made by WOODRUFF and GOODPASTURE[1] on the infecting capacity of "young" versus "old" inclusion bodies, as well as with the result of titration experiments[2], and of current opinions on virus synthesis.[3]

The evidence to date is that inclusions which are not caused by viruses never contain DNP. The inclusions found in gliomata[4] are eosinophilic and hyaline and therefore presumably Feulgen negative. The same applies to inclusions observed after extensive burns or after X-ray treatment[5]. Inclusions due to chemical agents such as bismuth and lead salts, aluminum hydroxide, ferric hydroxide were all reported to be Feulgen negative[6].

In order to eliminate the possibility that such inclusions might also contain DNP at some stage of their development, WOLMAN[7] has recently studied the staining characteristics of these inclusions at early stages of their formation. It has been found that these inclusions never contain DNP.

On the basis of the evidence presented above it is contended that viral inclusion bodies contain at some period of their development DNP and that the DNP present in them represents the virus. Pending corroboration from further studies on animal viruses, it is suggested that the presence of Feulgen positive inclusion bodies be considered as strong prima facie evidence for the viral nature of the process. The finding of Feulgen negative inclusion bodies should not be considered as excluding a viral etiology until the presence of a Feulgen positive stage in some phase of the development has been excluded.

It is suggested that the term "inclusion body" should be reserved for those structures which are either Feulgen positive, or which exist with other elements which are Feulgen positive, or for such inclusion bodies which are known to have been Feulgen positive at some stage of their development.

The term "inclusion" as distinct from "inclusion body" should continue to cover all the formed aggregates of passive temporary constituents of the cell. The former term is appropriate for the intranuclear glycogen deposit of the diabetic liver cell, the siderotic granule of the erythrocyte and the inclusions found in the kidney tubule cell in lead poisoning. The term "inclusion" is considered to be more time honored and less misleading than the term "Pseudo-inclusion" suggested by LIPSCHUETZ in 1932[8].

M. WOLMAN

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Zusammenfassung

Auf Grund der vorhandenen Information wird geschlossen, dass virale Einschlusskörper zu einer gewissen Zeit ihrer Entwicklung DNP, also elementäre Virusteilchen enthalten. In nichtviralen Einschlüssen wurde DNP niemals gefunden. Es wird ein Weg für die morphologische Unterscheidung zwischen Einschlusskörpern (Virus) und Einschlüssen anderer Natur vorgeschlagen.

Isolation of Actinophages

The existence of bacteriophages acting upon actinomycetes was first reported, in 1936, by WIEBOLS and WIERINGA[1]. Since then, no new investigations on the subject were published to our knowledge, but for the simultaneous isolation of a very specific actinophage, acting only upon certain streptomycin-producing strains of *Streptomyces griseus*, by SAUDEK and COLINGSWORTH[2] and by WOODRUFF, NUNHEIMER, and LEE[3]. This particular phage was frequently observed later on in many streptomycin plants, and was studied by REILLY, HARRIS, and WAKSMAN[4], by WALTON[5] and, more recently, by one uf us[6].

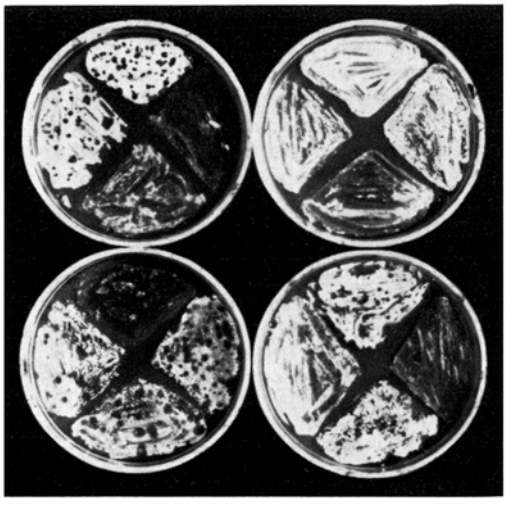

Fig. 1.–*Isolation of actinophages.* Sixteen different strains of *Streptomyces* were streaked on "manure-agar". About one half of them show typical plaques of bacteriophagy.

A systematic search for actinophages was undertaken in our laboratory, using the following method.

[1] G. L. WIEBOLS and K. T. WIERINGA, *Bacteriophagie een algemeen voorkomend verschijnsel* (H. Veenman and Zonen, Wageningen, 1936).

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Samples of soil are extracted with three to five volumes of sterile distilled water, during several hours at room temperature. During the extraction period, the flasks are frequently shaken. The supernatant liquid is then decanted, filtered through paper and finally Seitz-sterilized.

