of broth on each irradiation strip from .05 to .01 ml reduced this curvature from 20 or 30% survival to below 10%. Absorption in the broth film is the most probable explanation, for although an absorbing film of uniform thickness would still give nearly logarithmic inactivation (*4*), we were not able to obtain consistently uniform broth layers. The difficulty of measurement and transfer of these small volumes is the most likely cause of the dispersion of the points on our inactivation curves.

TABLE 1

λ, A	3664	3131	2967	2894	2803	2760	
37% dose, ergs/mm²	~100,000	30,000	1200	750	220	224	
λ, A	2700	2650	2540	2482	2400	2350	2250
dose	207	185	232	455	523	327	690

The calibration of intensity in absolute units must be considered less accurate than the relative intensities of various wavelengths. The energy doses in ergs/mm² for reduction to 37% survival are shown in Table 1. The action spectrum plot is given with *a* calculated for *It* expressed in quanta/mm².

The results are very similar to those obtained by Gates. The features to note are the minimum at 2425 A, which is rather long in wavelength for desoxyribose nucleic acid, and the rather high inactivation at 2800 A. The value of *a* appears to fall at 2250 A, but this may be apparent and not real, since there can be heavy broth absorption. We do not believe that there is a sharp rise corresponding to protein absorption, however, as the broth absorption as estimated by rough measurements could hardly have exceeded a factor of two.

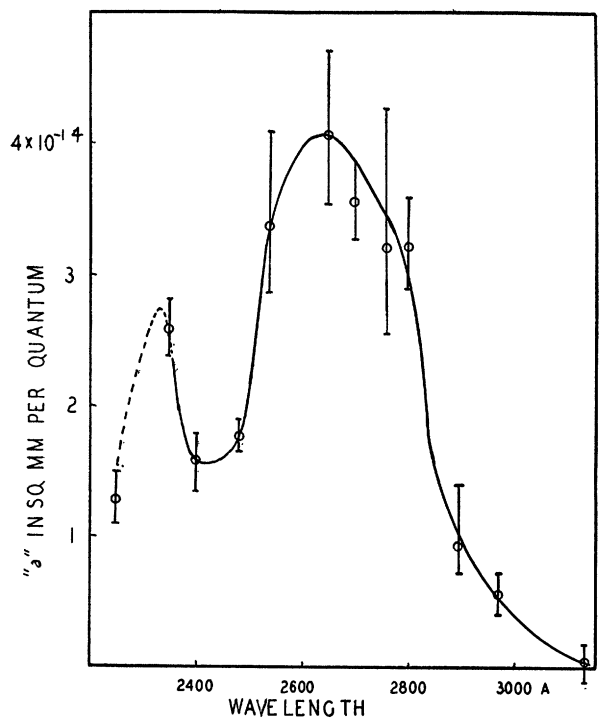

FIG. 2. Relative effectiveness of various wavelengths of ultraviolet light in causing inactivation of T1 bacteriophage.

A comparison with work on bacteria as tabulated by Hollaender can be made. Our figure for the 10% dose at 2540 A is 535 ergs/mm². This shows that dried T1 bacteriophage is less susceptible at this wavelength than vegetative bacteria, but more so than spores.

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Lysis of Formalinized Bacteria by Bacteriophage

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Recently it has been pointed out (*1, 2*) that many lysed cells are found in mixtures of young and actively growing cultures of the B strain of *E. coli* with suspensions of certain bacteriophages when such mixtures are chilled and prepared for electron microscopy after very short incubation. After longer incubation stages in the development of new bacteriophage, particles are evident in such masses of lysed protoplasm. We have now found that the same phenomena of bacterial lysis and bacteriophage production are observed using bacteria previously inactivated with formaldehyde.

The bacteria used were 1 to 1½-hr cultures in tryptose broth. They were inactivated by being held for 15–30 min at room temperature after the addition of the desired volume of commercial formalin. Then the cells were washed once or twice by putting them in fresh broth and sedimenting them in a centrifuge. The washed suspension was made up to have a turbidity approximately that of the original culture. Effectiveness of the formalinization was tested in each instance by streaking a loop of this suspension on an agar plate and incubating. Such test plates remained completely free of bacterial growth when a concentration of 0.2% or higher of formaldehyde was employed; a few isolated colonies sometimes developed if the formaldehyde concentration was 0.04 or 0.08%. Such suspensions of killed, washed bacteria become clear on incubation following the addition of a suitable bacteriophage suspension containing enough particles to infect the bacteria present. Samples for electron microscopy were withdrawn after periods of incubation up to 1 hr, immediately chilled in ice water, and centrifuged cold, and the sediment was washed by successive centrifugations first from 0.5% formalin-saline and then from water-formalin. Final suspensions in a small volume were spread and dried in the usual formvar-covered grids and

metal-shadowed for electron microscope examination.

