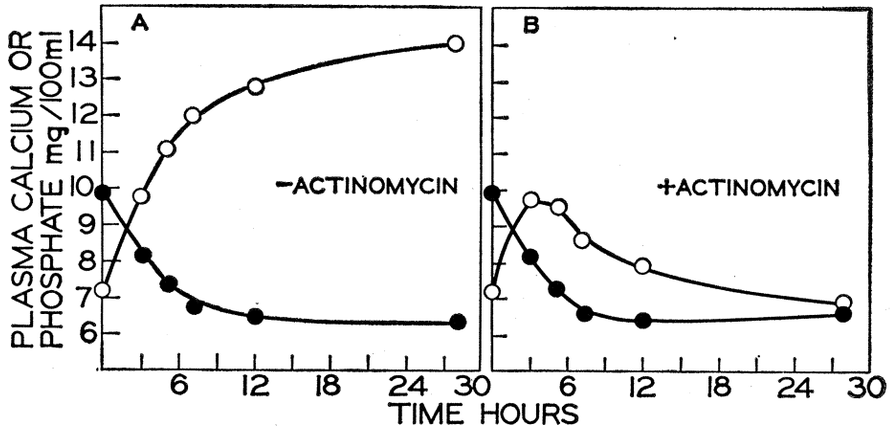

Fig. 1. The changes in plasma calcium (open circles) and plasma phosphate (closed circles) as a function of time after the intraperitoneal injection of 200 μg of purified bovine parathyroid hormone into parathyroidectomized rats weighing 150 g. The values on the left are for control animals, and those on the right for animals given 1 μg of actinomycin D per gram of body weight 2 hours before the injection of hormone. Each point represents the mean of the plasma values of 12 rats.

days before the study was done, and the rats were maintained on a high calcium diet. We used methods previously described (*10*).

The administration of hormone to the control animals (Fig. 1, left) produced the expected prompt and sustained fall in plasma phosphate and a slow but progressive rise in plasma calcium. The same dose of hormone to the animals given actinomycin D produced the same sustained fall in plasma phosphate; but after an early initial rise in plasma calcium, the concentration of this ion gradually declined in contrast to its progressive rise in the plasma of the control animals (Fig. 1, right). It is important to note that the plasma phosphate concentration in animals receiving actinomycin D but no hormone rose to concentrations higher than those observed in the animals given no hormone and no actinomycin. In spite of this, the amounts of phosphate in the blood of animals given both actinomycin D and parathyroid hormone were maintained, over the entire 28 hours, at the same low concentrations as that of animals given only hormone.

The early rise in the plasma calcium in the animals receiving both agents is probably due to the hormonal stimulation of the absorption of calcium from the gastrointestinal tracts of these animals maintained on a high calcium diet.

The results suggest that the renal response to this hormone is unimpaired in the presence of actinomycin whereas the osseous response to hormone is inhibited by this agent.

In order to verify further these suggestions, we studied the time course of the excretion of calcium and phosphate in the urine of parathyroidectomized animals maintained for long periods of time by the constant infusion of a glucose and salt solution into the tail vein (*11*). After an appropriate period of time, parathyroid hormone was added to the perfusion mixture and given at a rate of 7 μg/hr for 24 hours or more. Some animals were given actinomycin D (0.15 μg/g) before hormonal infusion. Typical responses observed in a control (left) and actinomycin-treated animal (right) are shown in Fig. 2.

