A preliminary calibration must be made with a sample containing a measured activity of $P^{33}$ with negligible $P^{32}$, under identical experimental conditions.

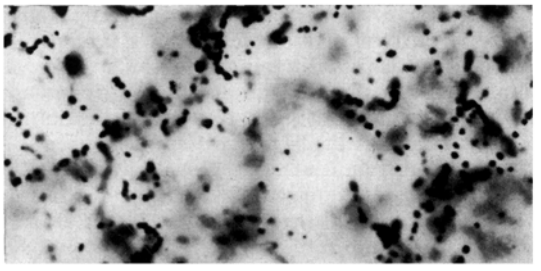

Fig. 2.

Then experiments are made, always under the same conditions, to estimate the amount of $P^{33}$ present in the mixtures, deducing from the counts of tracks in "pure" $P^{32}$ and in the mixtures on the basis of a linear relationship, referring to equal activities.

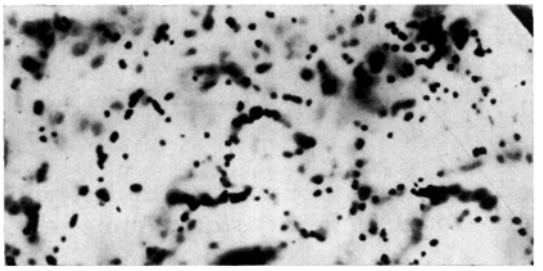

Fig. 3.

Particular attention was paid to the effects of self-absorption[1] by making comparison between specimens evaporated and spread on filter papers, under identical geometrical conditions.

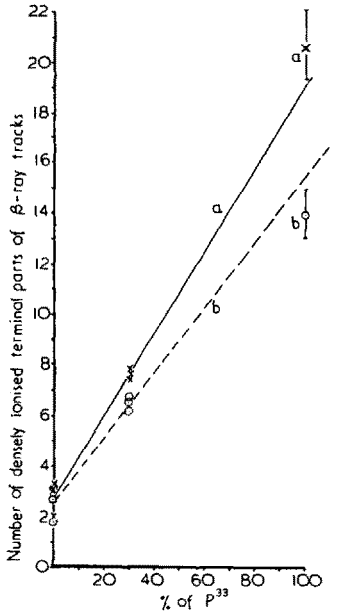


Fig. 4.–Experimental points.
× $a$ = from evaporated samples. ○ $b$ = from 8 mg/cm² filter paper samples

[1] LAPP and ANDREWS, *Nuclear Radiation Physics* (New York, Prentice Hall Inc., 1948), 236.

Three sets of specimens were used;
(1) Freshly prepared $P^{32}$ in solution;
(2) A solution containing about 30% $P^{33}$ together with $P^{32}$, and
(3) A very old solution containing 99% $P^{33}$.

All the materials were obtained from A.E.R.E., Harwell.

Direct measurements of the radioactivities were made and absorption curves in aluminium were made using a G.M.4 end window counter, with window and air corrections (Fig. 1).

Ilford G 5 nuclear emulsions, 200 $\mu$ thick were used in making the track counts. Weighting according to the number of points observed in the track was employed.

The photomicrographs show examples of $\beta$-ray tracks in equal areas corresponding to specimens of mixture of the same activity evaporated in an aluminium dish (Fig. 2) and when spread on filter paper of thickness 8 mg/cm² (Fig. 3).

The results are summarised in Figure 4. The sensitivity of this method is evident from the fact that the total activities used in the various experiments lay between 0·015 and 0·035 microcuries.

I wish to thank Professor J. S. MITCHELL for having suggested the problem of $P^{33}$ and for much advice.

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*Zusammenfassung*

Es wird eine Methode beschrieben, welche die Bestimmung kleiner Mengen $P^{33}$ in altem Radiophosphor erlaubt: $P^{33}$-Beta-Spuren werden ausgezählt in 200 $\mu$ dicken Emulsionsschichten, in denen sie in ihrer ganzen Reichweite enthalten sind.

