NOMENCLATURE AND CLASSIFICATION OF INSECT VIRUSES[1]

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Although there were earlier attempts to name and classify viruses, the subject gained new and critical attention when the sixth edition of *Bergey's Manual of Determinative Bacteriology* for the first time included a 160-page supplement titled "Order Virales, The Filterable Viruses." This supplement was prepared by Francis O. Holmes, and was an extension of an earlier preliminary presentation published by him in 1939. It included animal viruses, plant viruses, and bacterial viruses (bacteriophages). As organized by Holmes, the order Virales consists of 13 families, 32 genera, and 248 species.

One's philosophy as to whether or not the time is yet ripe to name and to attempt to classify the viruses may fall within a range from violent objection to at least sympathetic acceptance of the idea. There are those who feel that since we have little or no information as to how viruses multiply, these agents should not be treated in the same manner as are the bacteria which, in general, divide by fission. The fact that certain of the plant viruses have been isolated in the form of crystalline proteins also causes some to question the appropriateness of applying a binomial nomenclature to viruses. A more realistic approach, however, would seem to be the acceptance of the fact that there is a distinct need for some kind of generally accepted nomenclature for these agents as is evidenced by several past attempts at nomenclatures using numbers, letters, and other designations. Furthermore, the presentation made by Holmes in the Bergey *Manual* constitutes a formal and scientifically valid proposal which should be dealt with in a scholarly manner. On a general ideological basis, the writer is inclined to accept the Holmes classification as a provisionally satisfactory starting point. Furthermore, even though in this paper certain constructive criticism will be offered concerning points of pertinent detail regarding Holmes' treatment of insect viruses, the writer wishes to make clear his appreciation of the great amount of thought and work that this author has put into a commendable pioneer presentation.

So far as the appropriateness of applying the binomial system of nomenclature to the viruses is concerned, with certain possible exceptions (*e.g.*, the advisability of recognizing the viruses as a new kingdom), the writer finds himself in rather general agreement with the point of view expressed by R. E. Buchanan in a talk at the "Bergey's Manual Dinner" in Cincinnati, May 17, 1949, and since then circulated in mimeographed form. In his discussion, Buchanan acknowledges several inadequacies of Holmes' systematics but believes that none of them is fundamental and that there appears to be no good reason why the binomial system should be avoided. The writer also recognizes the statement in the In-

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ternational Bacteriological Code of Nomenclature: "Bacteriological nomenclature considers bacteria, related organisms, and the viruses."

The present paper is concerned with the nomenclature and classification of only those viruses which infect and cause diseases in insects; it does not deal with those viruses, of other animals and of plants, which are transmitted from host to host by insects. Furthermore, it is to be recognized that some of the generalizations made with regard to the insect viruses may not apply to plant and animal viruses in general.

HISTORICAL ASPECTS IN BRIEF

As it was with the virus diseases of plants and animals generally, so it was with the virus diseases of insects—the first ones observed were attributed to other causes. In addition to miasmic causes, early investigators assigned etiological roles to certain protozoa, bacteria, and fungi.

Probably the first recognized disease of insects to be caused by what is now known to be a virus was jaundice disease of the silkworm (*Bombyx mori* (Linn.)). Records as far back as the early 16th century refer to what is very probably this polyhedrosis. It was not until the early part of the 20th century that the virus nature of this disease was definitely acknowledged. Not only was silkworm jaundice the first known virus disease of insects to have been discovered, but even today probably more is known concerning it than any other of the more than a hundred reported virus diseases of insects. Similarly, the agent of silkworm jaundice has served as the center for the development of a nomenclature for the insect viruses.

Believing the agent of silkworm jaundice to be a protozoan, Bolle (1894) gave it the name *Microsporidium polyedricum*. It was Bolle's conception that the polyhedral inclusion bodies were the spores of the protozoan which multiplied in a manner similar to that of coccidia. Von Prowazek (1907) at first also believed that a protozoan was responsible for the disease, but that the polyhedra themselves were simply by-products of the infectious process. He gave to the agent the name *Chlamydozoon bombycis*. Prell (1918) considered the parasite to be located within the polyhedron and that the granules sometimes seen in the inclusion represented the nuclei of the causative organism for which he proposed the new generic name *Crystalloplasma* with the agent of silkworm jaundice, *Crystalloplasma polyedricum* (Bolle), as the type species. Later, Prell (1926) gave the name *Crystalloplasma monachae* to the agent of *Wipfelkrankheit*, a polyhedrosis of the nun-moth caterpillar (*Lymantria monacha* Linn.).

By the time of Prell's contributions, the idea that jaundice disease of the silkworm, as well as similar diseases of other insects, might be caused by a filterable virus was being advanced by several workers, notably Acqua (1918–19) and Paillot (1924 *a*, *b*, 1925, 1926 *a*, *b*, 1930). Paillot identified the virus with certain minute, ultramicroscopic granules which could be demonstrated with the aid of a darkfield microscope. To these granules, which he considered the true cause of the disease, Paillot gave the name *Borrellina bombycis*. The generic name *Borrellina* honored the name of the French bacteriologist, A. Borrel. This genus has retained its validity and includes those viruses which cause the formation of polyhedra in the tissues of the diseased host. According to Holmes (1948), Paillot's original spelling of the generic name with two l's is an error, and the name should be spelled *Borrelina*.

Mention should be made here of a lengthy paper by Del Guercio (1929) dealing with the taxonomy of what he believed to be the etiological agents of polyhedral diseases of numerous insects of Italy. Just what Del Guercio actually saw is difficult to say; they were unlike any known microörganism. He believed them to be organisms phylogenetically located between fungi and algae on the one hand and true bacteria on the other. He characterized them as being microscopic, thallus-shaped, arborescent vegetative growths, having a ramified, involved structure growing out of a stroma, and having fruiting bodies of polyhedric form with some species also having small coccus-like reproductive forms. Of 71 species reported, Del Guercio named 66. These species were separated into 12 genera. The type genus was *Entomococcus* of which the type species was *Entomococcus bombycinus*, the supposed cause of silkworm jaundice. The drawings which illustrate the paper indicate the incongruity of the work, and are difficult to interpret from a biological standpoint. As the writer has indicated elsewhere (1949), it seems unnecessary to give serious consideration to the names proposed by Del Guercio since it is quite obvious that whatever were the forms that he saw, they certainly were not viruses, and would appear to have no phylogenetic relation of any kind to the agents which cause typical polyhedroses.

This brings us to a consideration of the nomenclature proposed by Holmes (1948) in the sixth edition of *Bergey's Manual of Determinative Bacteriology*, and an evaluation of the generic categories.

GENERA OF INSECT VIRUSES

The classification of insect viruses as presented by Holmes groups these agents into two genera, *Borrelina* and *Morator* in the family Borrelinaceae, suborder Zoophagineae, order Virales. The genus *Borrelina* is characterized as consisting of "Viruses inducing polyhedral, wilt, and other diseases; hosts, Lepidoptera, so far as known." The genus *Morator* is given the following description: "Only one species at present, inducing the disease known as sacbrood of the honey bee."

The inadequacies of these descriptions are probably apparent to most students of insect viruses, although it should be admitted that the knowledge concerning these viruses was poorly organized even as recently as when Holmes prepared his classification. Nevertheless, enough information was at hand to have been more exacting in the delimitation of genera. Thus, Holmes' statement that members of the genus *Borrelina* are "known only as attacking lepidopterous insects," although true for the species he recognizes, does not take into account the polyhedroses of certain Hymenoptera and Diptera. His assertion that members of the genus *Morator* are "known only as attacking the honey bee, a hymenopterous insect," could perhaps have been broadened to include the virus (*Borrelina flacheriae* Paillot) responsible for initiating the diseases of gattine and true flacherie of the silkworm, a lepidopterous insect, although the uncertainty which surrounds the nature of this virus could reasonably justify its omission from this genus, at least for the time being. To be more logically questioned is the advisability of including *Borrelina brassicae* Paillot and *Borrelina pieris* Paillot in the genus *Borrelina*, as was done by both Paillot and Holmes. These viruses not only do not give rise to polyhedral inclusions (but rather "granules") in the infected cells of the host, but also produce a distinctly different type of disease from that caused by the socalled polyhedral viruses.

To assist in correcting and clarifying some of these systematic vagaries, the writer has recently proposed (Steinhaus, 1949) that the known insect viruses be considered tentatively as consisting of four principal groups according to the type of inclusion bodies formed when the latter are present. Thus, those viruses causing insect diseases characterized by the presence of polyhedral inclusions in the infected cells of the hosts are placed in the genus *Borrelina* Paillot; those viruses causing insect diseases characterized by the presence of refringent, polymorphic inclusions of very irregular shape and size in the cytoplasm of the infected cells of the host are placed in the genus *Paillotella* Steinhaus; those viruses causing insect diseases characterized by the presence, in large numbers, of very small but microscopically visible granular inclusions in the infected cells (particularly visible in the cytoplasm) of the host, are placed in the genus *Bergoldia* Steinhaus; and those viruses causing insect diseases in which no visible pathological inclusion body of any kind is produced are placed in the genus *Morator* Holmes.