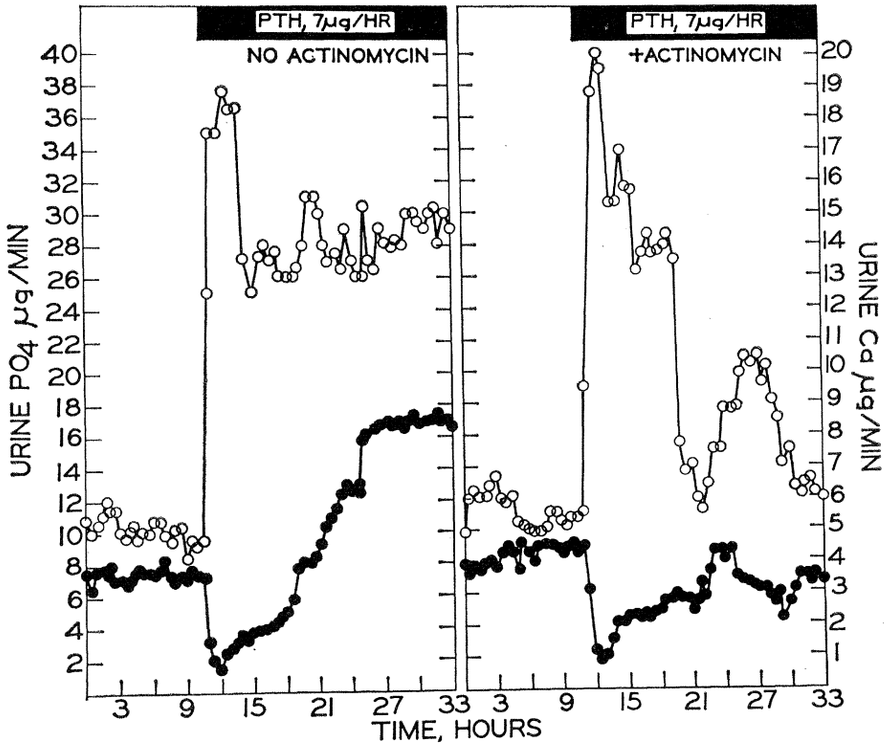

Fig. 2. The excretion of calcium (closed circles) and phosphate (open circles) in the urine during the constant infusion of calcium, magnesium, sodium, potassium, and glucose into a parathyroidectomized rat. After approximately 10.5 hours parathyroid hormone was added, and the infusion was continued for another 23 hours. The values on the left are for control animals, and those on the right for animals given 0.15 μg of actinomycin D per gram of body weight 12 hours before hormone infusion. Note that this dose of actinomycin D was approximately one-eighth of that given in the experiments shown in Fig. 1.

In both animals the immediate responses were the same: a prompt increase in phosphate, and a prompt decrease in calcium excretion. However, striking differences in response became apparent as hormone perfusion was continued. In the control animal, phosphate excretion remained elevated through the course of the experiment; and after 2 hours of hormone infusion, calcium excretion rose progressively until its rate of excretion was three times that observed before hormone treatment. On the other hand, phosphate excretion progressively declined in the animal treated with actinomycin D until it eventually reached the level of before treatment, and calcium excretion never rose above the level of before treatment in these animals even though it did tend to return to the values of before treatment after 2 to 3 hours of hormone perfusion.

Both the sustained phosphaturia and the progressive rise in calcium excretion observed in the control animal receiving hormone can be considered to result from mobilization of bone mineral. The lack of both these events in the actinomycin-treated animal indicates that the hormone was not effective in mobilizing these ions from bone in the presence of actinomycin.

The results shown in Fig. 2 fully confirm those recorded in Fig. 1 and lead to the conclusion that actinomycin D blocks the action of this hormone on bone but not its action on the kidney. Furthermore, the results suggest that the action of parathyroid hormone on bone cells leads to an increased synthesis of RNA and protein and that this response is necessary for the development of osteolytic activities upon the part of these cells.

Perhaps of most interest are the questions which are raised by these results. In recent years, there has been increasing interest in the possibility that many hormones act by regulating gene activity (*12*). In many of these cases, all the major effects of the hormone on a given tissue have been blocked by the administration of agents which block either protein or RNA synthesis (*12*). In other cases, such as insulin action on the rat diaphragm, these agents have been found to block some but not all the effects of the hormone on this particular tissue (*13*). In the present case, we have a situation where one of these agents appears

to block the effect of a hormone upon one receptive tissue, but has no apparent effect upon its action on a second receptive tissue. We are led to one of two alternatives. Either the actions of this hormone upon these two tissues are fundamentally different, or the hormone exerts the same basic action on both tissues; but because of the biochemical uniqueness of the respective tissues, the ultimate physiological expressions of its action are entirely different in the two tissues. The latter possibility seems more likely in the case of parathyroid hormone.

