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).

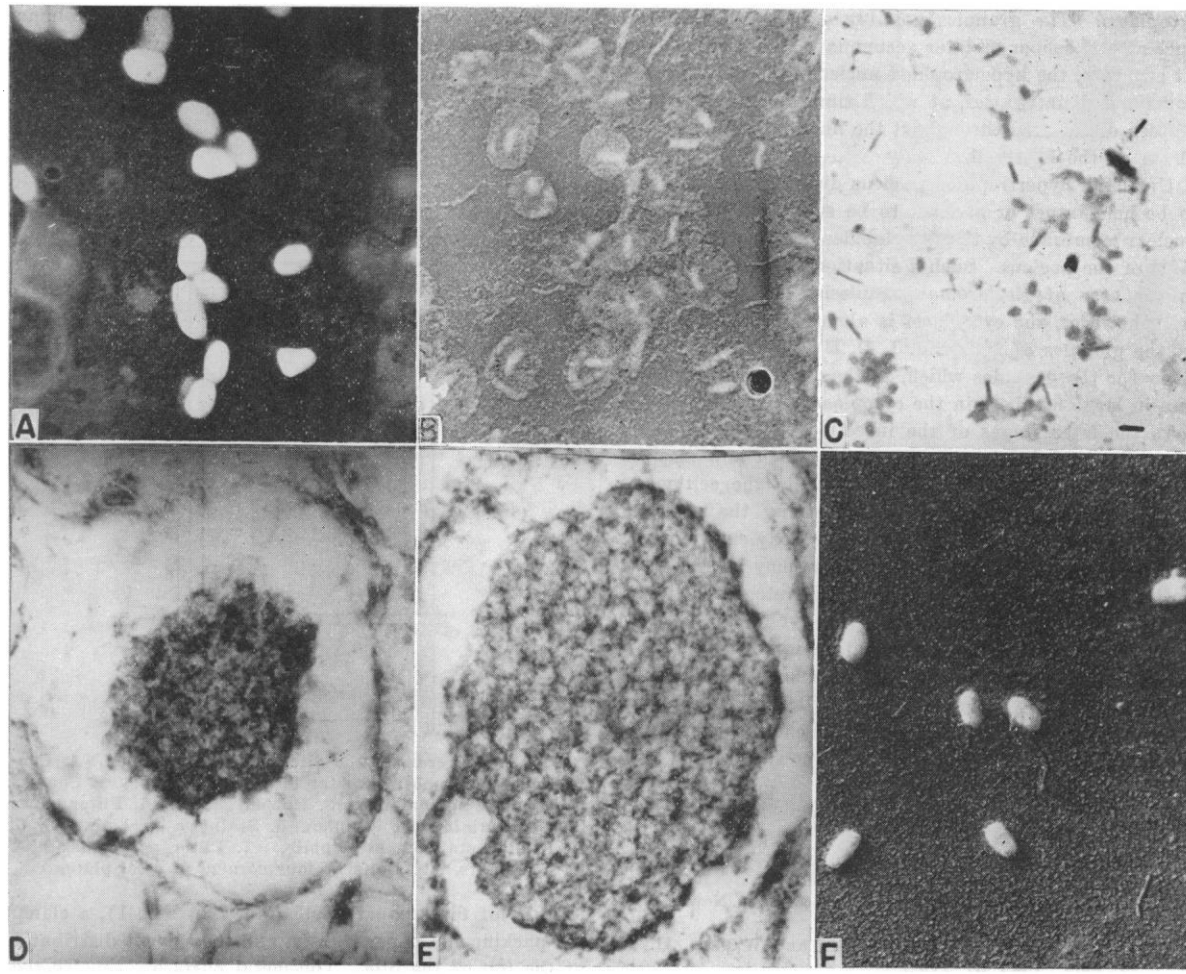

FIG. 1. A. Granules characteristic of the granulosis of the buckeye caterpillar, *Junonia coenia* Hübner. B. Granules partially dissolved in 0.05 M sodium carbonate solution, showing the location of virus particles, which are still covered with some of the granular material. C. Preparation in which the granular material has been completely dissolved, leaving the free rod-shaped virus particles. D. A single fat-tissue cell showing the nucleus slightly hypertrophied and beginning to disintegrate. The cytoplasmic area of the cell is occupied by large fat globules. E. Fat-tissue cell later in the disease, nucleus greatly hypertrophied. F. Granules from salt marsh caterpillar, *Estigmene acraea* (Drury), suffering from ganulosis. A, B, C, and F, electron micrographs, magnifications approximately 10,000 ×; A, B, F, gold shadowed. D and E, ordinary photomicrographs, magnification approximately 1,100 ×. (Photographs by K. M. Hughes and H. B. Wasser.)

In order to demonstrate the virus particle enclosed within the granule, the same technique as that used with *Peridroma* granules (*4*) was applied here. With the use of Na_2CO_3 in a concentration of 0.05 M for 3 hr, the granules dissolved, revealing a rod-shaped virus particle enclosed within each granule, approximately 40×300 mμ in size. With certain preparations electron micrographs were made during the dissolving process, showing the virus particle still surrounded by some of the granule material. Such preparations indicate that the virus particle is freed from the granule simply by the dissolving away of the granular material. Working with the granulosis virus of *Cacoecia murinana* Hüb, Bergold (*1*) believed that in some cases, at least, the virus slips out of the particle when under the influence of the alkaline solvent. The writers have not observed such action with either the *Peridroma* or *Junonia* granuloses viruses.