In all probability, the viruses causing diseases in insects are not limited to the four genera just mentioned. Indicative of this is the likelihood that the infection described by Weiser (1948) as occurring in larvae of *Camptochironomus tentans* (Fabr.) may be caused by a virus representative of a fifth genus. Indeed, we should be inclined herewith to recognize such a genus were it not for the fact that the virus itself has not yet been isolated or demonstrated with the electron microscope or other defining physical means. As will be explained shortly, the writer is of the opinion that henceforth and until other criteria are established, no new specific names should be proposed unless the viruses concerned have at least been demonstrated with the electron microscope or by other means which delineate their basic physical and morphological properties. In the present case, the virus not having been demonstrated, it is not possible to designate the required type species for the genus and at the same time adhere to the specifications just mentioned. It may be pointed out, however, that the disease described by Weiser differs from those caused by the viruses of the four named genera primarily by the fact that the relatively large oval inclusion bodies occur in the cytoplasm of the infected cell (the nucleus remaining undamaged and of normal size), and these bodies contain numerous small granules which can be seen in the inclusions even without alkaline treatment.

TYPE SPECIES

Of the four genera into which the insect viruses are grouped, two (*Borrelina* and *Paillotella*) have type species originally named by Paillot as members of the genus *Borrelina*. In neither of these instances is it certain that this French worker actually saw the virus. In the case of jaundice disease of the silkworm (caused by *Borrelina bombycis*) he observed certain minute granules, invisible by ordinary microscopy but visible with the aid of a darkfield microscope, in the blood and tissues of the diseased insect. Paillot believed these granules to be the causative agent of the disease and it was to them he gave the name *Borrellina bombycis* [*Borrelina*]. The exact relation these granules of Paillot's have to the virus still remains to be determined. Since then the actual virus has been isolated and demonstrated with certainty by means of the electron microscope (Bergold, 1947), and has been shown to be a rod-shaped particle approximately 40 by 288 millimicrons in size. In spite of the uncertainty as to the identity of the granules described by Paillot, it is acknowledged that this worker knew he was concerned with a filterable virus and that the name he gave the granules was intended for the etiologic agent of the disease which he believed to be identical with them. Names used prior to that of *Borrelina bombycis* were not favored with as acceptable an insight as to the true cause of the polyhedrosis concerned (most earlier writers thought they were naming a protozoan) and hence they have been generally ignored. Consequently the name proposed by Paillot has been accepted as the name of the virus. In this we follow Holmes' acceptance of Paillot's nomenclature for those species which he named and recognized as associated with certain of the insect virus diseases. It so happens therefore that the specific epithets proposed by Paillot have become those of the type species of each of the genera *Borrelina* and *Paillotella*. The two species concerned are *Borrelina bombycis* Paillot and *Paillotella pieris* (Paillot) Steinhaus. The exact nature of *Paillotella pieris* is unknown; it is the only species known for the genus, and the disease it causes is the only one of its kind so far reported.

At the time the writer (Steinhaus, 1949) proposed the name *Bergoldia* for the genus of viruses which cause those diseases provisionally known as granuloses, the only species bearing a name was *Bergoldia brassicae* (Paillot) Steinhaus (= *Borrelina brassicae* Paillot). Unfortunately, this species was so inadequately known as compared with certain others of the group that it would have been a very unsatisfactory type species for the genus. Furthermore, the genus could not be adequately characterized from what was known of this species alone. Accordingly, in defining the genus, the writer indicated as type species of the genus the virus responsible for the granulosis (Bergold's *Kapselvirus-Krankheit*) of *Cacoecia murinana* (Hbn.), which is the first one of the group to have been isolated in a free state (Bergold, 1948) and one about which relatively much is known. The species was named *Bergoldia calypta*.

The type species of the remaining genus, *Morator*, is *Morator aetatulae* Holmes, the cause of sacbrood in honeybees.

CLASSIFICATION OF INSECT VIRUSES

In recent years considerable progress has been realized in an understanding of the basic nature of the insect viruses. In order to bring the information pertaining to the classification and nomenclature of this group up to parallel these new advances, the arrangement which follows is presented. In making this presentation, however, the writer is under no illusions as to its infallibility. Indeed, it is offered strictly in a tentative and provisional sense, and with the hope that it will serve as a satisfactory starting point for more profound treatment by others.

With regard to the naming of new species of insect viruses, the writer wishes to make the proposal that henceforth new species be named only after the virus itself has been demonstrated as a morphologically distinct entity by accepted physical or visual means. It is admittedly difficult to decide on the criteria that should be used in naming species of viruses. It would seem, however, that there is something fundamentally wrong in describing as a new species an agent which has not even been seen or whose gross morphological aspects have not been determined. At any rate, so far as the insect viruses are concerned, the electron microscope has been able to reveal most species which have been diligently sought. In keeping with this belief, in the present paper the writer is assigning new names only to those species which have at least been demonstrated with the electron microscope. In order to preserve continuity, however, it is suggested that at least for the time being all those species of insect viruses which have been previously recognized as such by Holmes in *Bergey's Manual of Determinative Bacteriology* be accepted and retained even though some of them may not as yet have been seen with the electron microscope or demonstrated by a determination of their physical properties.

Another discipline the writer would like to suggest concerns the description of the various species. In the sixth edition of *Bergey's Manual*, properties (*e.g.*, thermal inactivation) are listed for certain of the polyhedral viruses (genus *Borrelina*) which are based on studies made of polyhedral suspensions and not on the virus itself. Since significant amounts of virus are contained within the polyhedral body and protected by it, a determination of the thermal and other survival properties, for example, of such protected virus would not represent a true picture so far as the virus itself is concerned. It is therefore suggested that henceforth proper care be taken in citing the properties of the virus concerned to differentiate between free virus and virus incorporated within the polyhedral or other inclusion body. The same applies to those viruses of the genus *Bergoldia* which are surrounded by a proteinaceous envelope which undoubtedly exerts a protective influence.

Although aware of the faults inherent in using the hosts as criteria for differentiating species of viruses and in constructing keys to species, the writer recognizes the difficulty in using any other criterion, especially under the present circumstances, and for this reason has provisionally adopted the procedure used by Holmes (1948). For the sake of uniformity, in presenting the following classification we shall follow the generally accepted form used by *Bergey's Manual* wherever this form is applicable to our subject. In so doing, however, we are not acknowledging the necessity of including such matters as symptomatology in the descriptions; this information is being incorporated primarily for the convenience of the reader.

Order **VIRALES** Breed, Murray, and Hitchens
(J. Bact., **47,** 1944, 421)
Suborder **Zoophagineae** Holmes
(Bergey's Manual, 6th ed., 1948, 1225)

I. Viruses causing insect diseases characterized by the presence of polyhedral inclusions in the infected cells of the host. The inclusions (each of which contains numerous virus particles) originate in the nuclei of the host cells. The size and shape of the inclusions may vary within certain limits, but usually they are relatively uniform in these respects and in each case a more-or-less typical form ordinarily predominates.
Genus I. **Borrelina** Paillot

II. Viruses causing insect diseases characterized by the presence of refringent polymorphic inclusions of very irregular shape and size in the infected cells of the host. The inclusions originate in the cytoplasm of the host cell.
Genus II. **Paillotella** Steinhaus

III. Viruses causing insect diseases characterized by the presence, in large numbers, of very small but microscopically discernible granular inclusions in the infected cells of the host. The inclusions are particularly visible in the cytoplasm of the cells, but in some cases at least also occur in the nuclei. [In all instances so far known, the size of the inclusions is less than 1.0 micron in their longest diameter.] In all known cases, a single virus particle is located within each granule.
Genus III. **Bergoldia** Steinhaus

IV. Viruses causing insect diseases in which no visible pathological inclusion body of any kind is produced.
Genus IV. **Morator** Holmes

Genus I. **Borrelina** Paillot
(Compt. Rend. Acad. Sci., Paris, **182,** 1926, 182)

Viruses causing insect diseases characterized by the presence of polyhedral inclusions in the infected cells of the host. The inclusions (each of which contains numerous virus particles) originate in the nuclei of the host cells. The size and shape of the inclusions may vary within certain limits, but usually they are relatively uniform in these respects and in each case a more-or-less typical form ordinarily predominates.

The type species is *Borrelina bombycis* Paillot.