What was seen depended on the strength of the formaldehyde used for inactivation. At all concentrations up to the highest tried (1% formaldehyde = 2.5% formalin) there was the same picture of extensive cell rupture after short incubation that was observed with living bacteria. The masses of bacteriophage particles in the ruptured bacteria of many preparations further demonstrate beyond question that bacteriophage has proliferated during incubation with killed organisms. Electron microscopic evidence for such proliferation has been clear for both T2 and T4 bacteriophages; none could, on the contrary, be found in numerous experiments with T3 bacteriophage, though lysis evidently had occurred. The number of new bacteriophage particles depends obviously on the strength of the inactivating formaldehyde. As far as can be judged by electron microscopy, bacteria treated with 0.04% formaldehyde support as extensive bacteriophage multiplication as do living bacteria; the number of new particles appears diminished at concentrations above 0.2% formaldehyde and is greatly reduced and perhaps completely arrested at the highest concentration used (1%). It is also greatly lowered if the killed bacterial suspension is allowed to stand for several hours before the addition of infecting bacteriophage.

It has been instructive to determine by plaque counting the number of viable bacteriophage particles in these lysed suspensions of inactivated bacteria. They depend, even more strikingly, on formaldehyde concentration. With all but the minimal amount of formalin, the recoverable particles were far fewer than those added to bring about lysis, but after inactivation with 0.04% formaldehyde the yield often greatly exceeded the input. Thus in one experiment where suspensions containing 10^8 killed bacteria per ml were inoculated with approximately 10^6 particles of T4 per ml, the yield was reduced to about 0.01 the inoculum when the killing concentration of formaldehyde was 0.2%; it about equaled the inoculum when it was 0.08%; and it was about tenfold greater when it was 0.04%. Evidently the newly formed bacteriophage particles that the electron microscope shows developing from the more strongly formalinized bacteria either do not fully mature as independent infectious units or they are themselves gradually killed by the formalin-treated protoplasm which they have consumed.

Light on this point can be drawn from studies we made of the relative sensitivities of different bacteriophages to formaldehyde. These demonstrated, for instance that whereas the titer of a suspension of T2 or T4 was reduced to much less than 10^{-5} of its initial value by contact for 15 min with 0.2% formaldehyde, most particles in a suspension of T1 withstood 1.5% formaldehyde for at least one hour. Notwithstanding this remarkable resistance of T1 bacteriophage and its ability to lyse formalinized bacteria, its titer after such lysis was as dependent on the concentration of formalin used for bacterial killing as was that in the corresponding experiment with formalin-sensitive T2 or T4 bacteriophages..

These experiments on the development of particles of bacteriophage at the expense of bacteria that were inactivated by formalin have a direct bearing on the nature of this inactivation, as well as on the nature of bacteriophage and its mode of multiplication. They do not yet give a sufficient answer to any of these questions, but they definitely prove that certain bacteriophages can proliferate on bacteria that have been killed in the sense of having been rendered unable to develop into visible colonies. Other methods of experimentation will be required to show whether in this case bacteriophage is actually multiplying on already dead or on dying cells.

These studies are being extended to other circumstances of bacterial killing and will be reported elsewhere.

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Granulosis Disease in the Buckeye Caterpillar, *Junonia coenia* Hübner

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The first reported instance of a granulosis disease of an insect in the Western Hemisphere was that found occurring in the variegated cutworm, *Peridroma margaritosa* (Haw.), which was being reared in an insectary in California (*2*). In September 1948, several specimens of the buckeye caterpillar, *Junonia coenia* Hübner, collected near Westley, California, and brought into the laboratory were found to be infected with what also appeared to be a granulosis virus. This observation was confirmed by the microscopic demonstration of characteristic granules in the diseased host tissues, and by electron microscope demonstration of the virus particles in the granules.

In general, the disease and the accompanying granules in *Junonia* are very similar to those of *Peridroma.* Cross infection experiments, however, showed that the two diseases were not identical, since neither host is susceptible to the virus of the other. The *Junonia* virus is also apparently distinct from those causing granuloses of several European insects (*3*). Diseased *Junonia* appear brownish in color and lose their characteristic metallic blue-black luster. The time from the beginning of the infection to the death of the insect varies considerably and may be rather prolonged. As a rule, death results in 6–12 days after infection. Dead larvae "hang up" on the food plant or die on the ground or on the bottom of the rearing cage. The integument usually remains intact.

When the integument is ruptured, the body fluid flows out rapidly and if the disease has progressed far enough the fluid has characteristic milky appearance. When examined with an ordinary compound microscope the fluid was seen to be filled with large numbers of small granules. Microscopic examination of pieces of the fat body showed granules present in cells of this tissue. Electron micrographs of these materials showed the granules to be ovoid bodies about 350×500 mμ in size (Fig. 1A).