into the pulsating vessel as it enters the axial gland. Motion picture analysis showed a unidirectional movement of pulsations. Since the converging vessels are so numerous at this point (Fig. 3), it is reasonable to attribute a valvular function to them. The pulsating vessel is much branched. The terminal processess of each branch appear to end blindly within the dense tissues of the axial organ. These penetrating branches create a multiplicity of small cavities, each communicating with the main axial gland lumen (Fig. 1). The gland surface is covered by a vascular network that eventually converges into a single vessel that leads to the periesophageal ring. From this ring another vessel leads along the surface of the esophagus. It eventually contributes to and forms the inner hemal sinus, which runs along the inner surface of the digestive tract, and leaves the rectum to terminate in the second contractile chamber. Thus, fluid can enter the contractile chamber by two routes, (i) the inner hemal sinus and (ii) the previously mentioned microscopic ostia.

Since microinjection of fluorescein dye into the cavity of the axocoel demonstrated movement of dye throughout the pulsating chambers, we conclude that injected fluid passes from the axocoel into the chambered vessels by way of the ostia and thence into the pulsating vessel within the axial gland lumen.

Injection of dye into the axial gland lumen was followed within seconds by its appearance in the surface network of vessels on the axial gland. The dye was traced into the stone canal and peripharyngeal ring and then throughout the water vascular system. This confirms our histological observation on the communication between the water vascular system and axial gland (*7*).

Analysis of motion pictures also revealed contractions of the stone canal. A long segment of this tube contracts simultaneously with the pulsating vessel. On close observation we noted a synchrony in the contraction of the stone canal and the pulsating chambers. When the latter contracts, the lumen of the stone canal is conspicuously open.

We found a direct communication between the lumina of the axial gland and stone canal. The rhythmic contraction of the pulsating vessel together with the pulsations of the stone canal may be important in moving fluids throughout the entire water vascular system.

Since acetylcholine inhibits pulsations and adrenaline accelerates them, the process appears to be myogenic in origin.

RICHARD A. BOOLOOTIAN
Department of Zoology, University of California, Los Angeles
JAMES L. CAMPBELL*
Los Angeles Valley College, Van Nuys, California

References and Notes

1. F. Tiedemann, *Anatomie der Röhren-Holothurie des Pomerantz-farbigen Seesterns und Stein-Seeigels* (Joseph Thomanschen Buchdruckerei, Landshut, 1816), pp. 1–98; G. Valentin, in *Monographies d'Echinodermes vivants et fossiles*, L. Agassiz, Ed. (Petitpierre, Neuchâtel, 1841), pp. 1–126.
2. M. Perrier, *Arch. Zool. Exptl. Gen.* **4**, 605 (1875); R. Koehler, *Mus. Hist. Natur. Marseille, Zool.* **1**, Mém. 3, 167 (1883); P. Sarasin and F. Sarasin, *Ergeb. Naturw. Forsch. auf Ceylon* **1** (3), 81 (1888).
3. L. Cuénot, in *Traité de Zoologie*, P. Grassé, Ed. (Masson, Paris, 1948), pp. 120–200.
4. H. Prouho, *Arch. Zool. Exptl. Gen.*, Ser. 2, **5**, 213-380 (1887).
5. K. C. Vogt and E. Yung, *Lehrbuch der prakischen Vergleichs-Anatomie* (Vieweg, Braunschweig, Germany, 1888).
6. L. Hyman, *The Invertebrates* (McGraw-Hill, New York, 1955), vol. 4, pp. 413–589.
7. The only evidence suggesting a connection between the water vascular system and the axial gland was reported by Hyman (*6*), who stated that colored water was forced through the madreporite and traced to the axial gland and through the water vascular system (*6*, p. 568). However, Hyman noted the disagreement of other workers on the existence of a connection between the water vascular system and axial gland (*6*, p. 473).
8. This work was supported by grant GB-1042 from the National Science Foundation and contract Nonr 233 (85) from the Office of Naval Research. We thank Miss Gerry Beye for illustrating Fig. 2.
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Ultraviolet Sensitivity of Escherichia coli Containing Heat-Inducible λ Prophages

Abstract. *The* c1-t *mutants of bacteriophage λ can form prophage at 36°C but cause lysis of sensitive bacteria at temperatures above 42°C. Growth of cultures at 42° to 46°C induces prophage replication and lysis in* Escherichia coli *K12* (λ c1-t); *lysogenic strains containing wild-type prophage are not induced to lyse at these temperatures. Heat induction is prevented by chloramphenicol. Strains containing heat-inducible prophage are much more sensitive to killing by ultraviolet light than is K12 (λ+).*

The *c1* gene of bacteriophage λ controls the production of a cytoplasmic factor that is required for the establishment and maintenance of prophage. This factor has been called "immunity substance" because it also prevents vegetative multiplication of related phages introduced into the lysogenic cell. According to Jacob and his co-workers (*1*) this cytoplasmic factor is a repressor that inhibits the synthesis of one or more of the "early" proteins essential for initiating growth of vegetative phage.