Key to the species of genus **Borrelina**[2]

I. Attacking the silkworm, *Bombyx mori* (Linn.)
1. *Borrelina bombycis* Paillot

II. Attacking the larva of the nun moth, *Lymantria monacha* Linn.
2. *Borrelina efficiens* Holmes

III. Attacking the larva of the gypsy moth, *Porthetria dispar* (Linn.)
3. *Borrelina reprimens* Holmes

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[2] Since this paper was written, Harriette B. Wasser and the writer have isolated the viruses causing polyhedrosis of the forest tent caterpillar, *Malacosoma disstria* Hbn., of the western tent caterpillar, *Malacosoma pluviale* (Dyar), and of the beet armyworm, *Laphygma exigua* (Hbn.). As determined from electron micrographs the size of the *M. disstria* polyhedron averages about 1.8 microns, while that of the virus particle is approximately 40 by 315 millimicrons. The polyhedron from *M. pluviale* averages about 1.6 microns in size, while the virus particle measures approximately 40 by 350 millimicrons. In the case of *L. exigua* the polyhedron has an approximate size range of 1.0 to 1.5 microns in diameter, and the virus particle is about 40 by 270 millimicrons in size. Recently, G. H. Bergold (Forest Insect Invest. Bi-monthly Prog. Rept., 1949, *5*, No. 3, p. 2) reported the isolation of a polyhedrosis virus from the spruce budworm, *Choristoneura fumiferana* (Clem.). The average dimensions of the virus particles are 28 millimicrons in width and 260 millimicrons in length.

IV. Attacking the western yellow-striped armyworm, *Prodenia praefica* Grote.
4. *Borrelina olethria* n.sp.
V. Attacking the alfalfa caterpillar, *Colias philodice eurytheme* Bdvl.
5. *Borrelina campeoles* n.sp.
VI. Attacking the California oakworm, *Phryganidia californica* Pack.
6. *Borrelina peremptor* n.sp.

Borrelina bombycis Paillot
(Compt. Rend. Acad. Sci., Paris, **182,** 1926, 182)

The following organisms and supposed organisms have been designated by their authors as the cause of silkworm jaundice: *Micrococcus bombycis* (Béchamp) Cohn, Beitr. z. Biol. d. Pflanzen, **1,** 1872, 165. *Microsporidium polyedricum* Bolle, Atti e Mem. dell'I. R. Soc. Agr. Gorizia, **33,** 1894, 193. *Micrococcus lardarius* Krassilstschik, Mém. Soc. Zool. de France, **9,** 1896, 513. *Chlamydozoon bombycis* von Prowazek, Arch. f. Protistenk., **10,** 1907, 363. *Crystalloplasma polyedricum* (Bolle) Prell, 1918, (see Verh. III Internat. Entomol.-Kongr., Zürich, **2,** 1926, 152.)

The Virus (Fig. 1): Rods, approximately 40 by 288 millimicrons, fairly uniform in size, occurring singly, in bundles of 2 and sometimes 4 units each. Comprise 3–5 per cent of polyhedron weight. Axial ratio 7.2; sedimentation constant s_{20} 1871; diffusion constant D_{20} 0.215 $\times 10^{-7}$; frictional ratio f/f_0 1.51. Particle weight (from $s_{20} - D_{20}$) 916 $\times 10^6$ (from length and diameter) 299 $\times 10^6$. Filterable through Berkefeld V and N, Chamberland L_1, L_2, L_3 filters. Chemical constituency: nucleoprotein (desoxyribonucleic acid type), relatively high phosphorus content. Inactivated (in polyhedral suspensions) by: boiling for 10 minutes. 60 C for 30 minutes, alcohol and ether, strong acids and strong alkalies, 1 per cent dodecyl sulfate, 36 per cent urea, 36 per cent guanidine, antiformin formalin. In polyhedral suspensions, resists action of weak acids and weak alkalies, drying, and is suspendible in water. (M.I.D. = 1.0 $\times 10^{-11}$ g/host animal.)

The Inclusion (Fig. 2): Polyhedron with 5 to 8 faces, usually 6 hexagonal rhombododecahedra; rather sharp, angular corners. Size ranges from 0.5 to 15 microns (average 3 to 5 microns) in diameter; usually fairly regular in size. Refractile crystallike, dense toward center, sometimes appears to have layers as though formed by accretion; may crack on pressure. Not optically active. Heavier than water. Stains rather poorly with ordinary aniline dyes; better when mordants are used. Has somewhat of a limiting "membrane." Soluble in weak alkalies (*e.g.*, 0.006M Na_2CO_3, 0.06 per cent KOH or NaOH; optimum solubility at pH

PLATE I

1. Electron micrographs of *Borrelina bombycis* Paillot, the cause of jaundice in the silkworm, *Bombyx mori* (Linn.). Top view shows individual virus particles, and bundles of two particles each; lower view (negative print) shows individual particles. The virus particles and bundles have been freed from the polyhedra by dissolution of the latter in dilute sodium carbonate. Approximate magnification, top view: 27,500 ×; lower view: 21,000 ×.
2. Polyhedra characteristic of silkworm jaundice, as seen with an ordinary light microscope. Magnification approximately 1,000 ×.
3. Electron micrograph of *Borrelina efficiens* Holmes, the cause of a polyhedrosis (*Wipfelkrankheit*) of the nun-moth caterpillar, *Lymantria monacha* (Linn.). Bundles predominate. Magnification approximately 16,800 ×.
4. Preparation showing the polyhedra characteristic of the polyhedrosis of the nun-moth caterpillar. Magnification approximately 460 ×.
5. Electron micrograph of *Borrelina reprimens* Holmes, the cause of a polyhedrosis ("wilt disease") of the gypsy-moth caterpillar, *Porthetria dispar* (Linn.). Magnification approximately 37,500 ×.
6. Polyhedra characteristic of the polyhedrosis of the gypsy-moth caterpillar. Magnification approximately 1,000 ×.

