which is relatively inaccessible to the predator, the prey can multiply in safety while releasing progeny into the hostile liquid phase. The wall of the culture vessel provides such a refuge. The onset of growth on the wall in the experimental populations was preceded by one complete oscillation of the population densities, that is, predation decreased the bacterial density from 2.5×10^8 cells per milliliter to a residual population of 2×10^5 cells per milliliter. Slight wall growth then appeared.

An electrostatic attractive force of about 10^{-8} dyne is required to hold a bacterium against the wall of a culture stirred at 300 rev/min (*11*). To dislodge adherent bacteria, *Tetrahymena* would have to increase the strength of its ciliary currents. Alternatively, attachment to the wall, plus an increase in ciliary action, might dislodge attached prey. Both adaptations would, however, involve major morphological and physiological changes akin to speciation.

The lag before wall growth first appears and the lack of wall growth in the control cultures strongly suggest that, in this particular system, adherence to the vessel wall is a specific adaptation avoiding predation. Although the control cultures did not exhibit significant wall growth for the duration of the experiments, the subsequent addition of *Tetrahymena* to these cultures resulted in the appearance of wall growth after about 250 hours. Bacteria isolated from the walls of the culture vessel on termination of the experiment showed a marked preference for growth on solid surfaces or in large cell aggregates. Attempts were made to measure the amount of wall growth by suspending glass slides in the culture vessel, scraping the adherent cells into buffer, and counting under the microscope. Densities of 10^5 to 10^6 cells per square centimeter were found.

Apparently genetic feedback (*12*) occurs within the populations ultimately stabilizing the predator-prey relationship at a level that ensures the survival of both species. Natural selection in predator-prey systems increases the efficiency of the predator in finding and eating its prey but it also favors those individual prey which escape being eaten. If adherence to the walls of the culture vessel enables a bacterium to survive and reproduce, these variants have significant selective advantage in the presence of the predator and eventually "take over" the bacterial population. This system provides a specific example of niche diversification, the creation and utilization of a separate niche in what was initially chosen to be a "homogeneous" experimental system.

P. VAN DEN ENDE

School of Plant Biology,
University College of North Wales,
Bangor, Caernarvonshire, North Wales

References and Notes

1. R. P. Canale, *Biotechnol. Bioeng.* **12**, 353 (1970).
2. R. M. May, *Science* **177**, 900 (1972).
3. L. B. Slobodkin, *Growth and Regulation of Animal Populations* (Holt, Rinehart and Winston, New York, 1961), chap. 13.
4. G. F. Gause, *The Struggle for Existence* (Williams & Wilkins, Baltimore, 1934).
5. P. H. Leslie and J. C. Gower, *Biometrika* **47**, 219 (1960).
6. C. B. Huffaker, K. P. Shea, S. G. Herman, *Hilgardia* **34**, 305 (1963).
7. S. Utida, *Ecology* **38**, 442 (1957).
8. J. L. Meers, *J. Gen. Microbiol.* **67**, 359 (1971).
9. This system was not a chemostat, but a continuous culture. As no attempt was made to control the chemical conditions existing in the mixed culture, any inhibitory or stimulatory by-products of the predator-prey interaction could affect the density of either one or both of the species.
10. C. L. Curds and A. Cockburn, *J. Gen. Microbiol.* **54**, 343 (1968).
11. R. J. Munson and B. A. Bridges, *ibid.* **37**, 411 (1964).
12. D. Pimentel, *Science* **159**, 1432 (1968).
13. Supported by a grant from the Science Research Council to Professor J. L. Harper. I thank Professor Harper for guidance at various stages of the work, and Elaine Jones for technical assistance.

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Intestinal Calcium Transport: Stimulation by Low Phosphorus Diets

Abstract. *Rats maintained on a low phosphorus diet supplemented with 25-hydroxyvitamin D_3 show high intestinal calcium transport activity as compared to rats similarly treated but fed a diet containing adequate phosphorus. This increased transport activity is correlated with an increased biosynthesis of 1,25-dihydroxyvitamin D_3, the probable metabolically active form of the vitamin in the intestine.*