There is considerable evidence which indicates that the fundamental action of this hormone is upon the translocation of ions across cellular and subcellular membranes (*3*). Kroeger has recently found that changes in gene activity, or at least chromosomal morphology, can be brought about by changes in the ionic environment of cells (*14*). The link between altered ion transport and gene activation in the present circumstance is unknown.

HOWARD RASMUSSEN
CLAUDE ARNAUD
CHARLES HAWKER
Department of Biochemistry, University of Wisconsin, Madison

References and Notes

1. R. O. Greep, in *The Hormones*, G. Pincus and K. V. Thimann, Eds. (Academic Press, New York, 1948), vol. 1, chap. 7.
2. H. Rasmussen, *Am. J. Med.* **30**, 112 (1961).
3. H. Rasmussen and H. F. DeLuca, *Ergeb. Physiol. Biol. Chem. Exptl. Pharmakol.* **53**, 108 (1963).
4. P. J. Gaillard, in *The Parathyroids*, R. O. Greep and R. V. Talmage, Eds. (Thomas, Springfield, Ill., 1961), p. 20.
5. D. B. Kroon, *Acta Morphol. Neerlardo-Scand.* **2**, 38 (1958); P. Goldhaber, *New Engl. J. Med.* **266**, 870 (1962).
6. J. P. Weinmann and H. Sicher, *Bone and Bones* (Mosby, St. Louis, 1955); H. T. Kirby-Smith, *Am. J. Anat.* **53**, 377 (1933).
7. N. Hancox, in *The Biochemistry and Physiology of Bone*, G. H. Bourne, Ed. (Academic Press, New York, 1956), chap. 8.
8. E. Reich, *Cancer Res.* **23**, 1428 (1963); *Science* **143**, 684 (1964).
9. H. Rasmussen and L. C. Craig, *Biochim. Biophys. Acta* **56**, 332 (1962).
10. H. Rasmussen, H. F. DeLuca, C. Arnaud, C. Hawker, M. Von Stedingk, *J. Clin. Invest.* **42**, 1940 (1963).
11. These perfusion studies were carried out with a modification of the method developed by E. Cotlove, *J. Appl. Physiol.* **16**, 764 (1961).
12. P. Karlson, *Perspectives Biol. Med.* **6**, 203 (1963); I. Edelman, R. Bogoroch, G. A. Porter, *Proc. Natl. Acad. Sci. U.S.* **50**, 1169 (1963).
13. D. Eboue-Bonis, A. M. Chambant, P. Volfin, H. Clauser, *Nature* **199**, 1183 (1963).
14. H. Kroeger, *ibid.* **200**, 1234 (1963).
15. Supported by USPHS grant A-5762. The actinomycin D was obtained either from Dr. Norman Brink, Merck and Company, Rahway, N.J., or the Cancer Chemotherapy National Service Center, National Institutes of Health. We thank Miss Judith Jacobsen and Miss Berit Johansson for expert technical assistance.

16 March 1964

Wheat Gibberellins

Abstract. *Two "gibberellin-like" substances were present in malted wheat, compared to only one in sound, unmalted wheat. Alcoholic extracts of wheat malt fractionated by paper chromatography and bioassayed for gibberellin-like activity by three methods, indicated a new gibberellin in malted wheat. This component increased particularly in the embryo section of the sprouted wheat during the first 3 to 4 days of germination.*

Substances inducing responses in plants similar to gibberellic acid have been isolated from many of the higher plants (*1*) including barley (*2*), wheat (*3*), and other grasses (*4*). While some plants contain more than one gibberellin (*1*), only one has been reported for wheat. We have isolated from malted wheat a second compound with gibberellin-like properties which differs chromatographically from the one previously reported (*3*).