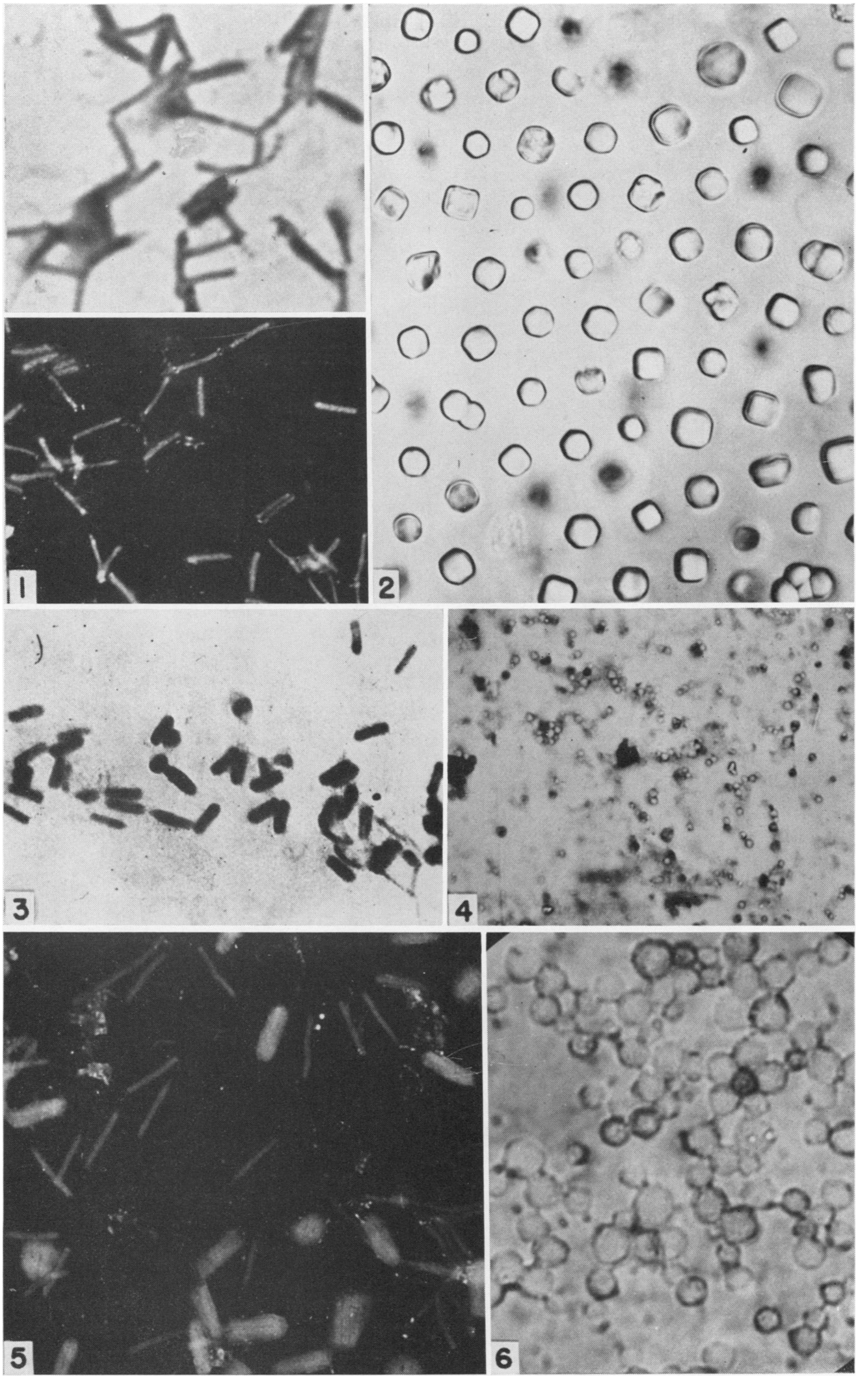

PLATE I

10.8 to 11.0) and in weak acids (optimum solubility at pH 1.0 to 0.5). Insoluble in hot or cold water, alcohol, chloroform, ether, or xylol. Chemical constituency: nucleoprotein. When dissolved in Na_2CO_3, the principal component has a molecular weight of about 378,000 and a diameter of about 10 millimicrons (sedimentation constant s_{20} 12.85); the molecular weight of the split components is about 60,500 (sedimentation constant s_{20} 3.16). Lower phosphorus content than virus. Contains no fat as indicated by its failure to stain with Sudan III and osmic acid. Amino acid content consists of following percentages; histidine 2.5, arginine 5.6, lysine 10.6, tyrosine 9.6, phenylalanine 6.7, tryptophan 3.3, cystine 0.52, methionine 3.3, and alanine 4.4. Small amounts of iron (0.5 per cent) are also detectable. The polyhedron protects virus activity from heat at 60 C for 30 minutes, sunlight irradiation 2 to 10 hours, putrefaction 22 months, 0 to 4 C in insect blood 1 year, storage in 1 per cent NaCl and glycerol in 1 per cent toluol, or in 1 per cent zepharol at 40 C for 22 months, drying in high vacuum (10^{-4} mm Hg) for 5 hours, drying in air at room temperature for months, and 15-minute treatments with the following: acetone, ether, 2.5 to 30 per cent formaldehyde, 5 per cent phenol, 5 per cent mercuric chloride, 70 per cent alcohol, and a 1:1 solution of 96 per cent alcohol and 1:1000 mercuric chloride. Virus activity completely inactivated when boiled in water for 10 minutes, or when treated for 15 minutes with trichloracetic acid.

Host: The silkworm, *Bombyx mori* (Linn.) (Lepidoptera, Bombycidae).

Disease Caused: Silkworm jaundice. (Synonyms: *grasserie, giallume, Gelbsucht.*) Symptoms: Infected larvae may exhibit loss of appetite and show general inactivity although frequently these symptoms are absent until late in the disease. The insect's body assumes a distended, swollen or fatlike appearance. Just before death the integument becomes opaque and assumes a shiny, yellow color. The larva is very flaccid because of the disintegration of internal tissues. The caterpillar is difficult to move without breaking the integument and liberating the liquefied contents. Incubation period 6 to 8 days. Polyhedra form in the nuclei of infected cells of hypodermis, fat body, tracheal matrix, and probably other tissues but not in the cells of the gut epithelium.

Pertinent Literature: Bergold, Biol. Zent., **63**, 1943, 1–55; Zeit. f. Naturforsch., **2b**, 1947, 122–143; Zeit. f. Naturforsch., **3b**, 1948, 25–26. Desnuelle, *et al.*, Ann. Inst. Pasteur, **69**, 1943, 75–86, 248–250; **71**, 1945, 264–272. Glaser and Chapman, Biol. Bull., **30**, 1916, 367–391. Glaser and Lacaillade, Am. J. Hyg., **20**, 1934, 454–464. Glaser and Stanley, J. Exp. Med., **77**, 1943, 451–466. Paillot, Traité des maladies du ver à soie, Doin, Paris, 1930, 137–184. Paillot and Gratia, Arch. f. Gesell. Virusforsch., **1**, 1939, 1920–1929. Steinhaus, Principles of Insect Pathology, McGraw-Hill, 1949, 425–449.

Borrelina efficiens[3] Holmes

(Bergey's Manual, 6th ed., 1948, 1226)

The following organisms and supposed organisms have at one time been designated by their authors as the cause of the polyhedrosis (*Wipfelkrankheit*) concerned: *Bacillus* "B" Hofmann, Die Schlaffsucht der Nonne, P. Weber, Frankfurt, 1891, 31 pp. *Bacterium monachae* v. Tubeuf, Forstl. Naturwiss. Z., **1**, 1892, 41. *Bacillus monachae* Eckstein, Z. Forst- u. Jagdwesen, **26**, 1894, 6. *Crystalloplasma monachae* Prell, Verh. III Internat. Entomol.-Kongr., Zürich, 1926, 152. By some authors: *Chlamydozoon prowazeki* Wolff, Mitt. Kais.-Wilh. Inst. Landw. Bromberg, **3**, 1910, 69 (named as causing polyhedrosis in larvae of *Bupalus piniarius* Linn.).

The Virus (Fig. 3): Rods, somewhat larger than *Borrelina bombycis* Paillot, occurring in bundles of 2 units each. Probably has the general physical and chemical properties similar to those of *Borrelina bombycis* Paillot.

The Inclusion (Fig. 4): Polyhedron more or less triangular to tetrahedral in shape. Size averages about 2.5 microns in diameter. Remaining characteristics similar to those described for polyhedra associated with *Borrelina bombycis* Paillot. The polyhedron protects

[3] As the result of an obvious typographical error, this specific epithet is spelled *efflciens* at the point where it is described. Elsewhere in the same publication it is spelled *efficiens*.

virus activity for 3 years when held in a dry state. Held moist, in glycerol, polyhedral suspensions retain virus activity for at least 5 days. Virus withstands putrefaction.

Host: Larva of the nun-moth, *Lymantria monacha* (Linn.) (Lepidoptera, Lymantriidae).

Disease Caused: Wipfelkrankheit (Synonyms: *Wipfelsucht*, nun-moth wilt, nun-moth polyhedrosis). Symptoms: Infected larvae show loss of appetite, become very flaccid, and if disturbed shortly before or after death the broken integument liberates the fluid, disintegrating internal tissues containing large numbers of polyhedra. Before dying the larvae tend to migrate to the tops of trees (*Wipfeln*). Incubation period from 13 to 15 days.

Pertinent Literature: Bergold, Biol. Zent., **63,** 1943, 1–55; Zeit. f. Naturforsch., **3b,** 1948, 25–26. Heidenreich, Arch. f. Gesell, Virusforsch., **1,** 1940, 582. Komárek and Breindel, Z. Angew, Entomol., **10,** 1924, 99–162. Wahl, Cent. Ges. Forstw., **35,** 1909, 164–172; 212–215; **36,** 1910, 377–397; **37,** 1911, 247–268; **38,** 1912, 355–378.

Borrelina reprimens Holmes

(Bergey's Manual, 6th ed., 1948, 1226)

At one time Glaser and Chapman (Science, **36,** 1912, 219) believed that the polyhedrosis of the gypsy moth was caused by a small gyrating micrococcus which they named *Gyrococcus flaccidifex*. A year later (J. Econ. Entomol., **6,** 1913, 479) they realized that this bacterium was only a secondary invader and that the true cause of the disease was a filterable virus.

The Virus (Fig. 5): Rods, approximately 41 by 360 millimicrons, occurring in bundles having an average size of 160 by 415 millimicrons. At certain points along each of the rod-shaped particles making up a bundle can sometimes be seen small nodes directly opposite each other when two or more particles are still hanging together. Sedimentation constant s_{20} 2500 to 4000 (depending on number of virus particles adhering together); diffusion constant D_{20} 0.175 $\times$ 10^{-7}; frictional ratio f/f_0 1.42; axial ratio 8.8. A particle weight of 1300 $\times$ 10^6 when calculated from the length and diameter of the virus particle as seen in electron micrographs. Filterable through Berkefeld N but apparently not through Chamberland F filters. Chemical constituency: nucleoprotein of the desoxyribonucleic acid type. (M.I.D. $= 1.0 \times 10^{-10}$ g/host animal.)

The Inclusion (Fig. 6): Polyhedron with 5 to 8 faces, crystalline in appearance, corners not regularly as sharp or as angular as in case of those associated with *Borrelina bombycis* Paillot. Average size 3.5 microns, although variations of from 0.5 to 15.0 microns have been reported. They are nucleoprotein in nature. The principal component has a sedimentation constant of s_{20} 12.57 and a molecular weight of 276,000. The split components have a sedimentation constant of 3.12 and a molecular weight of 47,250. In general properties, the polyhedra associated with *Borrelina reprimens* Holmes are very similar to those of the polyhedra associated with *Borrelina bombycis* Paillot (which see).