Vitamin D must be hydroxylated in the C-25 position by the liver (*1*, *2*) and in the C-1 position by the kidney (*3*) before it can function to stimulate both intestinal absorption of calcium (*4*) and mobilization of bone calcium (*5*). In addition, strong evidence indicates that the resulting 1,25-dihydroxyvitamin D_3 [1,25-$(OH)_2D_3$] does not require further metabolic alteration before it initiates both intestinal calcium absorption and bone calcium mobilization (*6*). Therefore, it appears that 1,25-$(OH)_2D_3$ is at least one of the hormonal forms derived from vitamin D responsible for the mobilization of calcium from these two organs. This concept was strengthened by the find-

Table 1. Stimulation of intestinal calcium transport and accumulation of 1,25-$(OH)_2D_3$ in blood and intestine of rats fed a low phosphorus diet. Weanling rats were fed a vitamin D–deficient diet containing 1.2 percent calcium and either 0.1 or 0.3 percent phosphorus for 2 weeks. At that time the rats receiving 0.1 percent phosphorus weighed 76 ± 6 g (24 rats) and those receiving 0.3 percent phosphorus weighed 88 ± 10 g (20 rats). At this stage a third of the rats in each group received 130 pmole of 25-OHD_3 orally each day in cottonseed-soybean (Wesson) oil; another third received 130 pmole of 25-OH[^{3}H]D_3 orally each day in Wesson oil, and the other third received only Wesson oil each day. All rats were killed by decapitation 2 weeks after this treatment was begun. The tissues from three or four rats receiving 25-OH[^{3}H]D_3 were pooled for each group, extracted, and chromatographed (*13*) to determine the amount of 1,25-$(OH)_2D_3$, while the other rats (at least six per group) were used to measure intestinal calcium transport by the everted sac method (*12*). Serum calcium and phosphorus were measured in all animals. Standard deviations are given.

Treatment	Body weight (g)	Serum calcium (mg/100 ml)	Serum phosphorus (mg/100 ml)	Calcium transport (inside/outside)	1,25-$(OH)_2D_3$ in Intestinal mucosa (pmole/g)	1,25-$(OH)_2D_3$ in Serum (pmole/ml)
		0.1 percent dietary phosphorus				
Oil	104 ± 10	10.7 ± 0.4	1.7 ± 0.3	1.6 ± 0.1		
25-OHD_3	116 ± 11	13.1 ± 0.7	6.5 ± 0.8	7.9 ± 0.6*	0.760	1.07
		0.3 percent dietary phosphorus				
Oil	154 ± 9	11.6 ± 0.4	3.9 ± 0.3	2.0 ± 0.2		
25-OHD_3	165 ± 16	11.7 ± 0.4	9.8 ± 0.7	3.9 ± 0.3†	0.322	0.395

* Significantly different from † ($P < .001$).

ing that the biosynthesis of 1,25-$(OH)_2D_3$ in the kidney is subject to strong feedback regulation in some manner by serum calcium, which is in turn determined to some degree by dietary calcium (*7*). Thus, the adaptation of intestinal calcium absorption to dietary calcium can be accounted for at least in part by regulation of 1,25-$(OH)_2D_3$ synthesis.

The parathyroid glands sense the serum calcium concentration and secrete parathyroid hormone in response to hypocalcemia. The parathyroid hormone plays a determining role in the synthesis of 1,25-$(OH)_2D_3$ (*8*). Hypophosphatemia also triggers 1,25-$(OH)_2D_3$ synthesis, and it appears that a low concentration of inorganic phosphorus in the renal tubule cell may actually be responsible for increased 1,25-$(OH)_2D_3$ synthesis in response to parathyroid hormone (*9*).

Morrissey and Wasserman demonstrated that chicks on low phosphorus diets show increased rates of calcium absorption and increased intestinal production of calcium-binding protein (*10*). It seemed likely that these observations might be accounted for by an increased production of 1,25-$(OH)_2D_3$, the hormonal form of vitamin D_3 responsible for increased intestinal calcium absorption.

