that the hydroxylation observed on the released peptides was not obscured by the hydroxylation that was taking place normally on the polysomes.

While ours is, we believe, the first direct demonstration that hydroxylation is not needed for release of nascent collagen chains, and that released chains can be hydroxylated, these conclusions were suggested by, and are consistent with, earlier work with chick embryos. Bhatnagar and Prockop had reported that, when hydroxylation was inhibited by dipyridyl, protocollagen continued to be synthesized for at least 4 hours at about two-thirds the normal rate of collagen synthesis (*4*). Furthermore, after reversal of the inhibition, the accumulated protocollagen was hydroxylated by the tissues at the same rate as newly synthesized collagen chains (*5*). It had also been shown (*4*) that apparently complete, unhydroxylated alpha chains could be isolated from chick embryo minces incubated with dipyridyl, a finding consistent with release as well as completion of nascent chains, in the absence of hydroxylation. Our work confirms these conclusions and leads to the realization that, while hydroxylation of proline normally occurs on nascent collagen chains, it can also occur on released chains when hydroxylation has been temporarily inhibited. It appears therefore that it is not crucial whether the collagen chain is nascent or released in order to have hydroxylation of proline take place.

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Isolation of Mycoplasmatales Viruses and Characterization of MVL1, MVL52, and MVG51

Abstract. *Eleven Mycoplasmatales viruses are now known. Burst size, burst time, sensitivity to ultraviolet and the host range of three viruses that were studied are different. However, all three are naked, rod-shaped particles of similar size. Plaque morphology and the isolation of immune cells suggest that both virulent and nonvirulent infections are possible.*

Gourlay (*1*) described the first isolation of a Mycoplasmatales virus, which infects *Mycoplasma laidlawii* strain BN1 [renamed *Acholeplasma laidlawii* BN1 (*2*)], and named this agent MVL1 (*2*). Using BN1 as an indicator strain, we have isolated two more viruses, designated MVL52 and MVG51, which have properties different from those of MVL1. During an examination of other possible host strains, eight additional virus isolations have been made; these eight have not yet been characterized.

Cells of BN1 were grown either in a liquid tryptose medium or on a solid tryptose medium containing 1 percent agar (*3*). The transfer to this medium from the richer GS medium of Gourlay (*1*) affected neither cell growth (the generation time is about 2 hours) nor MVL1 viral growth.

For plaque assays, viruses are added to plates containing 2- to 6-hour-old indicator lawns; plaques are counted after 24 hours' growth at 37°C (Fig. 1). Because a naladixic acid–resistant clone of BN1, designated BN1 Nal^r, gives more distrinct plaques than BN1, it is used for the indicator lawn. To prepare virus stocks, plates with confluent plaques are washed for 6 to 16 hours at 37°C with phosphate buffer (*p*H 7.4) and filtered through a Millipore filter (pore size, 0.22 μm); this yields virus titers of about 10^{12} per milliliter.

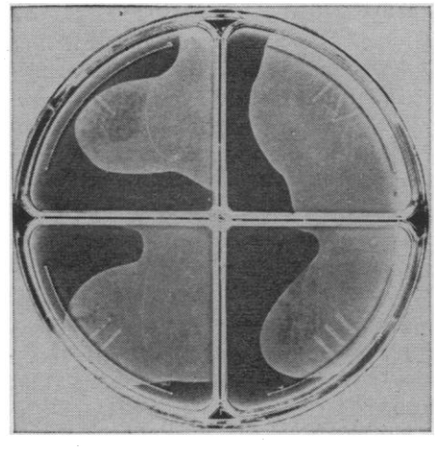

Fig. 1. Growth of BN1 Nal^r lawn (light areas) on 100-mm tryptose agar plates, showing Mycoplasmatales virus plaques. Sector I, lawn infected with MVL1; sector II, with MVL52; sector III, with MVG51; and sector IV, uninfected control lawn. Many plaques have a halo appearance; this can be seen particularly in sector III.

In an attempt to isolate more viruses, 24-hour lawns of three *Mycoplasma* were washed and plated on BN1. Plaques were observed from the washings from *A. laidlawii* B and *Mycoplasma* sp. strain 14 (goat) [these strains are described by Tourtellotte and Jacobs (*4*)]. The resulting plaque-forming units were designated MVL52 and MVG51, respectively. As an amendment to the nomenclature scheme of Gourlay, Bruce, and Garwes (*2*), MV, the Mycoplasmatales virus symbol, is followed by a letter designating the species origin of the virus (in our case, either G or L for goat or *laidlawii*) and then an isolation number (in our case, starting in the fifties).