Host: Larva of the gypsy moth, *Porthetria dispar* (Linn.) (Lepidoptera, Lymantriidae).

Disease Caused: Polyhedrosis of gypsy-moth caterpillar. (Synonyms: wilt disease, gypsy-moth wilt.) Symptoms: Infected caterpillars lose their appetite, become sluggish in movement. Diseased insects usually seek an elevated place on their host plant. Just before death they become soft, the internal tissues disintegrate and liquefy, and upon rupturing the integument a brownish liquid is liberated.

Pertinent Literature: Bergold, Biol. Zent., **63,** 1943, 1–55; Zeit. f. Naturforsch., **2b,** 1947, 122–143; Zeit. f. Naturforsch., **3b,** 1948, 25–26. Chapman and Glaser, J. Econ. Entomol., **9,** 1916, 149–167. Glaser, J. Agr. Research, **4,** 1915, 101–128; Science, **48,** 1918, 301–302. Glaser and Chapman, J. Econ. Entomol., **6,** 1913, 479–488. Steinhaus, Principles of Insect Pathology, McGraw-Hill, 1949, 454–464.

Borrelina olethria n.sp.

(From Greek *olethrios*, destructive)

The Virus (Fig. 7): Rods, occurring in bundles of several members each. Approximate size 50 by 290 millimicrons.

The Inclusion (Fig. 8): Polyhedron, appears to have 4 or 5 sides when seen in outline. Size ranges from 2.0 to 5.0 microns with an average diameter of about 3.0 microns. General properties probably similar to those described for the polyhedra associated with *Borrelina bombycis* Paillot.

Host: The western yellow-striped armyworm, *Prodenia praefica* Grote (Lepidoptera, Phalaenidae [Noctuidae]).

Disease Caused: Polyhedrosis. Symptoms: Infected larvae lose their appetites, become sluggish in movement. They may turn reddish brown before death, after which the body contents disintegrate into a dark watery mass within the integument.

Pertinent Literature: Blanchard and Conger, J. Econ. Entomol., **25,** 1932, 1059–1070. Steinhaus, Principles of Insect Pathology, McGraw-Hill, 1949, 467–468.

Borrelina campeoles n.sp.

(From Greek *kampe*, caterpillar + *olesai* to destroy)

The Virus (Fig. 9): Rods, approximately 40 by 300 millimicrons, occurring in bundles of several members each. Filterable through bacteriological filters of coarse to medium porosity. Most of the physical and chemical agents which destroy bacteria also destroy the virus. Incorporated within the polyhedron the virus is very resistant to drying and can retain its infectivity for at least a year.

The Inclusion (Fig. 10): Polyhedron, appears to have from 3 to 6 sides when seen in outline, varies considerably in shape. Corners are angular but somewhat rounded. Size varies from 1.0 to 3.0 microns in diameter, with an average width of about 1.5 microns. Exceptionally large polyhedra (4.0 to almost 5.0 microns) are seen occasionally. Most of the general properties of the polyhedra are essentially the same as those described under *Borrelina bombycis* Paillot.

Host: The alfalfa caterpillar, *Colias philodice eurytheme* Bdvl. (Lepidoptera, Pieridae).

Disease Caused: Polyhedrosis of the alfalfa caterpillar. (Synonyms: Wilt disease, wilt.) Symptoms: Infected larvae show loss of appetite, and decreased activity. The normally green color of the larva changes to a pale, yellowish, or grayish-green, sometimes giving the insect a mottled appearance. Body becomes very flaccid and somewhat darkened and usually dies about 7 days after infection. The internal tissues become liquefied and the caterpillar breaks down into a disintegrating mass. Pupa may also show symptoms of disease.

Pertinent Literature: Michelbacher and Smith, Hilgardia, **15,** 1943, 369–397. Steinhaus, J. Econ. Entomol., **41,** 1948, 859–865; Principles of Insect Pathology, McGraw-Hill, 1949, 477–484. Wildermuth, U. S. Dept. Agr., Circ. 133, 1911, 1–14; Bull. 124, 1914, 1–40.

PLATE II

7. Electron micrographs of *Borrelina olethria* n.sp., the cause of a polyhedrosis of the western yellow-striped armyworm, *Prodenia praefica* Grote. Top view shows virus bundles (dark bodies are salt crystals); lower view shows individual virus particles as seen in a gold-shadowed preparation. Magnification approximately 12,500 ×.
8. Polyhedra characteristic of the polyhedrosis of the western yellow-striped armyworm. Magnification approximately 1,000 ×.
9. Electron micrograph (gold-shadowed preparation) of *Borrelina campeoles* n.sp., the cause of a polyhedrosis of the alfalfa caterpillar, *Colias philodice eurytheme* Bdvl. Virus bundles as well as individual virus particles may be seen. Magnification approximately 12,500 ×.
10. Polyhedra characteristic of the polyhedrosis of the alfalfa caterpillar. Magnification approximately 1,000 ×.
11. Electron micrograph of *Borrelina peremptor* n.sp., the cause of a polyhedrosis of the California oakworm, *Phryganidia californica* Pack., showing individual virus particles. Magnification approximately 12,500 ×.
12. Polyhedra characteristic of the polyhedrosis of the California oakworm. Magnification approximately 1,000 ×.

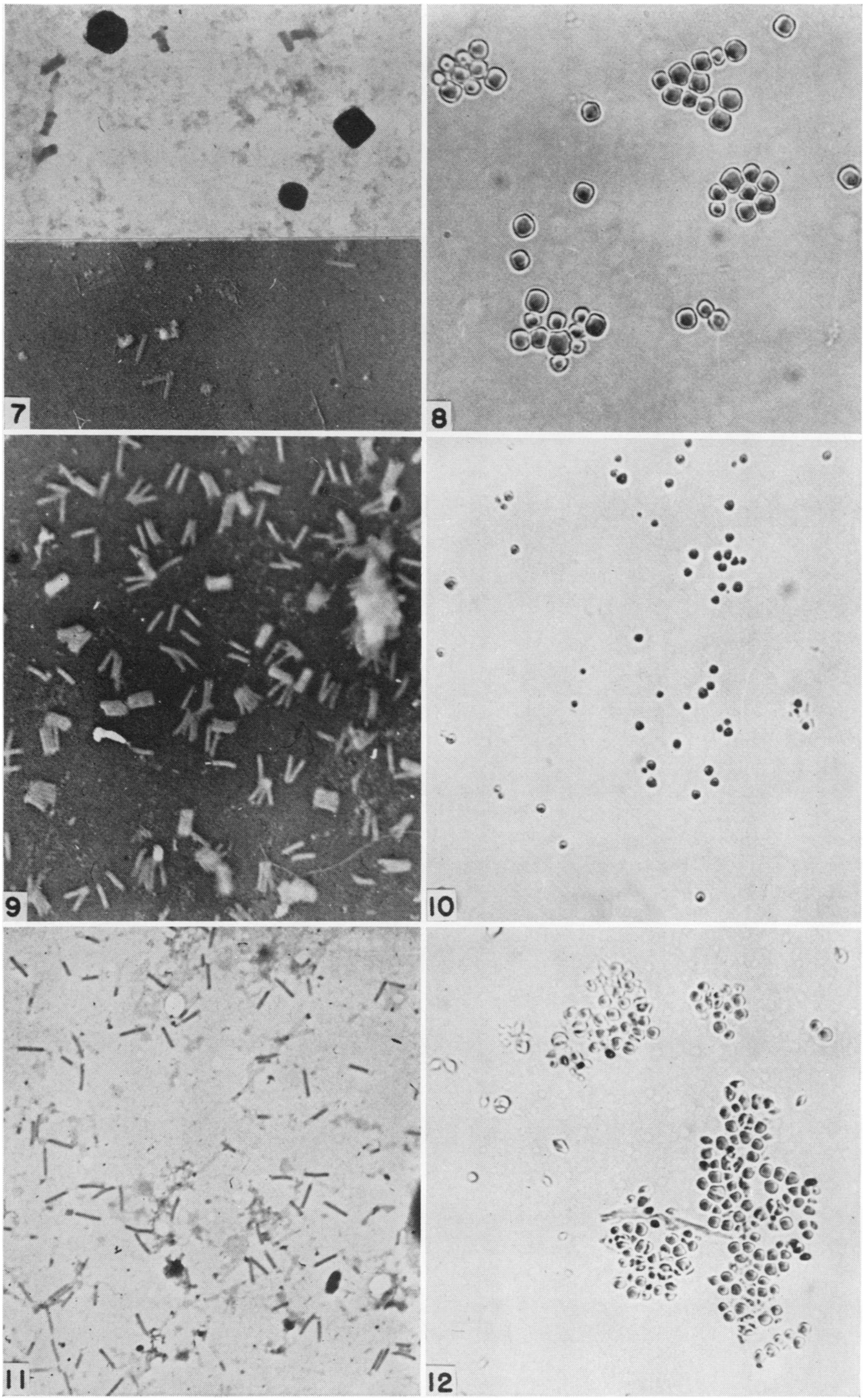

PLATE II

Borrelina peremptor n.sp.