Wheat malt was extracted with 80 percent methanol, 80 percent ethanol, or 50 percent acetone for 24 hours at 23° to 25°C. Extracts were partially purified by the ethyl acetate-buffer procedure (*3*). The concentrated extracts were chromatographed on paper with solvent systems as follows: *n*-amyl alcohol, pyridine, and water, 35:35:30; *n*-butanol and 1.5*N* ammonium hydroxide, 3:1; and *n*-butanol, acetic acid, and water, 95:5:30. The papers were dried, treated with sulfuric acid, and exposed to ultraviolet light to detect the gibberellin (*5*). Other similar papers were cut to appropriate sections at right angles to the flow of the solvents, and the gibberellins were extracted with ethanol.

Bioassays for gibberellin-like activity were of three types: the dwarf pea seedling test, wheat-leaf section test (*3*), and a wheat endosperm test. The wheat endosperm assay was based on the effect of gibberellins on the formation of alpha-amylase in wheat endosperm. Distal halves of wheat were incubated for 2 days between filter paper moistened with aqueous solutions of the eluated materials. Contamination by fungi was prevented by including formaldehyde (0.05 percent) in the liquid. Endosperm pieces were tested for alpha-amylase by placing them on starch-agar gels and incubating them for 18 hours at 30°C (*6*).

The R_F values obtained for gibberellic acid with the three solvent systems employed agreed very closely with those reported previously (*5*). Faint fluorescent spots (at R_F 0.85) were observed on a few chromatograms of ethanolic extracts of malt when the butanol–acetic acid–water solvent system was employed, but could not be detected routinely in agreement with Radley (*3*) and Phinney *et al.* (*5*).

Pea seedlings receiving treatment by the chromatographic fractions having R_F values of 0.2 to 0.3 and 0.8 to 0.9 and gibberellic acid exhibited significantly more growth than the control seedlings or those treated with the other chromatographic fractions. This was emphasized particularly by the 5th day of growth of the pea seedlings. The chromatographic mobility of gibberellic acid was identical to that fraction having R_F of 0.8 to 0.9 and agrees with that previously reported (*3*). The active fraction having R_F of 0.2 to 0.3 has not been previously reported and was found only in malted wheat. However, the amount isolated appeared to be less than that of the fraction having R_F of 0.8 to 0.9.

The presence of two active components in ethanol and methanol extracts was also demonstrated by the wheat-leaf section test. A single gibberellin-like substance, having an R_F equivalent to gibberellic acid was detected in the acetone extract. The

Table 1. Comparison of chromatographic mobility (R_F) of biologically active gibberellin-like fractions of wheat malt extracts and gibberellic acid. The solvent systems were: A, *n*-butanol and 1.5*N* ammonium hydroxide, 3:1; B, *n*-amyl alcohol, pyridine, and water, 25:25:30; C, *n*-butanol, *n*-acetic acid, and water, 95:5:30. F, fluorescence; PS, pea seedling; WL, wheat leaf; WE, wheat endosperm; x, slow-moving component; y, fast-moving component.

Test employed	Solvent system				
	A	B		C	
		x	y	x	y
Gibberellic acid					
F	0.25		0.60		0.85
Ethanol extract					
P S	0.25	0.19	0.62	0.26	0.85
W L	0.25	0.17	0.62	0.24	0.84
W E	0.27	0.17	0.62	0.24	0.86
Methanol extract					
P S	0.24	0.17	0.63	0.24	0.83
W L	0.26	0.15	0.59	0.23	0.85
Acetone extract					
W E	0.27	0.15	0.63	0.25	0.85
P S	0.27		0.63		0.86
W E	0.25		0.61		0.85
W L	0.27		0.61		0.83