In order to determine the degree of similarity of the three Mycoplasmatales viruses, a number of their properties were measured. Ultraviolet irradiation studies showed that MVL1, MVL52, and MVG51 inactivation follows one-hit kinetics (Fig. 2). The three viruses have different inactivation cross sections: 0.0020 mm^2/erg for MVL1, 0.0030 mm^2/erg for MVL52, and 0.0044 mm^2/erg for MVG51. Since, as discussed below, the three viruses are about the same size, the different cross sections may reflect differences in the virus nucleic acid structures.

To study virus growth, about 10^6 viruses and 10^6 cells were mixed in liquid media. After allowing 10 minutes for adsorption, a portion was filtered through a Millipore filter (pore size, 0.22 μm) to remove cells, and the filtrate was assayed for virus. Only about 0.1 percent of the virus was recovered in the filtrate. Corrections for the free virus titers were made in the studies below.

When BN1 Nal^r cells were mixed with MVL1, at a multiplicity of infection of 0.5, 32 percent of the cells were assayed as infective centers. When one considers a Poisson distribution of

Table 1. Relative titers of Mycoplasmatales viruses on *Acholeplasma laidlawii* lawns.

Strain of *A. laidlawii*	MVL1	MVL52	MVG51
BN1 Na1^r	1	1	1
B34P	3×10^{-6}	8×10^{-1}	6×10^{-7}
B28	9×10^{-7}	5×10^{-1}	4×10^{-7}
B52PH	2×10^{-6}	8×10^{-1}	8×10^{-7}
KD325NSB	0	1×10^{-7}	0
AKD416	8×10^{-9}	1×10^{-10}	6×10^{-9}
2009	3×10^{-4}	7×10^{-4}	4×10^{-4}

the virus, 61 percent of the cells received no virus, 30 percent had one, and 9 percent had two or more. Since the 32 percent infected cells is much greater than the 9 percent which received two or more viruses, but is close to the 39 percent which received one or more, it is concluded that a single MVL1 is the infective unit. Similar results were obtained in experiments with MVL52 and MVG51.

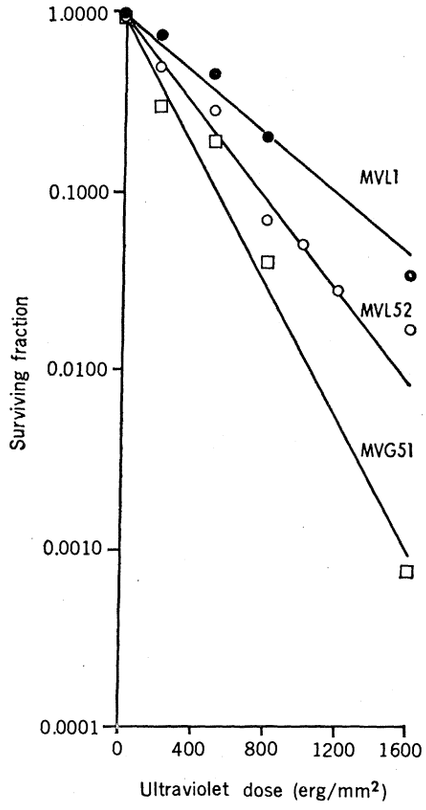

Fig. 2. Ultraviolet inactivation of MVL1, MVL52, and MVG51 viruses. The ordinate shows the logarithm of the surviving virus fraction after exposure to 257-nm light for the doses indicated on the abscissa. The dose rate was 10 erg mm^{-2} sec^{-1} and initial virus titers of 10^7 to 10^8 were used. After irradiation, the viruses were plated and counted on BN1 Na1^r. The lines are a least-squares fit of the data for one-hit kinetics.

One-step growth curves (Fig. 3) showed different growth patterns for the three viruses—MVL1 has definite latent and rise periods, MVL52 shows no measurable latent period, and MVG51 has a short latent period. Since these measurements began after a 10-minute period to allow virus adsorption, there could be up to ten additional minutes in the latent or rise periods. However, this does not change the basic differences in the one-step growth curves of the three viruses. The burst time (the time to the end of the rise period) and burst size, respectively, for each virus is MVL1, 60 minutes, 3; MVL52, 180 minutes, 80; and MVG51, 150 minutes, 5. These values are characteristic for the growth of each virus on BN1 Na1^r in liquid tryptose medium. The one-step growth curve of MVL1, with a defined latent period and short rise period, is similar to those of bacteriophage (*5*) and suggests that the new MVL1 viruses are released in a burst. In contrast, the short latent periods of MVL52 and MVG51 cannot be readily explained, and their rise periods suggest a gradual virus release similar to the filamentous bacteriophage and animal viruses (*6*).