Sussman and Jacob (*2*) and Thomas and Lambert (*3*) have described mutants of bacteriophage λ that can be maintained as prophage at temperatures below 37°C but are induced to become vegetative phage at higher temperatures. Both groups of investigators reported that mutations in the *c1* gene of the bacteriophage λ can be suppressed by mutations of the host bacterium; these suppressors also ameliorate the action of bacterial mutations which affect the synthesis of certain enzymes. These findings suggest that the repressor is a protein and that temperature-sensitive λ mutants produce an unstable protein that becomes nonfunctional at temperatures that do not affect the structure of the normal repressor. Suppressor mutations in the host bacterium presumably supply some mechanism that allows wild-type protein to be produced on a mutant messenger RNA.

It has been generally assumed that ultraviolet light induces vegetative multiplication of prophage by interfering with the synthesis or function of the repressor. This report concerns the sensitivity of lysogenic strains that contain temperature-sensitive prophages of bacteriophage λ to induction by ultraviolet light.

Wild-type bacteriophage λ that had incorporated 5-bromouracil into its DNA was plated on strain M3, a nonlysogenic strain of *Escherichia coli* K12, and the plates were incubated overnight at 43°C. Phage from clear plaques was replated at 43° and 35°C. Seven mutants giving clear plaques at 43°C but turbid plaques at 35°C were isolated. Genetic tests of the phage mutants are incomplete, but they have been designated as "*c1-t* mutants." An additional mutant used in these experiments, λ *c1-t1*, was the gift of J. J. Weigle. Each mutant was used to produce a lysogenic strain of M3. Cultures of the lysogenic strains were grown with aeration in broth containing 1 percent Difco tryptone + 0.5 percent NaCl to a concentration of 2 to 5×10^8 per milliliter. For studies of heat induction, the bacteria were diluted in the broth at the

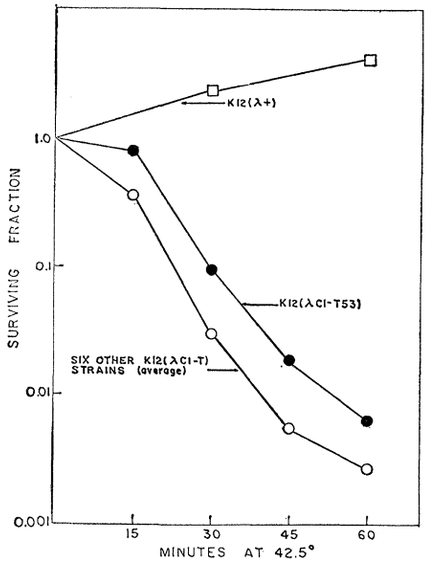

Fig. 1. Heat induction of lysogenic strains containing λ prophages.

desired temperature. During 45 minutes at 45°C, the percentage of bacteria that formed colonies gradually dropped to about 0.5 percent and the percentage forming plaques on M3 rose to almost 100 percent. After induction by heating at 46°C for 5 minutes, the latent period before any release of phage was 50 minutes and the yield of phage per bacterium about 100.

At 45° to 46°C, induction occurred at the same rate in all K12 (λ *c1-t*) strains, but at 42.5°C one strain gave an induction curve that was clearly different from the others shown in Fig. 1. At 41.5°C, K12 (λ *c1-t53*) can divide, while heat gradually kills bacteria carrying prophage of λ *c1-t1* and λ *c1-t59*. The process of heat induction requires that there be bacterial growth during the heating period. When K12 (λ *c1-t1*) was grown into stationary phase and then diluted in 0.9 percent NaCl or in tryptone broth containing 25 μg of chloramphenicol per milliliter, there was no induction at 45°C (Fig. 2). High concentrations of putrescine (0.3*M*) also inhibited heat induction as well as cell division. Bacteria containing a temperature-sensitive prophage can be protected from heat induction by superinfecting them with the original wild-type λ+ (*4*). Apparently, the normal repressor produced by the cytoplasmic phage takes over the functions of the inactivated repressor produced by the prophage.