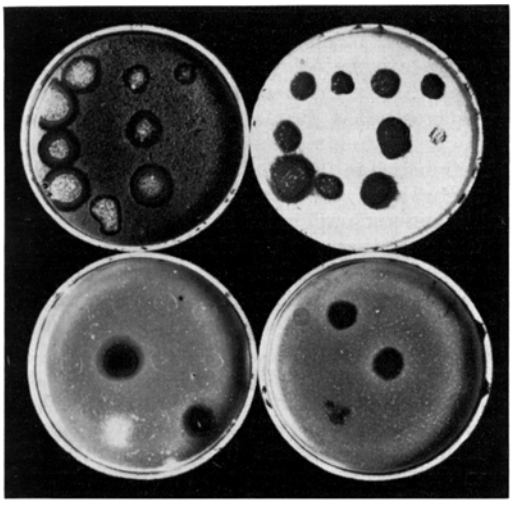

Fig. 2. *Spectrum of activity of actinophages.* Twelve phage-isolates, each from a single plaque, were spot-tested, in the same order (three rows of four), on cultures of different *Streptomyces*. Four of them are shown to illustrate actinophage specificity.

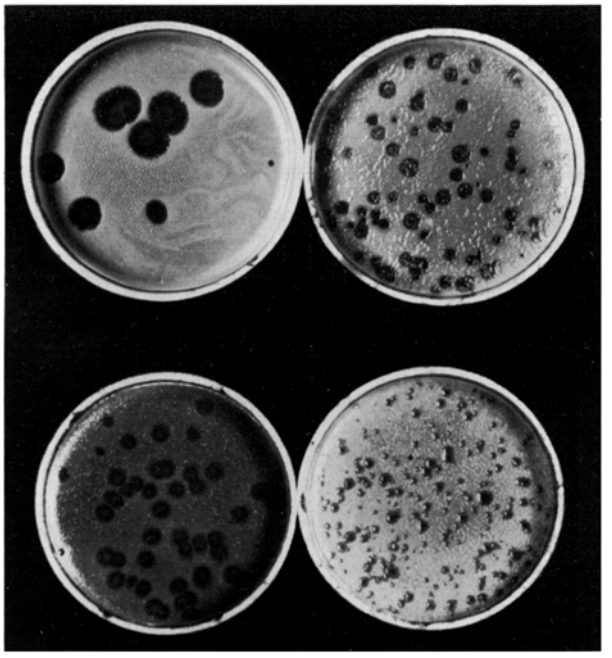

Fig. 3. *Morphology of the plaques formed by different pure actinophages on the corresponding sensitive strain.*

The sterile soil extract is then brought to a temperature of 50°C in a water-bath and aseptically combined with an equal volume of warmed melted agar (4% agar agar w/v in distilled water). The resulting mixture is immediately poured into sterile Petri dishes and allowed to harden.

Spore-suspension from a number of *Streptomyces* strains are then heavily streaked, each on one quarter of a dish. After incubation at a temperature of 25° to 30°C during 2 to 3 days, some of the strains may show characteristic plaques of lysis (Fig. 1). From these, the actinophages can be easily isolated by standard methods, propagated on sensitive *Streptomyces* cultures in liquid medium, and quantitatively studied by the now classical double-layer method of GRATIA[1], an adaptation of which was recently described in detail by one of us[2].

The occurence of one or more actinophages in several samples of soil was thus demonstrated and a number of strains were isolated. A similar method, using as a starting material a sample of stable manure, instead of soil, yielded several phages, differing one from the other by their spectrum of activity (Fig. 2) and by the size and morphology of their plaques (Fig. 3).

In contradistinction with the *S. griseus* phage, most of the actinophages isolated by us display a relatively low degree or specificity. In particular, a strain was obtained wich acts not only upon all the actinomycin-producing *Streptomyces* isolated in 1945 by one of us[3], but also on some strains producing no pigment. This phage has no action, however, upon the original actinomycin-producer: *S. antibioticus*[4].

In fact, none of the actinophages isolated so far has any action on the latter organism nor on the streptomycin-producing strains tested.

The methods here briefly described may be of value to ascertain the presence of actinophages in various natural substrates and to make a survey of their distribution in nature. They offer a mean of collecting a number of actinophages, some of which might become a useful tool for the classification and identification of species in the genus *Streptomyces*. Lastly, they have shown that the occurence of bacteriophagy is a widespread phenomenon among actinomycetes, a fact which is in perfect agreement with the concept, more and more widely held today, that those microorganisms are Bacteria and not moulds.

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Résumé

De nombreuses souches d'actinophages peuvent être mises en évidence et isolées, en ensemençant une série de *Streptomyces* spp. sur des milieux gélosés à base d'extraits aqueux de sol ou de purin préalablement stérilisés par filtration.

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