Weanling male rats from a low vitamin D colony (Holtzman, Madison, Wisconsin) were fed a vitamin D–deficient diet (*11*) containing 1.2 percent calcium and either 0.1 or 0.3 percent phosphorus for a total of 4 weeks. The animals were maintained in hanging wire cages and had free access to food and water. For each concentration of phosphorus, a third of the rats received 130 pmole daily of nonradioactive 25-hydroxyvitamin D_3 (25-OHD_3) orally in 0.1 ml of cottonseed-soybean (Wesson) oil for the last 2 weeks. Another third of the rats received 130 pmole of tritiated 25-OHD_3 orally each day, while the remaining third received the 0.1 ml of Wesson oil vehicle daily during the last 2 weeks. At this time, all rats were killed by decapitation. Serum was collected from each rat. The rats receiving the oil vehicle or the nonradioactive 25-OHD_3 were used for measurements of intestinal calcium transport by the everted sac method (*12*). Intestine, kidney, and serum were immediately collected from the rats receiving the 25-OH[^{3}H]D_3. Intestinal mucosa, kidney, and blood were homogenized with five volumes of distilled water, extracted with a methanol-chloroform mixture (*1*), and chromatographed on a Sephadex LH-20 column prepared and developed in chloroform, Skellysolve B (65:35, by volume) (*13*). Portions of tissue homogenates or blood were dissolved in Nuclear-Chicago solubilizer to determine total radioactivity. Column effluents were collected and dried under a stream of air. To each sample was added a toluene counting solution (*1*), and ^{3}H was determined in a Packard liquid scintillation counter (model 3375) equipped with automatic external standardization. The amount of metabolite in each tissue was calculated from the specific radioactivity of the 25-OH[^{3}H]D_3 given, since these animals had no other source of vitamin D.

Serum calcium concentration was determined with a Perkin-Elmer atomic absorption spectrometer (model 402) in the presence of 0.1 percent lanthanum chloride. Inorganic phosphorus was measured by the method of Chen *et al.* (*14*).

The 25-OHD_3 used was kindly supplied by John Babcock of the Upjohn Company, Kalamazoo, Michigan. The 25-OH[^{3}H]D_3 was prepared in this

Table 2. Dietary phosphorus and the metabolism of 25-OH[^{3}H]D_3. The rats were fed vitamin D–deficient diets containing 1.2 percent calcium and either 0.1 or 0.3 percent phosphorus for 2 weeks. At this time each rat received 135 pmole of 25-OH[^{3}H]D_3 orally in Wesson oil daily for 14 days. The rats were killed by decapitation for the radioactivity determinations. The samples of serum or tissue homogenate were solubilized in Nuclear-Chicago solubilizer to measure the total radioactivity in the tissues. For the determination of 1,25-$(OH)_2D_3$, the tissues were extracted and chromatographed (*13*). The tissues from four rats in each group were pooled and analyzed; dpm, disintegrations per minute.

Dietary phosphorus content (%)	Radioactivity in			Radioactivity as 1,25 = $(OH)_2D_3$ in	
	Intestinal mucosa (dpm per gram of tissue)	Serum (dpm/ml)	Kidney (dpm per gram of tissue)	Intestinal mucosa (dpm per gram of tissue)	Serum (dpm/ml)
0.1	11,650	31,425	16,125	2,867	4,044
0.3	11,125	31,620	16,875	1,216	1,489