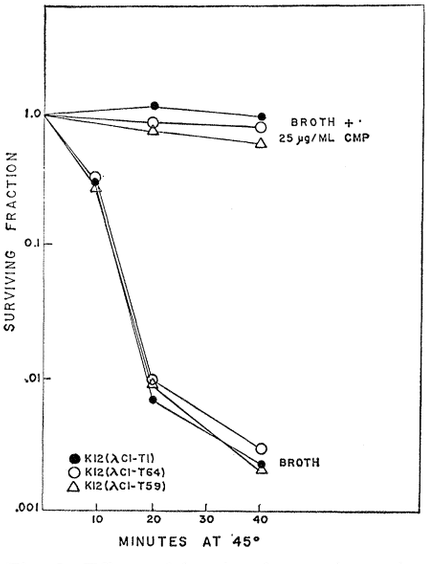

Fig. 2. Effect of heating lysogenic strains containing λ *c1-t* prophages in medium with or without chloramphenicol (CMP).

For ultraviolet irradiation experiments, cultures were grown in the tryptone broth to a density of 5×10^8 bacteria per milliliter and diluted 1:20 in cold 0.9 percent saline. Almost identical results were obtained with cultures grown at 30° and 36°C. Three-milliliter portions were placed in small petri dishes, and the dishes were agitated by hand under a Westinghouse Sterilamp delivering about 3 ergs/mm^2 sec^{-1}. In Fig. 3, induction of prophage by ultraviolet light is compared in strains carrying λ+ and various λ *c1-t* prophages. The highest dose of ultraviolet light killed less than 50 percent of nonlysogenic cells; killing is used as a measure of induction, even though not all of the nonsurviving cells formed plaques. The killing (induction) curve of K12 (λ *c1-t53*) at low doses was always less steep than killing curves of other M3 (λ *c1-t*) strains. Thus, there is a correlation between sensitivity to induction by heat and by ultraviolet light. The dose-reducing effect of photoreactivating light was at least as great in K12 (λ *c1-t*) strains as in K12 (λ+). Treatments given after ultraviolet irradiation that increase induction of K12 (λ+), such as fortifying the growth medium with proflavin or caffeine (*5*), also increased the induction of K12 (λ *c1-t*) strains. As free phages, λ *c1-t* mutants are not more sensitive to heat or ultraviolet light than wild-type λ.

The correlation between ultraviolet and heat sensitivity of mutant prophages suggests that both properties are altered by a single change in the *c1* gene. It

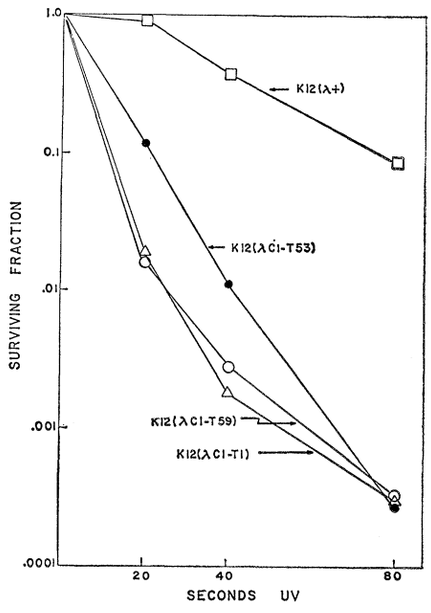

Fig. 3. Ultraviolet induction of lysogenic strains containing λ prophages.

is reasonable to assume that heating could inactivate a repressor that is a protein, but it appears unlikely that very low doses of ultraviolet light induce lysis by inactivating protein molecules. This dilemma can be solved by supposing that the repressor molecules produced by mutant prophages have an alteration in their structure which results in a lowered affinity for the site susceptible to repression. In that case, the concentration of repressor molecules required to prevent derepression would be higher in K12 (λ *c1-t*) than in K12 (λ+). Damage by ultraviolet light to the lysogenic cell that prevents formation of repressor substance, even for a short time, would be more likely to result in derepression in a cell containing a temperature-sensitive repressor than in a normal lysogenic strain.

MARGARET LIEB
Department of Microbiology, University of Southern California School of Medicine, and *Los Angeles County General Hospital, Los Angeles*

References and Notes

1. F. Jacob and A. Campbell, *Compt. Rend.* **248**, 3219 (1959); F. Jacob and J. Monod, *J. Mol. Biol.* **3**, 318 (1961).
2. R. Sussman and F. Jacob, *Compt. Rend.* **254**, 1517 (1962); F. Jacob, R. Sussman, J. Monod, *ibid.*, p. 4214.
3. R. Thomas and L. Lambert, *Bull. Acad. Roy. Belg.* **48**, 688 (1962).
4. M. Lieb, unpublished.
5. ———, *Genetics* **47**, 967 (1962); *Proc. XI Intern. Cong. Genet.* **I**, 27 (1963).
6. Supported by NIH grant AI-05367-01 and NSF grant G 24165.

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