

tumbling, but rather by bobbing in the membrane. Indeed, Blasie¹⁸ has interpreted the changes in X-ray scattering by the photoreceptor membrane that follow illumination as resulting from rhodopsin molecules sinking by about 10 Å into the hydrophobic region of the membrane.

The rhodopsin-containing model membranes have so far been short-lived, with an average lifetime of only about 1 min, making the search for light effects difficult. We have, however, investigated the effects of bright flash illumination on the capacitance of these membranes, and have not distinguished any effects within the resolution of our method. Future investigation will pursue the effects of light on the transport properties of rhodopsin in these model membranes.

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Fate of Bacteriophage Lambda in Non-immune Germ-free Mice

THE clearance of colloidal particles in animals has been studied in several experiments¹⁻¹⁶. The use of bacteriophage in such studies offers a sensitive tool to augment materials such as dyes, radioactive colloids and enzymes for analysing mechanisms of handling foreign particulate material. Furthermore, such use of bacterial viruses may enhance the general understanding of virus-host interactions.

Studies of the intracellular distribution of phages φX174 and T2 in the organs of rabbits and guinea pigs demonstrated that when they were taken up by the cells of the reticuloendothelial system (RES), most plaque-forming units (PFU) became localised in the larger granular fraction (lysosomes and mitochondria) of the cells². Inchley *et al.* studied the response of mouse macrophages to injected phage T4^{11,15,17}, finding that within 30 min of ¹³¹I-T4 injection, more than 99% of the phage was phagocytosed by liver Kupffer cells. Inchley also found that T4 PFU could be recovered from the spleen 5 d after the initial injection¹¹. Extensive studies have shown that elimination of colloidal particles is exponential with time regardless of whether these particles were phage^{11,15,17}, animal viruses¹⁸ or inert substances^{1,19}.

Our studies of the fate of phage λ, injected by several routes into NIH germ-free mice, differ from previous studies in several ways. First, previous investigations focused on elimination of foreign particles, either by phagocytosis or by antibody formation, while we were interested in the distribution of phage which survive rapid elimination. Second, most studies have used isotopic label to determine the location of the injected particles without showing whether the particles retained their biological activity, while we assayed for the ability to make plaques. Third, previous investigations concerned tissues known to be involved in the RES, while we were interested in any tissue accumulating phage. Fourth, our phage could be distinguished from possible contaminants by its genetic markers (Table 1). Fifth, the use of germ-free animals precluded interactions with microorganisms in the gastrointestinal tract, although the germ-free mouse system is not an ideally "clean" system for experimental purposes, for leukaemia viruses and other virus-like particles have been found in the thymus glands of such mice²¹⁻²⁴.

Table 1 Pattern of Test Results by which λ*pgal*₈₅-C/857 (ref. 20) can be distinguished from Possible Contaminating Phage

Phage	Plaques on pm+ host	Plaques on pm- host	Plaques at 34° C	Plaques at 40° C	Plaques on λ lysogen	Red plaques on MacConkey's gal with gal+ host
λ <i>pgal</i> ₈₅ -C/857	+	-	Turbid	Clear	-	+
Wild type λ	+	+	Turbid	Turbid	-	-
Wild type or virulent phage	+	+	Clear	Clear	+	-

The phage were checked periodically for their ability to transduce the galactose operon, for the presence of C/857 (production of a heat-sensitive repressor) as manifested by clear plaques at 40° C and turbid plaques at 34° C, and for sensitivity to λ immunity as demonstrated by the inability of the phage to plate on a *su*₁₁₁⁺ λ lysogen.

Figure 1 shows that the gross distribution of the virus was tissue-specific, regardless of the injection route. This, however, was not true with force-feeding, which failed to introduce significant amounts of virus to any tissues and spaces examined. Little, if any, virus was detected in the faeces after injection intraperitoneally, intramuscularly, intravenously or *per os*.

By 3 h after infection the blood contained considerably more virus than the peritoneal fluid even if the virus were given intraperitoneally. Within 48 h the virus was cleared from the blood (<10 PFU ml⁻¹) and the peritoneum, no matter where the virus was introduced.

