

would mask the effects of the adaptation. Any arrangement of the chemical environment so that ion exchange between the spores and the external medium would be minimal during lethal heating should also reduce the curvature of the death-rate curves. We have obtained fairly straight log survivor curves at moderate lethal temperatures for water suspensions of stripped spores neutralized to various degrees with hydroxides. As mentioned previously, heating hydrogen-form (stripped) spores in calcium buffer at moderately lethal temperatures gives a survivor curve with upward curvature. When heat adapted (calcium loaded) spores freshly inserted into an acidic buffer are subjected to similar moderate lethal heat, the logarithmic survivor curve shows an accelerating death rate or downward curvature.

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Vitamin D₃: Direct Action on the Small Intestine of the Rat

Abstract. Vitamin D₃ placed directly into loops of rat duodenum in vitamin D deficient animals increases markedly the subsequent transport of calcium by slices of the duodenal loop in vitro. Under similar conditions the same dose of vitamin given intravenously or placed in a jejunal loop has little or no effect on the duodenal tissue. Thus the vitamin acts directly on the small intestine without prior activation in another organ.

Studies with segments of small intestine in vitro demonstrate that vitamin D is required to maintain an active transport mechanism for calcium absorption in the mucosa (1, 2). Although this end result of the administration of vitamin D to intact animals is well-established, the biochemical mechanism and initial site of action of the sterol remain unknown. Addition of the vitamin to media containing intestinal segments in vitro has failed to affect the

calcium transport detectably. Sallis and Holdsworth have suggested that the vitamin must be activated in the adrenal prior to its intestinal action (3). This hypothesis is untenable in the case of the rat, for adrenalectomized, vitamin D deficient rats respond normally to administration of the vitamin (4). More generally, the following experiments demonstrate that vitamin D₃ acts directly on the small intestine of the rat, and the vitamin need not be activated in another organ.

Initial experiments compared the effects of vitamin D₃ given intravenously with equal doses placed in loops of duodenum tied *in situ*. The duodenum is the segment of maximal active transport of calcium in the rat, and the oxygen-dependent accumulation of Ca⁴⁵ (or Ca⁴⁷) by full-thickness slices of duodenum in vitro provides a sensitive method for the quantitative measurement of the action of the vitamin (2). When given to deficient rats, large doses of vitamin D act rapidly and small doses act slowly (2). Intermediate doses in the range of 100 to 400 I.U. of vitamin D₃ per rat yield suboptimal but detectable effects in 5 hours. In this period of time less than 5 percent of an intravenous dose of tritium-labeled vitamin D₃ accumulates in the duodenum, whereas over 80 percent of the same dose placed in a duodenal loop is absorbed into the mucosa (5). Under these conditions, therefore, vitamin D₃ placed in the loop would be much more effective than an intravenous dose if the action were direct and less effective if the sterol required prior activation in another organ.

The results of four experiments demonstrate that the action is direct (Table 1). Weanling male rats were maintained on the USP rachitogenic diet No. 2 (6) for 4 to 5 weeks in cages shielded from light. In each experiment three groups of eight depleted rats were anesthetized with ether. In one group (controls) a duodenal loop was tied in each rat (7) and the loop was filled with 0.25 ml of 0.85 percent NaCl and 0.01M sodium taurocholate (8); the common bile duct was ligated. The second group was treated similarly but given 100 to 400 international units (I.U.) of vitamin D₃ intravenously in 0.25 ml of the saline-taurocholate vehicle. The solutions were clear. The third group was treated similarly but the dose of vitamin D₃ was included in the solution used to fill each duodenal loop. Five hours after the operation the ani-

Table 1. Effect of vitamin D₃ given intravenously or placed directly in a loop of duodenum on the oxygen-dependent accumulation of calcium by slices of duodenum in vitro.

Dose of D ₃ (I.U./rat)	Oxygen-dependent accumulation of calcium (mμmole/g of slices per hour)		
	No D ₃	Intravenous D ₃	Loop D ₃
100	63	55	102
200	76	78	177
200	86	62	114
400	108	148	175

mals were sacrificed, and slices were prepared from the duodenal loops and tested for calcium accumulation in vitro (2). At 100 and 200 I.U., a distinct increase in calcium transport over the control values was observed only in loops exposed directly to the vitamin, with no increase after intravenous administration. At 400 I.U., some increase followed the intravenous dose, but a greater effect was observed in the loops exposed directly (Table 1).

A second type of experiment confirmed the foregoing results. Inasmuch as vitamin D is absorbed from the intestine into the chylomicron fraction of lymph (5), it was desirable to compare the effects on calcium transport in the duodenum when vitamin D₃ was placed either in a duodenal or a mid-jejunal loop of intestine. Absorption of vitamin D₃ from mid-jejunal loops is more rapid than from duodenal loops (5). Three groups, each containing eight vitamin-D deficient rats, were tested in each of two experiments. A duodenal and a mid-jejunal loop were tied in each rat

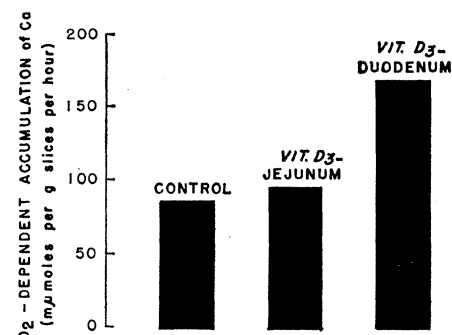


Fig. 1. Oxygen-dependent accumulation of calcium by slices of duodenum in vitro. The slices were prepared from duodenal loops tied 5 hours before the assay. When the loops were tied, the control rats received no vitamin D₃, the "vitamin D₃-jejunum" group was given 200 I.U. per rat in a jejunal loop, and the "vitamin D₃-duodenum" group was given 200 I.U. directly in each duodenal loop.