(From Latin *peremptor*, a destroyer)

The Virus (Fig. 11): Rods, approximately 30 by 270 millimicrons, occurring in bundles of several members each (in electron microscope preparations so far examined, the bundle formation has been seen only rarely). Most physical and chemical agents which destroy bacteria also destroy the virus. Within the polyhedron the virus is protected from these agencies to a considerable degree.

The Inclusion (Fig. 12): Polyhedron, appears to have from 3 to 5 sides when seen in outline, varies considerably in shape and may be very irregular; corners rounded. Size ranges from 1.0 to 3.5 microns in diameter; averages about 2.0 microns.

Host: The California oakworm, *Phryganidia californica* Pack. (Lepidoptera, Dioptidae).

Disease Caused: Polyhedrosis of the California oakworm. Symptoms: Infected larvae become sluggish in movement and lose their appetites. Natural coloration becomes less intense; recently dead larvae may assume a pink or faint reddish color. Internal tissues liquefy; the darkened dead larvae lose their normal form and hang limp from host plant.

Pertinent Literature: Chapman and Glaser, J. Econ. Entomol., **8,** 1915, 140–150. Steinhaus, Principles of Insect Pathology, McGraw-Hill, 1949, 472.

Genus II. **Paillotella** Steinhaus

(Principles of Insect Pathology, McGraw-Hill, 1949, 422)

Viruses causing insect diseases characterized by the presence of refringent, polymorphic inclusions of very irregular shape and size in the infected cells of the host. The inclusions originate in the cytoplasm of the host cell.

The type, and only, species is *Paillotella pieris* (Paillot) Steinhaus.

Key to species of genus **Paillotella**

I. Attacking the larva of the cabbage butterfly of Europe, *Pieris brassicae* (Linn.)

1. *Paillotella pieris* (Paillot) Steinhaus

Paillotella pieris (Paillot) Steinhaus

(Compt. Rend. Acad. Sci., Paris, **182,** 1926, 182; Principles of Insect Pathology, 1949, 422 and 498.)

The Virus: Not yet demonstrated with certainty. Paillot has described certain small granules less than 0.1 micron in diameter which appear to be associated with infectiousness. Electron microscope observations not yet made. Granules retained by Chamberland filter of fine porosity and the infectivity of the blood is thus destroyed. Infectivity also destroyed by heating at 75 C for one-half hour.

The Inclusion (Fig. 22): Polymorphic. Refringent. Of irregular size and shape: annular, spherical, globoid, and elongated structures. Form in cytoplasm of infected cell. Believed to arise from the mitochondria of the cell. Occur in fat and blood cells.

Host: The larva of the cabbage butterfly of Europe, *Pieris brassicae* (Linn.) (Lepidoptera, Pieridae).

Disease Caused: Polymorphic-inclusion disease. Symptoms: Very few outward signs. The blood of diseased larvae is viscous and milky in appearance. Mortality varies, may be low to fairly high. Pupae also susceptible. Experimentally, virus difficult to establish in insects when given *per os*. Direct inoculation succeeds. Virus also transmitted from one generation to the next in association with the egg.

Pertinent Literature: Paillot, Compt. Rend. Acad. Sci., Paris, **182,** 1926, 180–182; Ann. Inst. Pasteur, **40,** 1926, 314–352. Steinhaus, Principles of Insect Pathology, McGraw-Hill, 1949, 497–500.

Genus III. **Bergoldia** Steinhaus

(Principles of Insect Pathology, McGraw-Hill, 1949, 422.)

Viruses causing insect diseases characterized by the presence, in large numbers, of very small but microscopically discernible granular inclusions in the infected cells of the host. The inclusions are particularly visible in the cytoplasm of the cells, but in some cases at least also occur in the nuclei. [In all instances so far known, the size of the inclusions is less than 1.0 micron in their longest diameter.] In all known cases, a single virus particle is located within each granule.

The type species is *Bergoldia calypta* Steinhaus.

Key to the genus **Bergoldia**

I. Attacking the fir-shoot roller, *Cacoecia murinana* (Hbn.)
1. *Bergoldia calypta* Steinhaus

II. Attacking the variegated cutworm, *Peridroma margaritosa* (Haw.)
2. *Bergoldia daboia* n.sp.

III. Attacking the buckeye caterpillar, *Junonia coenia* Hbn.
3. *Bergoldia lathetica* n.sp.

IV. Attacking the salt-marsh caterpillar, *Estigmene acraea* (Drury)
4. *Bergoldia thompsonia* n.sp.

V. Attacking the larva of the cabbage butterfly of Europe, *Pieris brassicae* (Linn.).
5. *Bergoldia brassicae* (Paillot) Steinhaus

Bergoldia calypta Steinhaus

(Principles of Insect Pathology, McGraw-Hill, 1949, 422 and 512.)

The Virus (Fig. 13): Rods, 50 by 262 millimicrons, occurring singly and enclosed in a proteinaceous envelope or "capsule." Svedberg sedimentation constant s_{20} 1324; diffusion constant D_{20} 0.278×10^{-7}; a frictional ratio f/f_0 1.49; axial ratio 5.2. Particle weight 460×10^6 when calculated from sedimentation and diffusion constants, and 435×10^6 when calculated from the length and diameter of the particle as seen on electron micrographs.

The Inclusion (Fig. 14): Proteinaceous envelope or "capsule" which surrounds the virus particle. Approximate size: 0.23 by 0.36 micron. Dissolves in weak alkali giving a main component with a sedimentation constant of s_{20} 11.8, and a molecular weight of about 300,000. The split components have a sedimentation constant s_{20} 3.45, and a molecular weight of about 60,000.

Host: The fir-shoot roller, *Cacoecia murinana* (Hbn.) (Lepidoptera, Tortricidae).

Disease Caused: Granulosis. (Synonym: *Kapselvirus-Krankheit.*) Symptoms: Few external symptoms until shortly before death at which time the normal yellowish-green insects become thickly swollen and are colored a pale greenish hue. Upon puncturing the integument of a diseased larva, a milky-white fluid oozes out. This fluid contains the small granules which harbor the virus particles.

Pertinent Literature: Bergold, Zeit. f. Naturforsch., **3b**, 1948, 338–342. Steinhaus, Principles of Insect Pathology, McGraw-Hill, 1949, 510–514.

Bergoldia daboia n.sp.

(From Hindu *daboya*, that lies hidden)

The Virus: (Fig. 15): Rods, approximately 40 by 340 millimicrons, occurring singly and enclosed within ovoid envelope; occasionally somewhat curved. Sometimes several rods arrange themselves end to end in chainlike fashion.

The Inclusion (Figs. 16 and 21): Ovoid envelope covering virus particle, approximately 0.25 by 0.45 micron. Occasionally rather long coalesced forms seen. Soluble in low concentrations of Na_2CO_3.

Host: Variegated cutworm, *Peridroma margaritosa* (Haw.) (Lepidoptera, Noctuidae).

Disease Caused: Granulosis. Symptoms: Two or three days after infection the larvae begin to eat less food than normally; they remain slightly smaller in size than normally developing insects, have a somewhat languid appearance, and usually die before pupating. The larvae are flaccid but the body wall remains relatively firm. The fat tissue of the insect is especially affected. Histopathology of the disease under discussion is characterized by the accumulation of the granular inclusions in the diseased cell; they are particularly visible in the cytoplasm.

Pertinent Literature: Steinhaus, Science, **106,** 1947, 323–324; Principles of Insect Pathology, McGraw-Hill, 1949, 508–511. Steinhaus, Hughes, and Wasser, J. Bact., **57,** 1949, 219–224.

Bergoldia lathetica n.sp.

(From Greek, *lathētikos*, likely to escape notice.)

The Virus (Fig. 17): Rods, approximately 40 by 300 millimicrons, occurring singly and enclosed within an ovoid envelope.

The Inclusion (Fig. 18): Ovoid envelope, covering virus particle, approximately 0.30 by 0.45 micron. Soluble in low concentrations of Na_2CO_3.

Host: The larva of the buckeye, *Junonia coenia* Hübner (Lepidoptera, Nymphalidae).