## The Nature of Viral Inclusion Bodies and their Differentiation from Non-viral Inclusions

The nature of inclusion bodies has been the subject of much debate during the past 50 years, and is still not fully understood[1]. The purpose of this paper is (1) to show that all the available evidence can be explained by a single theory, (2) that the theory will help morphologists to distinguish viral inclusion bodies from non-viral inclusions, and finally, (3) to propose terminology which will eliminate the present confusion of terms.

The early discoverers of inclusion bodies considered them as living organisms, most often as Protozoa. That was the opinion of PATERSON[2], GUARNIERI[3], NEGRI[4], RIVOLTA and DELPRATO[5], and others. Filtration experiments which showed that the causative agent of all the above mentioned diseases can pass through bacteriological filters made the protozoan theory, at least in its primitive form, untenable. Since that time two schools of thought have held opposed views and brought forward valuable evidence in support of their opinions.

[1] T. M. RIVERS, *General Aspects of Viral and Rickettsial Infections*, in: *Viral and Rickettsial Infections of Man* (T. M. Rivers, Lippincott, 1948).

[2] R. PATERSON (1841), cited by RIVERS (1928).

[3] G. GUARNIERI. Arch. Sci. Med. *16*, 402 (1892).

[4] A. NEGRI (1903), cited by COVELL and DANKS (1932).

[5] RIVOLTA and DELPRATO (1880), cited by WOODRUFF and GOODPASTURE (1930).

According to the one theory, inclusion bodies are the product of altered metabolism caused by viral or other agents. This theory was advocated by SANFELICE[1], LUGER and LAUDA[2], COWDRY[3], HAAGEN[4], LEPINE and SAUTTER[5], OLITSKY and HARFORD[6], SCHILLER[7], ZOLLINGER[8], and others.

According to the other theory, inclusion bodies are made of the virus elements (elementary bodies), possibly in an aggregated form comparable to a bacterial colony, suspended within a matrix made of the host-cell's material. This theory was advocated by von PROWAZEK[9], LIPSCHUETZ[10], GOODPASTURE[11], and WOODRUFF and GOODPASTURE[12].

Further confusion is caused by the lack of proper definitions. Cytologists[13] reserve the term inclusions, as opposed to organoids, to describe those particles which are not present in all cells and which are "passive, lifeless, temporary constituents of the cell". There are thus inclusions of protein, glycogen or other carbohydrates, pigment, etc. On the other hand, most workers in the field have not considered intranuclear glycogen, for example, as an inclusion body and have accepted for the identification of the intranuclear inclusion bodies the criteria proposed by COWDRY[14] which have also been applied with slight modifications to the cytoplasmic inclusion bodies. These criteria are (a) acidophilia; (b) presence of a clear halo around the inclusion body, and (c) margination of the nuclear chromatin (obviously the third criterion cannot be applied to cytoplasmic inclusion bodies).

It is being generally assumed that many (if not all) animal viruses contain DNP[15]. This protein is closely associated with the transmission of hereditary characteristics and is found in the metazoan cells only in the chromatin matter. The substance appears to be of primary importance and its presence in any living cell cannot be considered as a casual finding. The finding of DNP outside of the chromatin network of a metazoan cell means therefore, either one of two things: (a) presence of nuclear remnants, or (b) presence of an extraneous organism which has the main characteristics of life i.e. the potentials of multiplication and of transmission of hereditary characteristics.

The inclusion bodies occurring in many viral diseases are Feulgen positive (i.e. they contain DNP) at some stage of their development. This has been shown for the following viral diseases: variola[1], vaccinia[2], herpes[3], psittacosis and lymphogranuloma venereum[4], B-virus[5], yellow fever[6], rabies[7], trachoma[8], verruca vulgaris[9], salivary gland virus (the so called cytomegalic disease of infants[10], as well as rabbit papilloma and human and swine influenza[11].