The rapidity of virus adsorption, the short latent periods observed, and some preliminary experiments which show that well over 300 MVL1 viruses can adsorb to each cell, indicate that virus-mycoplasma interaction probably does not involve specific adsorption sites, but instead follows the animal virus models, in which each cell can "take up" viruses at its membrane surface.

In order to get some idea of the virus morphology, viruses in phosphate buffer were banded in 5 to 20 percent sucrose gradients. The three viruses were recovered at about the same gradient fraction, which indicates that they are about the same size and that their sedimentation coefficients are in the 40*S* to 100*S* range, based on calculations for the sedimentation conditions used (*7*). If the viruses were suspended in phosphate buffer containing 0.2 percent of the nonionic detergent Triton X100, no change was observed in the sedimentation pattern, indicating that the viruses do not have a membrane-type envelope (*6*).

In order to confirm the absence of a membrane virus envelope, the ether sensitivity of the viruses was measured. For these studies, a virus solution was mixed with an equal volume of cold ethyl ether, shaken, and centrifuged at low speed to separate the phases. The ether phase and interface were pipetted off, and the procedure was repeated six times. All three viruses are ether-resistant; the MVL1 resistance confirms the finding of Gourlay (*1*).

Table 2. Sources of Mycoplasmatales viruses.

Virus*	Source
MVL1	Bovine nasal isolate*
MVG51	*Mycoplasma* sp. strain 14 (goat)†
MVL52	*A. laidlawii* B†
MVL53	*A. laidlawii* 2009‡
MVL54	*A. laidlawii* B34P‡
MVL55	*A. laidlawii* B79‡
MVL56	*A. laidlawii* B80P‡
MVL57	*A. laidlawii* KD325‡
MVL58	*A. laidlawii* AKD416‡
MVL59	*A. laidlawii* OKD336‡
MVL60	*A. laidlawii* PG8‡

* MVL1 described by Gourlay (1970); other viruses described in this report. † Cells obtained from Dr. M. E. Tourtellotte, University of Connecticut. ‡ Cells obtained from Dr. J. Fabricant, Cornell University.

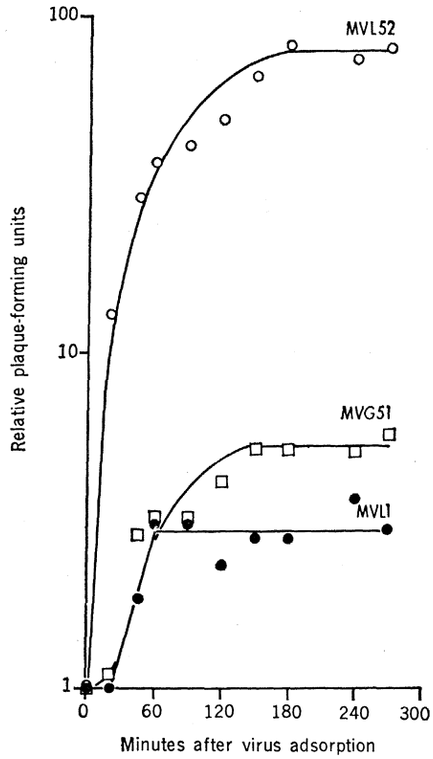

Fig. 3. One-step growth curves (*5*) of MVL1, MVL52, and MVG51. Exponentially growing BN1 Na1^r were mixed with virus at a multiplicity of infection of one. After adsorption for 10 minutes at room temperature, the mixtures were diluted 10^5-fold, incubated at 37°C, and plated at various times to assay for plaque-forming units. The ordinate is the logarithm of the titer, relative to the initial number of infective centers. In this experiment, the initial titers (after dilution) were (per milliliter) 260 for MVL1, 950 for MVL52, and 390 for MVG51. The abscissa is the time after the 10-minute adsorption period.

We noted that all three viruses gave turbid plaques on BN1 lawns, which suggested that some cells were immune to lytic infection. From such turbid plaques on BN1 lawns infected with MVL1, two resistant clones were isolated and designated BN1 v^{r1} and BN1 v^{r2}. Although selected for resistance to MVL1, both clones were also resistant to MVL52 and MVG51. Similarly, cells selected for resistance to MVL52 or MVG51 are also resistant to the other viruses. Adsorption studies show that the viruses do adsorb to the resistant cells. The possible lysogenic nature of these clones is indicated by preliminary studies which show that virus release from BN1 v^{r2} can be induced by ultraviolet irradiation.