All the organs examined in the infected animals contained viable phage. Of these organs, the spleen had the highest titre. With increasing time, the titre of phage within the liver decreased rapidly. The results were similar with the thymus. Within 3 h after intraperitoneal, intramuscular, and intravenous administration of virus the titre of virus in the spleen in PFU g⁻¹ of wet tissue was higher than any other organ examined. By 48 h

after infection, the blood titre had decreased to zero while the spleen titre still exceeded 10^4 PFU. This difference between the spleen and other organs increased with time.

The titres of phage found in any of the organs and in the two body spaces analysed were higher if the phage were injected intraperitoneally. This might indicate that the phage within the

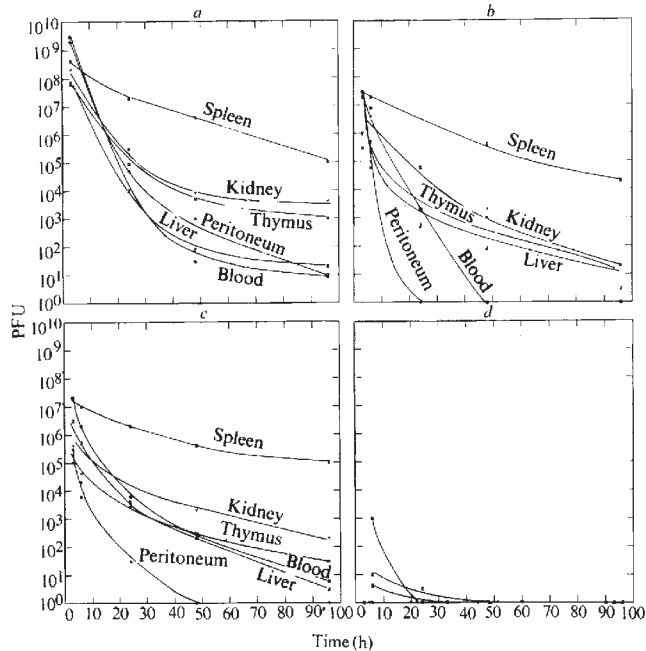


Fig. 1 Plot of the number of PFU g^{-1} wet tissue detected in: blood (\circ); peritoneum (\diamond); spleen (\times); liver (\square); thymus (\otimes); and kidney (γ); as a function of time after injection of phage by several routes: intraperitoneal (a); intramuscular (b); intravenous (c), and *per os* (d). λ *ppgal*₆₅, C1857, grown vegetatively on *E. coli* C600 (ref. 25), was purified using a CsCl gradient²⁶. NIH albino germ-free mice of mixed sexes were maintained in a plastic Trexler-type isolator unit provided by the Laboratory Aids Branch at NIH²⁷⁻³⁰. Sterility checks were made by streaking samples of organs, faeces, urine and blood onto Tryptone B1 plates to demonstrate the absence of bacteria and by titrating the samples against several strains of *E. coli* to demonstrate the absence of coliphage. All experiments with the germ-free animals were done within the isolator, and not with animals removed from the gnotobiotic environment. All materials passed into the isolator through an air-lock were either autoclaved for 20 min at 121°C, or swabbed with peracetic acid. The acid destroyed the coliphage used in these experiments. 2×10^{12} PFU of *E. coli* phage λ *ppgal*₆₅, C1857 were injected intramuscularly, intravenously, intraperitoneally or *per os*. Phage samples in 0.2 ml phosphate-buffered saline (PBS) containing 0.01 M $MgSO_4$ were injected intravenously into the tail vein or intramuscularly into the quadriceps femoris muscle group. The intraperitoneal injections of phage in 0.5 ml PBS were given in the right middle quadrant caudal to the liver. A mouse restrainer (Fisher) was used to facilitate injection procedures. A polyethylene tube 0.03 inch in diameter, forced down the oesophagus, was used to force-feed the phage sample in 0.2 ml PBS. At 3, 6, 24, 48 and 96 h after injection, mice from each injection group were anaesthetised with ether, the common carotids and jugular veins were cut and the blood was collected in a small test tube. The animals were pinned to a wooden board within the isolator and 3 ml of PBS was injected into the peritoneal cavity. After 1-2 min, the cavity was physically massaged and 0.5-1.5 ml of fluid was withdrawn. Faecal samples were collected when available. The animals were then dissected to remove the spleen, one kidney, the left lobe of the liver and (usually) the left lobe of the thymus. Each of the organs was washed thoroughly with PBS, and final washings were saved and titred to demonstrate that there was little or no phage remaining on the surface of the organs. This indicated that the virus was firmly associated with the organ samples and was not virus from the peritoneal fluid that had coated the organ. All samples were passed out of the isolator for further analysis. A 0.5 ml sample of PBS was added to each organ which was then ground with a sterile sintered glass pestle. Faecal samples were treated in the same way. The samples were titered against DB1 using an agar overlay method³¹. The presence of contaminating viruses was ruled out by tests described in Table 1.