It has been shown that the amount of DNP in some inclusion bodies[12] rises steadily until a certain peak is reached, after which it may diminish until the inclusion is Feulgen negative, eosinophilic and hyaline. It is clear, therefore, why the same inclusion bodies have been described as Feulgen positive by some authors and as negative by others. Conflicting reports have been published on the Feulgen staining of the inclusion bodies of rabies (compare PAUL and SCHWEINBURG[13] to COVELL and DANKS[14] and WOLMAN and BEHAR[15], of the cytomegalic disease of infants (compare CAPPELL and MCFARLANE[16] with BURMESTER[17], and of herpes simplex (compare COWDRY[18]) and LEPINE and SAUTTER[19] with CROUSE *et al.*[20], and WOLMAN and BEHAR[21].

On the assumption that the Feulgen positive material within the inclusion bodies represents the virus particles[22], the aforementioned studies seemed to indicate that an inclusion body undergoes a definite cycle in which an initially small number of virus particles multiplies within the inclusion body until a certain limit is reached. In some viral diseases the number of virus particles later begins to decrease, until finally the particles disappear. This is in complete agreement with the observations

[1] F. SANFELICE (1913), cited by WOODRUFF and GOODPASTURE (1930).

[2] A. LUGER and E. LAUDA, Z. ges. exp. Med. *24*, 289 (1921); *39*, 1 (1924).

[3] E. V. COWDRY, Arch. Path. *18*, 527 (1934); Amer. J. Clin. Path. *10*, 133 (1940).

[4] E. HAAGEN (1937), cited by BLAND and ROBINOW (1939).

[5] P. LÉPINE and V. SAUTTER, Ann. Inst. Past. *72*, 174 (1946).

[6] P. K. OLITSKY and C. G. HARFORD, Amer. J. Path. *13*, 729 (1937).

[7] E. SCHILLER, Z. Zellf. mikr. Anat. *34*, 356 (1949).

[8] H. U. ZOLLINGER, Schweiz. Z. allg. Path. Bakt. *14*, 446 (1951).

[9] V. PROWAZEK (1905), cited by BLAND and ROBINOW (1939).

[10] B. LIPSCHUETZ, Arch. Dermat. Syph. Orig. *136*, 428 (1921); Jadassohns Handb. Haut- u. Geschlechtskrankheiten, vol. 2 (Springer, Berlin, 1932).

[11] E. W. GOODPASTURE, Amer. J. Path. *1*, 1 (1925).

[12] C. E. WOODRUFF and E. W. GOODPASTURE, Amer. J. Path. *5*, 1 (1929); *6*, 713 (1930).

[13] A. A. MAXIMOW and W. A. BLOOM, *Textbook of Histology* (Saunders, 1942).

[14] E. V. COWDRY, Arch. Path. *18*, 527 (1934).

[15] T. F. ANDERSON, *Pathogenesis and Pathology of Viral Diseases* (Ed. J. G. KIDD, Columbia University Press, 1950). – H. HYDEN, *The Nucleoproteins in Virus Production*. Cold Spring Harbour Symposia on Quantitative Biology, vol. 12 (1947).

[16] D. F. CAPPELL and M. N. MCFARLANE, J. Path. Bact. *59*, 385 (1947).

[17] F. BURMESTER, Virch. Arch. *317*, 165 (1949).

[18] E. V. COWDRY, Science *68*, 40 (1928).

[19] P. LÉPINE and V. SAUTTER, Ann. Inst. Past. *72*, 174 (1946).

[20] H. V. CROUSE, L. L. CORIELL, H. BLANK, and T. F. MCNAIR SCOTT, J. Immunol. *65*, 119 (1950).

[21] M. WOLMAN and A. BEHAR, J. Inf. Dis. *91*, 63 (1952).

[22] T. F. MCNAIR SCOTT, H. BLANK, L. L. CORIELL, and H. CROUSE, *Pathology and pathogenesis of the cutaneous lesions of variola, vaccinia, herpes simplex, herpes zoster, and varicella*. In: *The Pathogenesis and Pathology of Viral Diseases*, ed.: J. G. KIDD (Columbia Univ. Press, 1950). – M. WOLMAN and A. BEHAR, J. Inf. Dis. *91*, 63, 69 (1952).