Four antibiotic-resistant cell populations were selected by the addition of antibiotics to the liquid culture medium. The cells isolated were resistant to (per milliliter) 400 μg of naladixic acid (BN1 Nal^r), 100 μg of kanamycin (BN1 Kan^r), 40 μg of novobiocin (BN1 Nov^r), or 500 μg of streptomycin (BN1 Sm^r). When the three viruses were plated on these cells, it was found that BN1 Nal^r and BN1 Kan^r supported viral growth, but none of the viruses was able to form plaques on lawns of BN1 Nov^r or BN1 Sm^r. The plaques on BN1 Nal^r were clear, as opposed to the turbid plaques on the parental BN1; hence, BN1 Nal^r was used as the indicator lawn for measurements of virus titer. The mechanism of resistance to virus infection of BN1 Nov^r and BN1 Sm^r, which were selected for antibiotic resistance, is not understood. Furthermore, the virus-resistant strains BN1 v^{r1} and BN1 v^{r2} have proved to be resistant to streptomycin. This suggests that perhaps Sm^r, v^{r1}, and v^{r2} may be ribosome protein mutations.

The viruses are able to form plaques on lawns which are 2 to 13 hours old. By 24 hours, a lawn is no longer able to support viral growth. No plaques were ever seen on uninfected lawns. However, if old lawns (over 24 hours) were washed and plated on 6-hour lawns, a few plaques were sometimes seen. The numbers are too small to allow a statistical estimate of this frequency; for example, four of the washes from 36 old BN1 lawns gave rise to plaques on young BN1 lawns. Hence, host-carried virus must be considered in studies of Mycoplasmatales viruses. However, such viruses must not interfere with titer measurements due to the low frequency of spontaneous virus release, the time required for virus growth, and the short period during which the cells are susceptible to lytic attack. These same reasons probably account for the stability of the virus-cell relationship and for the difficulty in isolating the viruses.

In an effort to find other hosts, the ability of the three viruses to form plaques was examined on lawns of 73 Mycoplasmatales strains, consisting of 62 *A. laidlawii*, one *M. hominis*, one *Mycoplasma* sp. (from goat), five *M. gallisepticum*, and four *Mycoplasma* sp. strains avian serotype I. Plaques were observed only on 13 *A. laidlawii* lawns. The titers of the three viruses on six of these lawns, relative to their titers on BN1 Nal^r lawns, is in Table 1. These data indicate a similar host range for MVL1 and MVG51, which is different from that of MVL52.

When lawns of the 13 strains, chosen for ability to grow MVL1, were washed, eight of the strains gave plaque-forming units. These data are summarized in Table 2. These eight new viruses give plaques on lawns of BN1 Nal^r but not on BN1 v^{r1}, BN1 v^{r2}, or BN1 Sm^r.

Of the 14 *A. laidlawii* strains examined thus far, we have isolated viruses from nine, or about 65 percent, of the strains. We do not know whether viruses could be isolated from the remaining strains by the use of other indicator lawns and we do not know whether such a high percentage of other species are carrying virus. Strain MVG51, isolated from a goat source, shows that other species can carry a virus. The original host of MVL1 is unknown. We also do not know whether the 11 Mycoplasmatales viruses (Table 2) are all different. At least three of them have different properties, although the three all appear to have the same morphology. Micrographs have also shown that MVL59 and MVL60 are also rod-shaped particles of similar size.

The prevalence of Mycoplasmatales viruses means that they must now be considered in investigations of *Mycoplasma* pathogenicity, both as etiologic agents and for their possible involvement in lysogenic conversion of the host *Mycoplasma*.

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Radiohalos: Some Unique Lead Isotope Ratios and Unknown Alpha Radioactivity

Abstract. *Previously unreported lead isotope ratios, that is, values for the lead-206/lead-207 ratio ranging from about 20 to 60, primarily radiogenic in origin but unsupported by uranium decay, have been determined in the inclusions of certain polonium halos by means of ion microprobe techniques. Evidence for radiogenic lead-208 unsupported by thorium decay may also be inferred from the existence of a composite polonium halo type with rings from the radioactive precursors of lead-208. Several new dwarf halo sizes seem to indicate the existence of unknown, very low-energy alpha-emitters. Furthermore, the three-ring "X halo" also provides evidence for an unknown series of genetically related alpha-emitters with energies in the range from 3 to 7 million electron volts.*

An intriguing aspect of the study of radioactive halos (radiohalos) is the occurrence of unusual halo varieties (*1–3*), some of which remain unassociated with known α-emitters. There has been speculation of late that certain of these variant halos may be related to the existence of superheavy