peritoneal cavity were taken up preferentially by the liver and then the spleen, whereas phage injected intramuscularly or intravenously were distributed more uniformly. This agrees with earlier findings³² that compounds administered intraperitoneally are taken up by the portal circulation. Thus the liver maintains the highest concentration of injected substances. Particles injected intramuscularly and subcutaneously were distributed by the heart; because 28% of the cardiac output reaches the liver, the liver should have theoretically fewer particles within its substance when injected by other than the intraperitoneal route. Our results were similar. Therefore the effects, the metabolism and the excretion of phage might be different when injected by varied routes, for varying percentages of injected phage came into contact with the liver.

The spleen retained high titres of injected intact phage (10^6) for prolonged periods of up to 7 d. Inchley also found intact phage in the spleen up to 5 d after infection¹¹.

Retention of phage in the spleen was previously suggested to be due to the fact that "inactivation of antigen occurs much more rapidly within Kupffer cells than within splenic macrophages"¹¹. This interpretation predicted that the spleen, well-endowed with macrophages, would retain lower titres of phage than other tissues deficient in macrophages, such as the kidney. Our data (Fig. 1) suggested an alternative interpretation that the spleen was engaged in non-destructive capture of antigens. The spleen can carry out phagocytosis, but recently a non-phagocytic capturing method utilizing the Schweigger-Seidel capillary sheaths was described³³. This latter mechanism might be responsible for the non-destructive capture of phage that we observed. This ability to non-destructively retain antigen might allow the spleen to serve as a continued source of antigen for the stimulation of antibody formation.

Although it was suggested that non-phagocytic antigen capture by the Schweigger-Seidel reticulum cells required interaction with antibody³³, in our experiments such an interaction was remote, for we were unable to detect neutralising antibody to λ ($K < 10^{-2}$)³¹.

Antigen trapping in the spleen may be closely coupled to antibody formation. An intact spleen is necessary for maximal antibody response¹⁷. The removal of the spleen severely depresses the antibody response in mice, and this defect in antibody formation following splenectomy cannot be corrected by injection of splenic elements¹⁷. Furthermore, in neonatal rats and mice the spleen is an alymphoid organ^{3,4}. The ability to trap antigen appears 2 weeks after birth^{3,4}. Multiple injections of antigen are required during this first 2-week period to stimulate antibody formation, whereas no single injection during this time produces an antibody response⁴. Following the development of the lymphoid elements in the spleen a single injection of antigen stimulates formation of antibodies. These observations indicate that the structural integrity of the spleen might allow cell-to-antigen and cell-to-cell interaction for antibody formation^{2,5,8,13-15,34-39}.

The rapid elimination of phage in intact animals may explain the limited success of phage treatment of infectious diseases⁴⁰ and may interfere with attempts to observe prokaryotic viral gene expression^{41,42} in whole animals. This rapid rate of elimination may be slowed by overwhelming the RES with inert colloidal particles such as Thorotrast (thorium dioxide)^{18,43}.

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osmotica in the ratio of 1:4 to give the final concentrations of enzymes shown in Table 1, and incubated at 28° C in the dark.

The arrangement of microspores in a few tetrads of *D. metel* (about 5%) was isobilateral but the predominant organisation was tetrahedral. Initially, the four microspores within the massive enveloping wall of the tetrad (Fig. 1a) were naked, but they subsequently developed thin cellulose walls, at which stage they could easily be released from the envelope by gentle pressure. Conventional methods were then used to obtain free protoplasts. The cell walls of both immature and mature pollen grains were resistant to the enzymes used in this study. The mature pollen grains germinated readily and the pollen tubes were then susceptible to enzyme action. Their contents, however, were released as sub-protoplast fragments and not as entities.