Disease Caused: Granulosis. Symptoms: Similar to those caused by *Bergoldia daboia* in the variegated cutworm. The fat body of the buckeye is not as prominent as is that of the cutworm so that the white, opaque aspect of this structure is not as noticeable as in the latter insect. Buckeye caterpillars may be almost moribund without showing much external evidence of disease, except a general lack of activity and loss of appetite. Usually, however, the diseased larvae appear brownish in color and lose most of their blue metallic luster. Presence of the granules in the nuclei of the fat cells is more evident than in the cutworm. Granules may also be present in cytoplasm. The nuclei hypertrophy greatly before breaking down.

Pertinent Literature: Steinhaus, Principles of Insect Pathology, McGraw-Hill, 1949, 501. Steinhaus and Thompson, Science, 1949, **110,** 276–278.

Bergoldia thompsonia n.sp.

(Named for Clarence G. Thompson who first observed the infection in salt-marsh caterpillars collected in the field.)

The Virus (Fig. 19): Rods, approximately 40 by 270 millimicrons, occurring singly and enclosed within an ovoid envelope.

The Inclusion (Fig. 20): Ovoid envelope covering virus particle, approximately 0.25 by 0.40 micron. Soluble in low concentrations of Na_2CO_3.

Host: The salt-marsh caterpillar, *Estigmene acraea* (Drury) (Lepidoptera, Arctiidae).

Disease Caused: Granulosis. Symptoms: Loss of appetite and lessened general activity. The hairy covering of the larvae obscures any other external symptoms. Upon death the larvae are flaccid and soft.

Pertinent Literature: Steinhaus, Principles of Insect Pathology, McGraw-Hill, 1949, 501. Steinhaus and Thompson, Science, 1949, **110,** 276–278.

Bergoldia brassicae (Paillot) Steinhaus

(Paillot, Compt. Rend. Acad. Sci., Paris, **182,** 1926, 182. Steinhaus, Principles of Insect Pathology, McGraw-Hill, 1949, 502.)

The Virus: Not yet demonstrated with the electron microscope. Probably similar to the other viruses of this genus.

PLATE III

13. Electron micrographs (negative print) of *Bergoldia calypta* Steinhaus, the cause of a granulosis of the fir-shoot roller, *Cacoecia murinana* (Hbn.). Each of the virus particles has been freed from a granular inclusion body (as shown in fig. 14) by dissolution of the latter by dilute sodium carbonate. Magnification approximately 27,000 ×.
14. Electron micrograph (negative print) of the granular inclusions characteristic of the granulosis of the fir-shoot roller. The large form probably represents a combined grouping of granules. Magnification approximately 27,000 ×.
15. Electron micrograph of *Bergoldia daboia* n.sp., the cause of a granulosis of the variegated cutworm, *Peridroma margaritosa* (Haw.). Magnification approximately 12,500 ×.
16. Electron micrograph of the granular inclusions characteristic of the granulosis of the variegated cutworm. Magnification approximately 12,500 ×.
17. Electron micrograph of *Bergoldia lathetica* n.sp., the cause of a granulosis of the buckeye caterpillar, *Junonia coenia* Hbn. Magnification approximately 12,500 ×.
18. Electron micrograph (gold-shadowed preparation) of the granular inclusions characteristic of the granulosis of the buckeye caterpillar. Magnification approximately 12,500 ×.

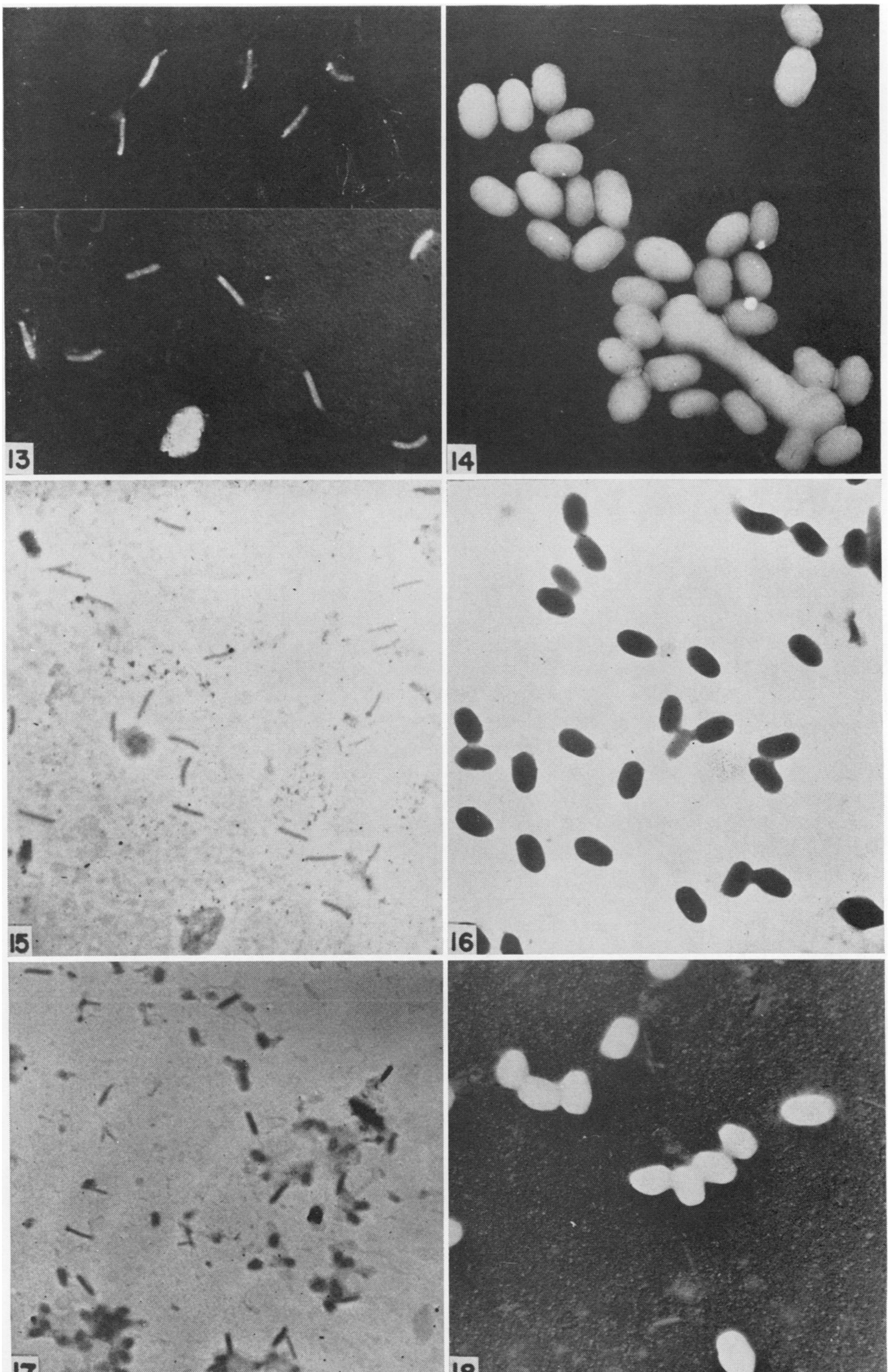

PLATE III

The Inclusion: Similar to that of other granuloses. Size averages about 0.2 to 0.3 micron "in diameter" (Paillot) although they are probably ovoid in shape as are the inclusions of the other granuloses.

Host: Larva of the cabbage butterfly of Europe, *Pieris brassicae* (Linn.) (Lepidoptera, Pieridae).

Disease Caused: Granulosis. Symptoms: Infected insects exhibit a whitish-yellow color on their ventral surface, lose their appetite and are less active. Upon death the body is soft and flaccid.

Pertinent Literature: Paillot, Compt. Rend. Acad. Sci., Paris, **182,** 1926, 180–182; *ibid.*, **198,** 1934, 204–205. Steinhaus, Principles of Insect Pathology, McGraw-Hill, 1949, 532.

Genus IV. **Morator** Holmes

(Bergey's Manual, 6th ed., 1948, 1227.)

Viruses causing insect diseases in which no visible pathological inclusion body of any kind is produced.

The type, and only reported species is *Morator aetatulae* Holmes.

Key to species of genus **Morator**

I. Attacking the honeybee, *Apis mellifera* Linn.

1. *Morator aetatulae* Holmes

Morator aetatulae Holmes

(Bergey's Manual, 6th ed., 1948, 1227.)