Histopathological studies of diseased *Junonia* caterpillars showed the fat tissue to be the principal one affected by the disease, although at times other tissues such as the hypodermis and tracheal matrix were also involved. Sections of diseased larvae were fixed in Bouin's or in Carnoy's fixative and stained with iron hematoxylin or with Mallory's triple stain. In such preparations, the nuclei of the fat cells were prominently altered in appearance. As the infection progressed, the nuclei became hypertrophied, usually to a marked degree. The chromatin material of such nuclei underwent karyorrhexis and karyolysis and became diffused throughout the nuclear area. Granules, characteristic of the disease, seemed to be forming in the enlarging nuclei, and sometimes the greatly enlarged nuclei appeared to be liberating granules into the cytoplasm of the cell. At other times the nucleus appeared intact and yet some granules were present in the

cytoplasm. The granules possibly originate within the nucleus and sooner or later accumulate in the cytoplasm. At any rate, the hypertrophied nucleus eventually breaks down and disintegrates, at which time the granules may be seen distributed throughout the nucleoplasm and cytoplasm of the host cell.

Until the hypertrophied nucleus disintegrates it seems to be held intact or at least to be supported within the nuclear membrane by the fat globules pressing against all sides of the nucleus. Such a situation is not so apparent in the case of *Peridroma* granulosis. In the *Junonia* cells, however, the cytoplasm is almost entirely occupied by the globules of fat (Fig. 1, D, E) leaving very little room for the granules which, in the *Peridroma*, accumulate in large numbers in the cytoplasm of the cell.

In the later stages of the infection there seem to be fewer fat globules in the cytoplasm, which now may be fairly well filled with granules. Finally the cell membranes themselves break down, liberating the granules and other contents of the cells into the body cavity of the host insect. A few chromatin remnants may be seen scattered about in the almost completely disintegrated tissue. It is at this time that the insect dies.

Shortly after the *Junonia* granulosis was found, a similar infection was discovered in specimens of the salt-marsh caterpillar, *Estigmene acraea* (Drury) collected in Albany, California. Whether the disease is caused by the same or another virus is not yet known.

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A Filter Paper "Chromatopile"[1]

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The application of one- and two-dimensional paper chromatography to the separation of small amounts of many kinds of compounds has become a well-established and useful technique (*1, 2, 3, 5, 6*). In general these methods do not handle sufficiently large quantities of materials for isolation and chemical identification. Large columns of starch and other materials have been used satisfactorily for handling larger quantities of mixtures (*4*). Starch columns proved to be unsatisfactory, however, for isolation of certain substances under investigation in this laboratory. As a consequence, a new and simple apparatus and technique have been developed, using a pile of filter paper disks as the absorbing column.

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A diagrammatic sketch of a cross section of the column is shown in Fig. 1. It consists essentially of three parts:

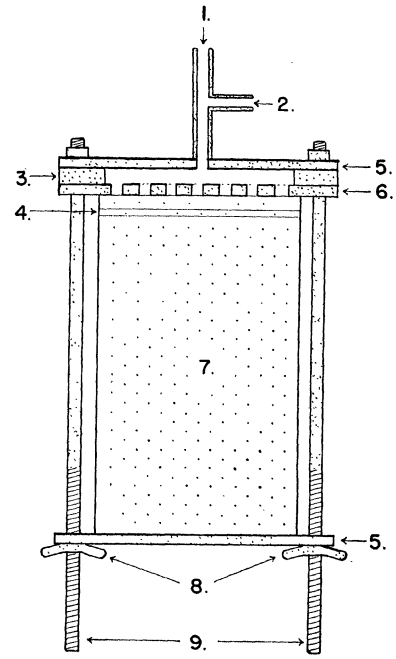

FIG. 1. Diagram of the filter paper pile column. 1. Connection for a rubber tube for filling the siphon. 2. Connection for the siphon tube. 3. Rubber gasket. 4. Filter paper disks containing the sample. 5. Stainless steel plates. 6. Perforated stainless steel plate. 7. Filter paper disk pile. 8. Wing nuts. 9. Bolts at four corners of steel plates.

the pile of filter paper itself (No. 7 in Fig. 1), a clamp for packing the paper tightly, and the solvent distributor at the top of the pile. The metal parts are constructed from stainless steel. The solvent distributor is connected by a siphon of rubber tubing to a 2-l flask containing about 1 l of solvent mixture. The flask is adjusted so that the level of the solvent is even with the top of the paper pile. In operation the column is placed upright on the ends of the clamp bolts in a 9 in. × 12 in. battery jar containing about 200 ml of solvent mixture. The jar is covered, with a hole left for the siphon tube.

The results of a trial run on known compounds will serve to illustrate the utility of the apparatus. Fifty mg each of adenine, tryptophane, phenylalanine, *p*-aminocinnamic acid, and anthranilic acid was dissolved in 20 ml of 0.1 N HCl and the solution was placed in the lid of a 100-mm Petri dish. Disks of 9-cm Whatman No. 1 filter paper were immersed in the solution, allowed to drain, and hung up to dry in air at room temperature. Twenty-five sheets were required to take up the solution and rinsings. To prepare for packing the column, the bottom plate of the clamp was removed and the clamp placed in an inverted position with the solvent distributor down. Forty filter paper disks (9 cm diam) were then placed carefully in the center of the perforated plate, followed by the 25 dried sheets containing the sample. The remainder of the column was made up of a pile of