Table 1 Effect of Enzyme-Osmotica on Release of Protoplasts from Pollen Tetrads of *Datura metel**

No.	Treatment †	Period of incubation (h)		
		1	4	6
1	CSS	—	++	+++
2	CR	—	+	++
3	CA	— ‡	++	+++
4	H	—	—	—
5	P	—	—	—
6	SDJ	+	+++	+++
7	CSS+H	— ‡	++	+++
8	CR+H	—	+	++
9	CA+H	—	++	+++
10	CSS+P	— ‡	++	+++
11	CR+P	—	+	++
12	CA+P	— ‡	++	++
13	CSS+SDJ	++	+++	++++
14	CR+SDJ	+	++	+++
15	CA+SDJ	++	+++	++++
16	H+P	—	—	—
17	H+SDJ	+	+++	+++
18	P+SDJ	+	+++	+++
19	CSS+H+SDJ	++	+++	++++
20	CR+H+SDJ	+	+++	+++
21	CA+H+SDJ	++	+++	++++
22	CSS+P+SDJ	++	+++	++++
23	CR+P+SDJ	+	++	+++
24	CA+P+SDJ	++	+++	++++

* Percentage yield; —, 0; +, <20; ++, 20–50; +++, 51–80; and +++++, >80. † CSS, Cellulase Onozuka SS (from *Trichoderma viride*), all Japan Biochemicals, 5%; CR, Cellulase Type III (from *Rhizopus* mould), Sigma, 10%; CA, Cellulase Type II (from *Aspergillus niger*), Sigma, 10%; H, Hemicellulase grade II (from *Rhizopus* mould), Sigma, 5%; P, Pectinase, Nutrition Biochemicals, 5% (all concentrations as w/v); and SDJ, snail digestive juice (from *Helix pomatia*), Koch-Light, 2% (v/v). ‡ Occasionally, 1 to 2% of tetrads released their protoplasts.

Nuclear Divisions in Protoplasts isolated from Pollen Tetrads of *Datura metel*

THE significance of haploid protoplast research has been discussed by Bhojwani and Cocking¹. They have succeeded in isolating protoplasts from microspores and bringing about regeneration of cell walls. The occurrence of nuclear divisions in protoplasts isolated from pollen tetrads of *Datura metel* L., a plant in which androgenesis could be experimentally induced^{2,3}, is described here.

Anthers from several buds were pooled in three groups, each of which contained either pollen tetrads, immature microspores or mature pollen grains. Segments of about 5 mm were excised from the central region of anthers and their contents gently squeezed out into 10% sucrose solution. The suspension volume for each group containing an entirely homogeneous cell population was adjusted to about 25 × 10⁴ cells ml⁻¹. Enzymes were prepared as stock solutions in 0.05 M potassium acetate buffer, pH 5.4, containing 10% sucrose. The cell suspensions were mixed with the enzyme-

Protoplasts from pollen tetrads were isolated in twenty-one of twenty-four enzyme-osmotica tested. Mixtures containing a cellulase (irrespective of its origin) or snail digestive juice (helicase) were effective, but pectinase and hemicellulase were ineffective (Table 1). The release of protoplasts in osmotica containing only cellulase (used as controls) was unexpected and contrary to earlier findings¹. This could not have resulted if callose (which is resistant to cellulase) were the only constituent of the tetrad wall. The snail enzyme complex which contains β-1,3- and β-1,4-glucan glucohydrolases that degrade callose and cellulose, respectively, was comparable in its action with that of cellulase. Cellulase in conjunction with helicase, however, accelerated the rate at which protoplasts were released. The final yield was also higher. Similar observations have been recorded for *Atropa belladonna*, *Lycopersicon esculentum* and *Solanum xanthocarpum* (unpublished data). These findings suggested that the tetrad walls of *D. metel*, and possibly those of the others, contained cellulase in addition to callose. Lysis of either component in the cell wall could bring about a structural