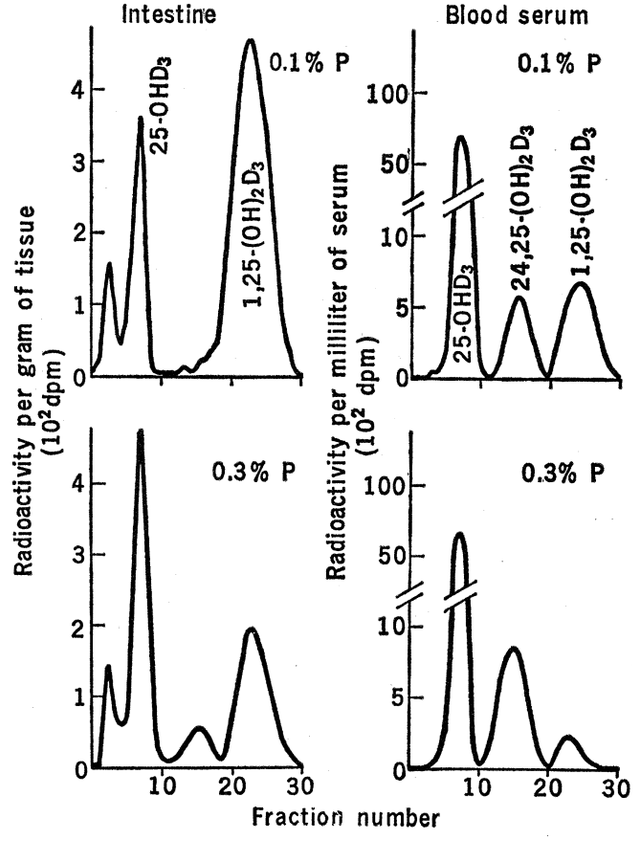

Fig. 1. Chromatographic profiles of methanol-chloroform extracts of intestinal mucosa and serum of rats fed a diet containing low phosphorus (0.1 percent) or normal phosphorus (0.3 percent). The 2 by 12 cm column contained 10 g of Sephadex LH-20 and was eluted with chloroform, Skellysolve B (65 : 35) (Skellysolve B is a light petroleum ether fraction redistilled at 67° to 69°C) as described by Holick and DeLuca (*13*). Fractions of 5.5 ml were collected, and the radioactivity in each was determined; dpm, disintegrations per minute.

laboratory (*15*), and Sephadex LH-20 was purchased from Pharmacia, Piscataway, New Jersey.

Rats on the low phosphorus diet develop severe hypophosphatemia, which is partially corrected by the administration of 25-OHD_3 (Table 1). These animals have marked intestinal calcium transport activity, even more marked than that observed in rats on low calcium diets (*16*). Rats fed a normal amount of phosphorus (0.3 percent) show a hypophosphatemia that is corrected by the 25-OHD_3 treatment. Although calcium transport is high, it is approximately half that of rats on the low phosphorus diet. Thus, in agreement with Morrissey and Wasserman (*10*), low phosphorus diets result in high rates of calcium absorption in rats as well as in chicks.

Because low phosphorus diets that result in hypophosphatemia have been shown to result in increased biosynthesis of 1,25-$(OH)_2D_3$ (*9*), the vitamin D–derived hormone responsible for the initiation of intestinal calcium absorption, it was of interest to determine if the increased transport of calcium could be correlated with the production of 1,25-$(OH)_2D_3$ and its accumulation in the intestine. The low phosphorus diet stimulates the accumulation of 1,25-$(OH)_2D_3$ in blood and intestine (Fig. 1); there is more than twice as much 1,25-$(OH)_2D_3$ in the intestine and blood of rats on the low phosphorus diet than in those of animals on the normal phosphorus diet (Table 1). More 24,25-dihydroxyvitamin D_3 [24,25-$(OH)_2D_3$] is observed in the chromatographic profiles of rats on the 0.3 percent phosphorus diet than in those from rats on the 0.1 percent phosphorus diet.

Dietary phosphorus does not affect the accumulation of radioactivity in blood serum, kidney, and intestine, whereas it markedly affects the amount of that radioactivity appearing as 1,25-$(OH)_2D_3$ (Table 2). Thus, low phosphorus diets and the resulting hypophosphatemia stimulate 1,25-$(OH)_2D_3$ production, which is probably responsible for increased calcium transport and may contribute further to the hypercalcemia. The increased accumulation of 1,25-$(OH)_2D_3$ under conditions of hypercalcemia and hypophosphatemia, in which there should be little or no parathyroid secretion, demonstrates that parathyroid hormone is not necessary for 1,25-$(OH)_2D_3$ production in the hypophosphatemic animal. It also provides support for the idea that the inorganic phosphate concentration in renal cells is an important determinant of 1,25-$(OH)_2D_3$ production.

Y. TANAKA
H. FRANK
H. F. DELUCA

Department of Biochemistry, College of Agricultural and Life Sciences, University of Wisconsin, Madison 53706