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

[1] C. E. WOODRUFF and E. W. GOODPASTURE, Amer. J. Path. *6*, 713 (1930).

[2] H. B. MAITLAND and A. W. LAING, J. Path. Bact. *53*, 419 (1941). – R. W. SCHLESINGER, J. Exp. Med. *89*, 491 (1949). – F. M. DAVENPORT and T. FRANCIS, Jr., J. Exptl. Med. *93*, 129 (1951). – F. C. ROBBINS and J. F. ENDERS, Amer. J. Med. Sci. *220*, 316 (1950). – F. L. HORSFALL, Jr., and H. S. GINSBERG, J. Expt. Med. *93*, 139 (1951). – J. D. AINSLIE, J. Exptl. Med. *95*, 1 (1952). – C. S. STULBERG and R. SCHAPIRA, J. Immunol. *70*, 51 (1953). – C. A. MILLER, Proc. Soc. Exptl. Biol. Med. *82*, 450 (1953).

[3] S. S. COHEN, *The synthesis of bacterial viruses in infected cells*, Cold Spring Harbor Symposia on Quantitative Biology. *12* (1947). – H. HYDEN, *The nucleoproteins in virus reproduction*. Cold Spring Harbour Symposia on Quantitative Biology *12* (1947).

[4] D. S. RUSSELL, J. Path. Bact. *35*, 625 (1932).

[5] H. U. ZOLLINGER, Schweiz. Z. allg. Path. Bakt. *14*, 446 (1951). – T. H. BELT, J. Path. Bact. *48*, 493 (1939). – A. M. LUCAS and W. H. RISER, Amer. J. Path. *21*, 435 (1941).

[6] S. S. BLACKMAN Jr., Bull. Johns Hopk. *58*, 384 (1936). – M. WACHSTEIN, Personal communication (1952). – H. U. ZOLLINGER, Schweiz. Z. allg. Path. Bakt. *14*, 446 (1951). – P. K. OLITSKY and C. G. HARFORD, J. Amer. J. Path. *13*, 729 (1937).

[7] M. WOLMAN, J. Path. Bact. *66*, 159 (1954).

[8] B. LIPSCHUETZ, Jadassohn's Handb. Haut- u. Geschlechtskrankheiten, vol. 12 (Springer, Berlin, 1932).

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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].

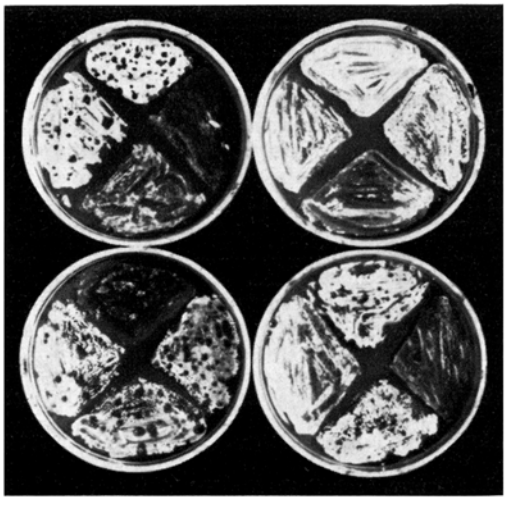

Fig. 1.–*Isolation of actinophages.* Sixteen different strains of *Streptomyces* were streaked on "manure-agar". About one half of them show typical plaques of bacteriophagy.

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).

[2] E. C. SAUDEK and D. R. COLINGSWORTH, J. Bacter. *54*, 41 (1947).

[3] H. B. WOODRUFF, T. D. NUNHEIMER, and S. B. LEE, J. Bacter. *54*, 535 (1947).

[4] H. C. REILLY, D. A. HARRIS, and S. A. WAKSMAN, J. Bacter. *54*, 451 (1947).

[5] R. B. WALTON, Antib. and Chemother. *1*, 518 (1951).

[6] M. WELSCH, C. r. Soc. Biol. *148*, 726 (1954).