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 D_3 in each jejunal loop and vehicle alone in the duodenal loops; (iii) with 200 I.U. of vitamin D_3 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 D_3 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 D_3 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 P22*h *have short tails with hexagonal base plates, whereas P221 has a long, flexible tail without a base plate. P22*h *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 P22*S *at a frequency of* 10^{-4} *and* 10^{-5}, *respectively. Unlike P22*h, *P22*S *gives clear plaques on both* S. typhimurium, *St, and its mutant, St/22, but is morphologically and serologically indistinguishable from P22 and P22*h. *The use of single-clone technique on P22-221Hy proved that a P22-221Hy genome consists of a P22*h *and a P221 genome and is carried by the P22*h *capsid. However, it seems probable that the information for synthesizing the P221 capsid may be suppressed by the information for synthesizing the P22*h *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 P22*h*, 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 P22*h* 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_{2t} 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_{2t} 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 P22*h* and P221 produces masked genomes, that is, particles carrying P22*h* genomes in P221 capsids, and P221 genomes in P22*h* capsids. Moreover, P22*h* markers within the homologous region (c, g, and h_{2t}) 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 P22*h* 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 P22*h* and P221. However, in the single-burst experiments of the doubly lysogenic strain St/22(P22*h*, 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

Table. 1. Genetic markers contributed to P221 by P22.

P22 stock*	Markers found in P221 isolated from stock
P22$c_2h_{21}^+$	P221$c_2h_{21}^+$
P22$c_3h_{21}^+$	P221$c_3h_{21}^+$
P22c_1h_{21}	P221c_1h_{21}
P22$m_3c_3h_{21}^+$	P221$c_3h_{21}^+$
P22$m_3c + h_{21}$	P221$c + h_{21}$
P22g	P221g

* Stocks of the various P22 mutants listed in the left hand column were prepared and plated on St/22 for the isolation of P221 strains.

found by the following procedure. The lysate of mixed infection of St/22 with P22*h* and P221 was filtered through a 0.45-μ Millipore filter to remove bacteria and debris. The filtrate was then treated for 30 minutes with rabbit anti-P221 serum diluted to give $k_e = 5$ min^{-1}, the velocity constant of neutralization. The treated lysate was then assayed on St/22 with top agar containing anti-P221 serum diluted to give $k_e = 0.8$ min^{-1} to inhibit the development of the P221 phage which was masked by the P22*h* capsid. After overnight incubation at 37°C, clear, pinpoint plaques were found. The size of surviving plaques seemed to be suppressed by anti-P221 serum in top agar. The ratio of these survivors to the original titer of the bacteriophage was about 10^{-6}. Among the 2498 clones of these survivors, isolated and tested with antisera, 2496 clones were P221. Two clones were antigenically P22*h* and showed no cross reaction with the anti-P221 serum. Also, they were morphologically indistinguishable from P22*h*. They were named P22-221Hy. P22-221Hy forms clear, pinpoint plaques on St/22 but no plaques on St. However, St adsorbs P22-221Hy rapidly. From the foregoing it is concluded that St is immune to P22-221Hy. In addition, St/22(P221) is immune to P22-221Hy.

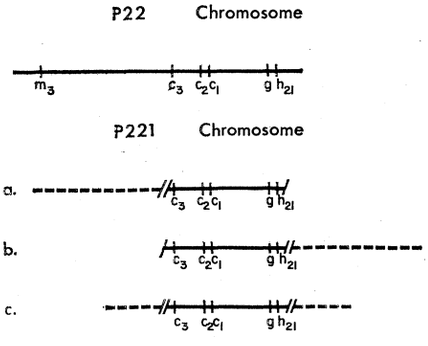

Fig. 1. Three possible structures of P221 chromosome. Solid line indicates region homologous to P22 chromosome. Dashed line indicates region nonhomologous to P22 chromosome. The symbols (/ and //) indicate uncertain location of end of the homologous region. The P22 chromosome was mapped by Levine and Curtiss (*3*).

A number of stocks was prepared from confluent-lysis plates originating from many single clones of P22-221Hy. Every stock of P22-221Hy contained a few percent of both P221 and P22*S*. Unlike P22*h*, P22*S* gives clear plaques on both St and St/22 strains, but it is morphologically and serologically indistinguishable from P22 and P22*h*. P22-221Hy is a stable hybrid rather than a heterozygote, because it does not segregate to P221 and P22*h* by passage through host cells. To further verify this phenomenon, the single-plaque clone technique (*5*) was applied. P221 and P22*S* were found in every plaque clone of P22-221Hy in a frequency of 10^{-4} and 10^{-5}, respectively. There is no explanation for the high frequency of P221 and P22*S* in stocks prepared from confluent-lysis plates.

To eliminate the possibility of P221 contamination, anti-P221 serum was used both in dilution test tubes and in top agar for single cloning technique of P22-221Hy. A number of isolates from a few passages with this treatment still gave the same results. A total of 120 clones of P22-221Hy were tested. All of them bred true, producing P221 and P22*S* in a frequency of 10^{-4} and 10^{-5}, respectively. P221 and P22*S* derived from P22-221Hy carried the same *c* marker and h_{21} color indicator marker. From these results, it may be concluded that P22-221Hy mutates to P221 and P22*S* in a high frequency.

It seems probable that P22-221Hy phage carries a genome which is capable of synthesizing two different phages (or two different phage capsids) but which is masked by only P22*h* capsid. The genetic information for making P221 capsid may be suppressed by that for making P22*h* capsid. To my knowledge, a similar hybrid has not been previously demonstrated for any other phage system.

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Fluoroacetate Inhibition of Amino Acids during Photosynthesis of Chlamydomonas reinhardti

Abstract. *Fluoroacetate inhibited respiration in* Chlamydomonas reinhardti *and greatly decreased the photosynthetic incorporation of* $C^{14}O_2$ *into glutamate and aspartate. This suggests that the Krebs cycle is important in the light-mediated synthesis of glutamate and aspartate in this alga.*

Although the photosynthetic incorporation of $C^{14}O_2$ into amino acids of *Scenedesmus* and *Chlorella* has been previously studied (*1*, *2*), many basic aspects of this problem have not been clarified for algae. Kinetic experiments on $C^{14}O_2$ fixation in *Chlorella pyrenoidosa* (*2*) during steady-state photosynthesis indicate that the formation of glutamate by way of glutamic dehydrogenase may not be the primary route of ammonium incorporation into the amino acids. Two lines of evidence were presented which favor this conclusion: (i) the rate of alanine synthesis was greater than that which could be accounted for by the transamination of pyruvate with glutamate, and (ii) the rate of synthesis of citrate was too low to account for the observed rate of synthesis of glutamate. It was, therefore, suggested that alanine might be synthesized directly from the reductive amination of a 3-C compound and that glutamate might be synthesized by a pathway not involving the Krebs cycle or α-ketoglutarate.

The enzyme alanine dehydrogenase, in the unicellular green alga *Chlamydomonas reinhardti* (*3*), catalyzes the formation of alanine from pyruvate and ammonium but is inactive with phosphoenolpyruvate as the substrate. This enzyme could, therefore, account for much of the synthesis of alanine in *Chlamydomonas*. We also reported the presence of glutamic dehydrogenase (*3*).

The purpose of the present investigation was to assess the importance of glutamic dehydrogenase in the photo-