The Virus: Not yet demonstrated with the electron microscope.[4] Passes Pasteur-Chamberland and Berkefeld filters. Virus is rendered inactive when heated in water at 59 C for 10 minutes, and in honey at approximately 70 C for 10 minutes. It withstands drying at room temperature for approximately three weeks. Dried virus exposed to the direct rays of the sun is destroyed in from 4 to 6 hours; when it is suspended in honey it is destroyed in from 5 to 6 hours. When suspended in honey and shielded from the direct sunlight the virus remains virulent for slightly less than one month at room temperature during the summer. In the presence of fermentative processes taking place in a 10 per cent cane sugar solution at room temperature, the virus is destroyed in about 5 days—the same period of survival as in a 20 per cent honey solution at outdoor temperatures. In the presence of putrefactive processes the virus remains virulent for approximately 10 days. The virus will resist 0.5, 1.0, and 2.0 per cent phenol for more than 3 weeks. Dead larvae which have remained in the brood comb more than one month are usually noninfectious. A single, freshly dead larva, contains enough infectious material to kill at least 3,000 healthy larvae in one week. Trans-

[4] Working with certain purified materials from honeybee larvae dying of sacbrood, Harriette B. Wasser and the writer have obtained electron micrographs of small particulate bodies which may possibly represent the virus of sacbrood although their identity has by no means been proved. Similar bodies have not as yet been found in material from healthy larvae. On the other hand, the infectivity of the minute particles remains to be demonstrated. The particles are spherical to slightly oval in shape and are approximately 60 millimicrons in diameter.

PLATE IV

19. Electron micrograph of *Bergoldia thompsonia* n.sp., the cause of a granulosis of the saltmarsh caterpillar, *Estigmene acraea* (Drury). Two "granules" may also be seen. Magnification approximately 12,500 ×.
20. Electron micrograph (gold-shadowed preparation) of the granular inclusions character istic of the granulosis of the saltmarsh caterpillar. Magnification approximately 12,500 ×.
21. Wet-mount preparation (as seen with an ordinary light microscope) of the granular

(*Continued on opposite page*)

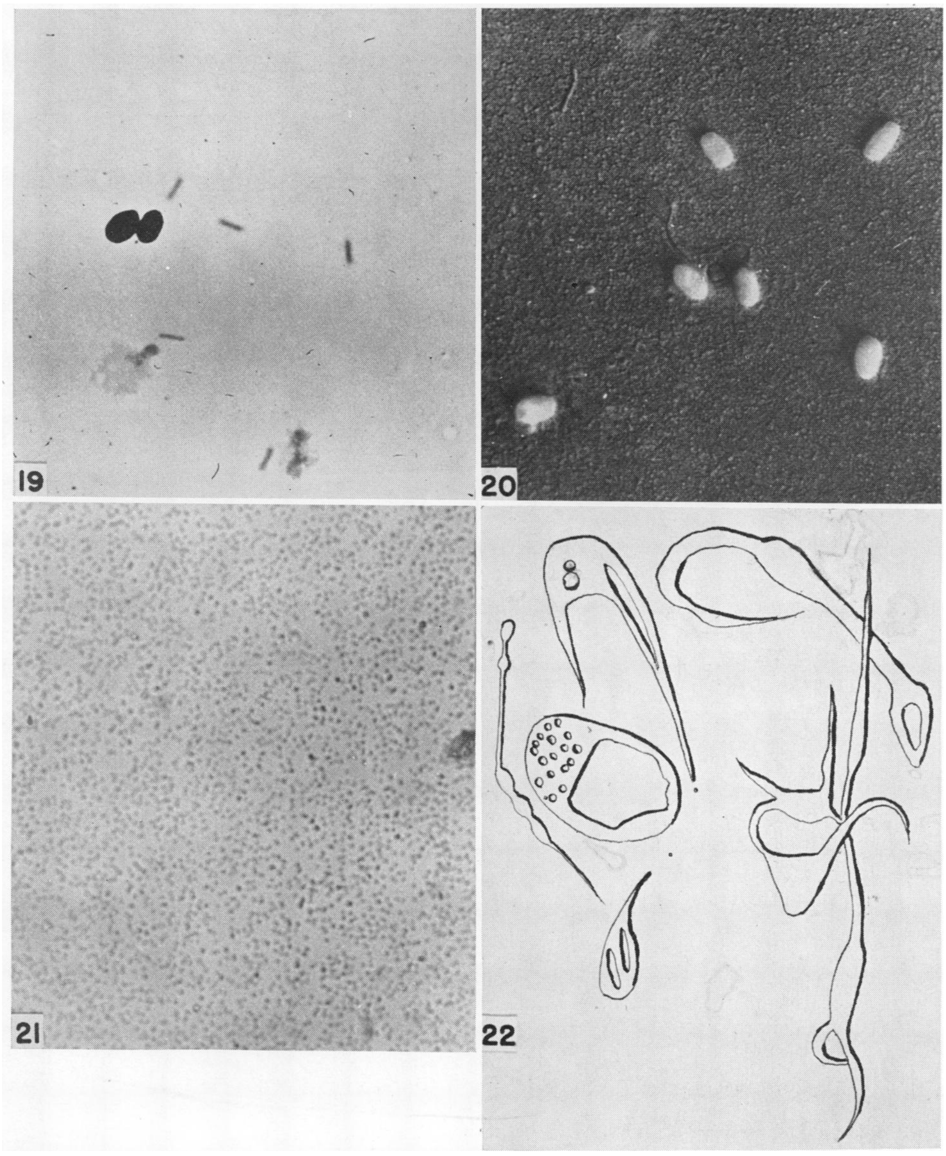

PLATE IV

inclusions from the fat tissue of a variegated cutworm, *Peridroma margaritosa* (Haw.), infected with *Bergoldia daboia* n.sp. Magnification approximately 650 ×.

22. Drawing of the refringent polymorphic inclusion bodies which characterize the disease of the larva of the cabbage butterfly of Europe, *Pieris brassicae* (Linn.), caused by *Paillotella pieris* (Paillot). (Redrawn from Paillot, 1926.)

(Photographs 1, 3, 5, 13, and 14, are from publications by Dr. G. H. Bergold and were obtained through the courtesy of Dr. Bergold. Photographs 2, 4, and 6 were obtained through the courtesy of the late Dr. R. W. Glaser. Photographs 7, 8, 9, 10, 12, 15, 16, 18, 20, and 21 were made for the author by Mr. K. M. Hughes; and photographs 11, 17, and 19 were prepared by Mrs. H. B. Wasser.)

mission occurs through the agency of contaminated food and probably water supply of insects.

Host: The larva of the honeybee, *Apis mellifera* Linn. (Hymenoptera, Apidae).

Disease Caused: Sacbrood, Symptoms: In his taxonomic account, Holmes (1948) described the disease as follows: "In the honey bee, immature stages only are susceptible; infected larvae die, usually after capping, some of the dead brood being uncapped by the bees. Occasionally caps are punctured. Affected areas of comb are usually small and scattered. Each larva is extended along its cell, head turned upward toward the roof. A larva recently dead appears light yellow, light gray, or light brown, soon darkening to brown or almost black. Cuticle of dead larva tough, permitting extraction of the saclike mass without rupture; contents watery with many suspended, fine brown particles. There are no characteristic intracellular bodies in affected tissues. Dead larvae eventually dry down to form scales that are black and roughened, that separate readily from the cell wall, and that may be lifted out intact."

Pertinent Literature: Bergey's Manual, 6th ed., 1948, 1227–1228. White, U. S. Dept. Agr., Bur. Entomol., Circ. 169, 1913, 1–5; U. S. Dept. Agr., Bull. 431, 1917, 1–54. Steinhaus, Insect Microbiology, Comstock Publ. Co., 1946, 426–430; Principles of Insect Pathology, McGraw-Hill, 1949, 514–523.

RECAPITULATION

Recent attempts to devise a satisfactory system of classifying plant and animal viruses have made it necessary to give further consideration to the systematic aspects of insect viruses if they are not to be left behind in the development of virus taxonomy. To assist in bringing this about, the writer has followed his previous proposal that the known insect viruses be separated provisionally into at least four generic groups (*Borrelina*, *Paillotella*, *Bergoldia*, and *Morator*) according to the type of inclusion body formed in the tissues of the diseased host, and the type of disease produced. In the present paper these groups have been further delineated, descriptions of species have been revised, and the following new species named: *Borrelina olethria*, *Borrelina campeoles*, *Borrelina peremptor*, *Bergoldia daboia*, *Bergoldia lathetica*, and *Bergoldia thompsonia*.

Although more than a hundred different insect hosts are known to be susceptible to probably as many different viruses, it is suggested that in the future no name be given to a virus until the agent has been demonstrated by physical or visual means in such a manner that at least its approximate size and shape are known. For instance, all new species described and named in the present paper have been demonstrated at least with the electron microscope. It is further recommended that the properties and characteristics of those insect viruses contained within inclusion bodies be determined on the basis of tests on the free virus rather than, as in the past, on the virus protected by the inclusion.

As the techniques of virus research are refined the characteristics and properties of the insect viruses will undoubtedly become more accurately known. The keys, descriptions, and groupings proposed in this paper are advanced as suggestions that may assist future virus systematists in better appraising the several kinds of viruses which cause infections in insects.

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