and filled as follows: (i) with 0.25 ml of the saline-taurocholate vehicle in all loops (controls); (ii) with 0.25 ml of vehicle containing 200 I.U. of vitamin D₃ in each jejunal loop and vehicle alone in the duodenal loops; (iii) with 200 I.U. of vitamin D₃ in each duodenal loop and vehicle alone in the jejunal loops. The common bile duct, which enters the duodenal loop, was ligated in each animal. Five hours after the operation, slices were prepared from the duodenal loops and tested for calcium transport in vitro. The results of both experiments were similar and the mean values are illustrated in Fig. 1. As compared to the control values, vitamin D₃ added to the jejunal loops did not significantly increase the calcium transport observed with duodenal slices, whereas a marked increment was observed with the duodenal tissues exposed directly to the vitamin.

The experimental results demonstrate clearly that the action of vitamin D₃ on calcium transport in the small intestine of the rat is not mediated by another organ. If the time lag required to observe the effect of the vitamin (2)

represents metabolism or intracellular translocation of the sterol molecule, these processes occur within the intestinal wall.

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Bacteriophage: An Unusual Hybrid of Serologically Unrelated Phages P22 and P221

Abstract. *Serologically unrelated bacteriophages P22 and P221 that grow on Salmonella typhimurium are described. The phage P22 and a mutant P22h have short tails with hexagonal base plates, whereas P221 has a long, flexible tail without a base plate. P22h and P221 can recombine to exchange genetic markers. In the course of the recombination experiment, an unusual hybrid, P22-221Hy, was found. It mutates to P221 and P22S at a frequency of 10^{-4} and 10^{-5} , respectively. Unlike P22h, P22S gives clear plaques on both S. typhimurium, St, and its mutant, St/22, but is morphologically and serologically indistinguishable from P22 and P22h. The use of single-clone technique on P22-221Hy proved that a P22-221Hy genome consists of a P22h and a P221 genome and is carried by the P22h capsid. However, it seems probable that the information for synthesizing the P221 capsid may be suppressed by the information for synthesizing the P22h capsid. P22-221Hy is a stable hybrid rather than a heterozygote, because it does not segregate by passing through host cells. Thus, one bacteriophage can carry the genetic information for two distinct bacteriophages.*

The bacteriophage P22 has a short tail with a hexagonal base plate but no contractile sheath. It does not form plaques on a mutant strain (St/22) of its host *Salmonella typhimurium* LT-2 (St). A mutant P22h, forming very faint plaques on St/22 and clear plaques on St, is morphologically and serologically indistinguishable from P22. Many preparations of P22 contain a small portion (about 10^{-10}) of a long-tailed phage, P221, which forms plaques on St/22 but not on St (1).

Three possibilities for the origin of P221 were considered, namely that P221 might be a mutant of P22, a prophage in St, or a defective prophage in St. Experiments were done to determine the most probable origin. The results may be summarized as follows (1, 2): (i) Neither head nor tail antigens of P221 are related to those of P22. (ii) All attempts to induce P221 formation from St were unsuccessful. (iii) The phage P221 was found in P22 stocks grown on St or its mutants

but it was not found in stocks grown on 20 other strains of *Salmonella* or in P22h stocks grown on St/22. (iv) P221 was found in stocks of four other phage strains (grown on St) that proved to be serologically related to P22. (v) P221 always has the same $c(c_1, c_2, c_3)$ markers, and g and h_{21} color markers as the P22 strain which gives rise to it. However, the m_3 color marker (3) of P22 never appeared in P221 (Table 1). From these results, it seems probable that a defective prophage exists in St but not St/22, and that P22 supplies the entire region containing $c(c_1, c_2, c_3)$, g , and h_{21} markers to complete the P221 genome.

Since the two phages are unrelated serologically yet partially related genetically, it seemed interesting to see whether progeny and their hybrids could be produced by the mixed infection of St/22. The mixed infection of St/22 by P22h and P221 produces masked genomes, that is, particles carrying P22h genomes in P221 capsids, and P221 genomes in P22h capsids. Moreover, P22h markers within the homologous region (c , g , and h_{21}) can be transferred to P221 genomes and vice versa (1, 2). However, the m_3 color marker of P22, situated outside the homologous region, has never been transferred to P221 genomes (2). Therefore, three possible structures for the P221 chromosome were suggested on the basis of the relation between the homologous and nonhomologous regions (Fig. 1). The frequencies of recombination (in the homologous region) were very small. This may be due to a partial exclusion (4). An explanation for the partial exclusion could be that P22 and P221 have different latent periods, of approximately 30 minutes and 40 minutes, respectively. From the lysate of the doubly lysogenic strain of St/22 for P22h and P221, the frequencies of recombinations are an order of magnitude larger than those obtained in the lysate of mixed infection of St/22 with P22h and P221. However, in the single-burst experiments of the doubly lysogenic strain St/22(P22h, P221), an indication of partial exclusion was observed. Some of the bacteria produced one of these phages (2). In such a case, frequency of recombination may not tell the true map structure. It would therefore be premature at this time to draw the definite map structure of the P221 chromosome.

While studying recombination, an unusual hybrid called P22-221Hy was