References and Notes

1. J. W. Blunt, H. F. DeLuca, H. K. Schnoes, *Biochemistry* **7**, 3317 (1968).
2. G. Ponchon, A. L. Kennan, H. F. DeLuca, *J. Clin. Invest.* **48**, 2032 (19(M. Horsting and H. F. DeLuca, *Biochem. Biophys. Res. Commun.* **36**, 251 (1969).
3. M. F. Holick, H. K. Schnoes, H. F. DeLuca, *Proc. Nat. Aacd. Sci. U.S.A.* **68**, 803 (1971); ———, T. Suda, J. Cousins, *Biochemistry* **10**, 2799 (1971); D. R. Fraser and E. Kodicek, *Nature* **228**, 764 (1970).
4. I. T. Boyle, L. Miravet, R. W. Gray, M. F. Holick, H. F. DeLuca, *Endocrinology* **90**, 605 (1972).
5. M. F. Holick, M. Garabedian, H. F. DeLuca, *Science* **176**, 1146 (1972); R. G. Wong, A. W. Norman, C. R. Reddy, J. W. Coburn, *J. Clin. Invest.* **51**, 1287 (1972).
6. C. A. Frolik and H. F. DeLuca, *Arch. Biochem. Biophys.* **147**, 143 (1971); *J. Clin. Invest.* **51**, 2900 (1972).
7. I. T. Boyle, R. W. Gray, H. F. DeLuca, *Proc. Nat. Acad. Sci. U.S.A.* **68**, 2131 (1971); I. T. Boyle, R. W. Gray, J. L. Omdahl, H. F. DeLuca, in *Endocrinology 1971*, S. Taylor, Ed. (Heinemann, London, 1972), pp. 468–476.
8. M. Garabedian, M. F. Holick, H. F. DeLuca, I. T. Boyle, *Proc. Nat. Acad. Sci. U.S.A.* **69**, 1673 (1972); H. Rasmussen, M. Wong, D. Bikle, D. B. P. Goodman, *J. Clin. Invest.* **51**, 2502 (1972).
9. Y. Tanaka and H. F. DeLuca, *Arch. Biochem. Biophys.* **154**, 566 (1973).
10. R. L. Morrissey and R. H. Wasserman, *Amer. J. Physiol.* **220**, 1509 (1971).
11. T. Suda, H. F. DeLuca, Y. Tanaka, *J. Nutr.* **100**, 1049 (1970).
12. D. L. Martin and H. F. DeLuca, *Amer. J. Physiol.* **216**, 1351 (1969).
13. M. F. Holick and H. F. DeLuca, *J. Lipid Res.* **12**, 460 (1971).
14. P. S. Chen, Jr., T. Y. Toribara, H. Warner, *Anal. Chem.* **28**, 1756 (1956).
15. T. Suda, R. B. Hallick, H. F. DeLuca, H. K. Schnoes, *Biochemistry* **9**, 1651 (1970).
16. D. V. Kimberg, D. Schachter, H. Schenker, *Amer. J. Physiol.* **200**, 1256 (1961).
17. Supported by PHS grant AM-14881, AEC contract AT(11-1)-1668, and by the Food Research Institute of the University of Wisconsin, Madison.

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Virus-Induced Cholesterol Crystals

Abstract. *One of the crystal types induced in cell cultures by a new feline herpesvirus was identified as cholesterol by crystal structure, polarized light microscopy, and mass spectroscopy.*

During our studies on virus-induced feline urolithiasis, we isolated a virus which produced cytopathic effects of the adenovirus type in cell cultures (*1*). This virus has been identified as a new feline herpesvirus. Intracellular and extracellular chemical crystals were observed in cell cultures infected with this virus. The crystals varied in size, shape, and structure, and some were birefringent when examined in polarized light (*1*).

The crystal formations were first observed in cell cultures derived from tissues of spontaneously obstructed cats. Similar formations have been seen in cell cultures of the stable Crandell feline kidney (CRFK) cell line infected with this herpesvirus. The crystals have not been observed in the uninfected control CRFK cells. The initial demonstration of the various crystal types in infected cells made with a microscope with either conventional or polarized light was confirmed by examination of ultrathin sections of similar cells with an electron microscope.

During the examination of unstained, living cell cultures derived from kidneys, bladders, and hearts of cats with either spontaneous or experimental cases of urolithiasis, many fat globules were observed in supernatant fluids within 24 hours after the initiation of culture. These fat globules persisted. On occasion a cell was seen to release fat globules into the culture fluids. Many cells in these cultures contained

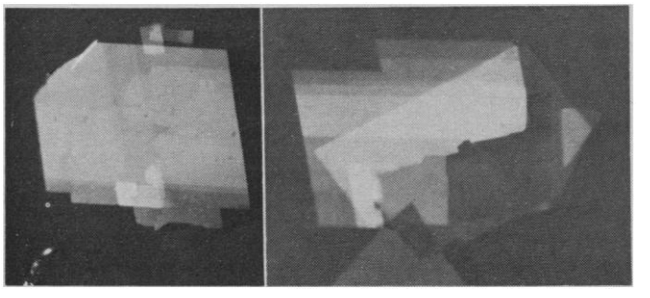

Fig. 1. Cholesterol crystals from a cell culture infected with a new feline herpesvirus photographed with polarized light (× 310).