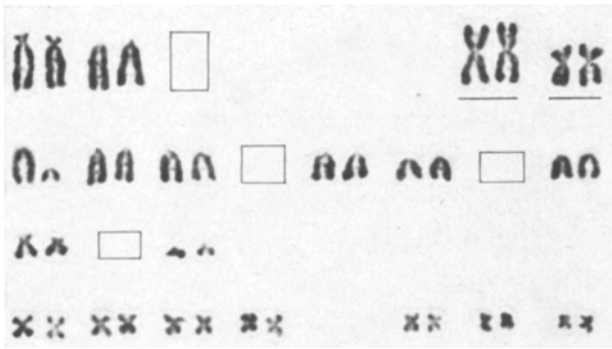


Fig. 1. Karyotype of male *R. rattus* with 38 chromosomes. The first pair in the second row are the *XY* chromosomes. Squares point out the absence of pairs 3, 6, 9 and 12 of *R. norvegicus*; underlined pairs are the new chromosomes not observed in *R. norvegicus* (compare with karyotype in Figure 2).

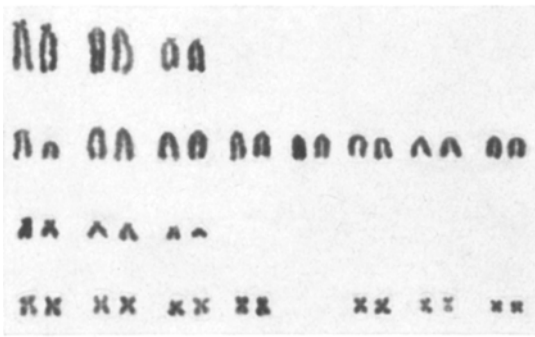


Fig. 2. Karyotype of male *R. norvegicus* with 42 chromosomes. The first pair in the second row are the *XY* chromosomes (chromosomes are arranged according with NOWELL et al.[4]).

Since no *R. rattus* with 42 chromosomes was found, we decided to compare the karyotype of our sample with the karyotype of a sample of *R. norvegicus* collected in a neighbouring area. Consequently, chromosome spreads from 5 wild *R. norvegicus* (2 ♂ and 3 ♀) trapped in La Plata city (Province of Buenos Aires) were prepared and studied. Diploid number in these animals was 42. Karyotypes were constructed according to the system proposed by NOWELL et al.[4] (Figure 2).

The comparison of the 2 complements showed that the karyotype of 38 chromosomes differed from that of 42 in the following features: (a) absence of the subterminal pairs 3 and 12; (b) absence of 2 pairs of acrocentric chromosomes of the group 4–10; (c) existence of 2 pairs of large metacentric chromosomes (Figures 1 and 2).

The diploid number of 42 chromosomes is probably the most general for rats. Therefore, it is possible to assume that the complement of 38 chromosomes has arisen from that of 42 by means of a process of chromosomal rearrangement, the following being the most probable mechanism: (a) deletion of the short arms of pairs 3 and 12, or alternatively, pericentric inversion transforming the subterminal 3 and 12 chromosomes into acrocentric elements; (b) centric fusion between the rearranged pairs 3 and 12 and 2 pairs of acrocentric chromosomes.

The external appearance of our specimens does not seem to differ from that habitually described in *R. rattus*. However, the lack of animals with 42 chromosomes prevented us from determining whether or not the 2 types of *R. rattus* have the same phenotype.

Finally, the existence of a similar chromosome complement in 2 *R. rattus* populations separated by more than 1200 km poses a very interesting question: Is the *R. rattus* with 38 chromosomes widespread all over the South American continent? Further rat samplings involving different regions of South America will be necessary to answer the question.

Resumen. Se estudiaron los cromosomas de 16 ejemplares de *Rattus rattus* provenientes de la Provincia de Buenos Aires (Argentina) y del Estado de Rio Grande do Sul (Brasil). En todos los animales el complemento estuvo formado por 38 cromosomas. Considerando que en las ratas el complemento de 42 cromosomas es probablemente el mas generalizado, se sugiere que el complemento de 38 cromosomas ha derivado del de 42 mediante un proceso de reordenamiento cromosómico con fusión céntrica.

N. O. BIANCHI[14], J. PAULETE-VANRELL[15] and L. A. DE VIDAL RIOJA[14]

Comisión de Investigaciones Científicas de la Provincia de Buenos Aires, La Plata (Argentina) and Universidad de São Leopoldo, Facultad de Filosofía, Ciências e Letras, São Leopoldo (R. G. do Sul, Brasil), 3 March 1969

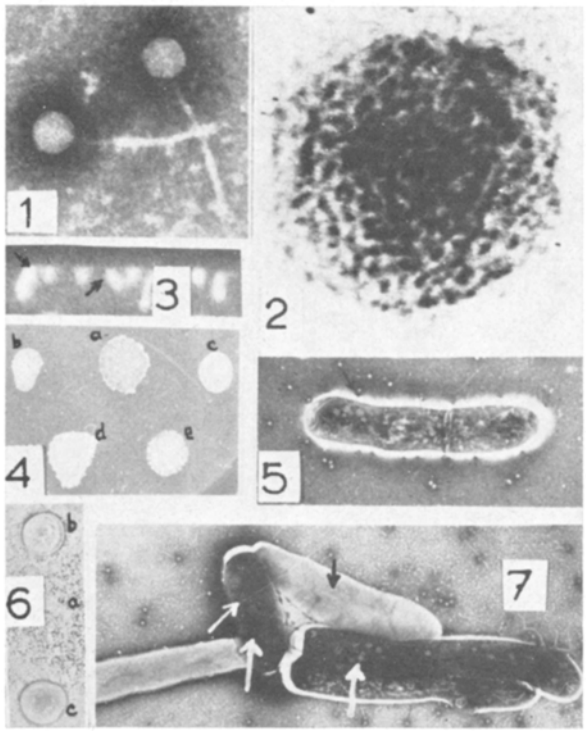
[14] Comisión de Investigaciones Científicas de la Provincia de Buenos Aires, La Plata (Argentina).

[15] Universidad de São Leopoldo, Facultad de Filosofía, Ciências e Letras, São Leopoldo (R. G. do Sul, Brasil).

Properties of Mycobacteriophage C2[1]

Mycobacteriophage C2 was isolated by MANKIEWICZ[2] in 1965 from sputum of a patient with alveolar cell carcinoma. This communication describes studies on the morphology, nucleic acid characterization of C2 and phage-*Mycobacterium smegmatis* relationship.

Experimental. Phage C2 and its host, *M. smegmatis*, strain ATCC 607, were supplied by Dr. E. MANKIEWICZ. Electron microscopy: phage was propagated on ATCC 607 grown in YOUMANS and KARLSON[3] medium, purified by differential centrifugation and suspended in 1% ammonium acetate. A drop of phage suspension or phage-infected bacterial culture was placed on a Formvar-carbon-coated grid, and negatively stained with 2% neutral potassium phosphotungstate (PTA). Specimens were examined with a Phillips EM 200 electron microscope. Nucleic acid studies: phage C2 was propagated, harvested and purified by the method of SELLERS and RUNNALS[4] modified by the use of RVA agar medium[5] instead of Leventhal's agar. Acridine orange staining of phage nucleic acid was done by the procedure of MAYOR and HILL[6]. Phage DNA was extracted with phenol at room temperature, purified[7] and its infectivity tested against *M. smegmatis*, BCG, *C. hoffmani*, *E. coli* and mouse L cells. DNA concentration was measured by spectrophotometry (Beckman DB-G) and diphenylamine reaction[8] and its buoyant density in CsCl was kindly



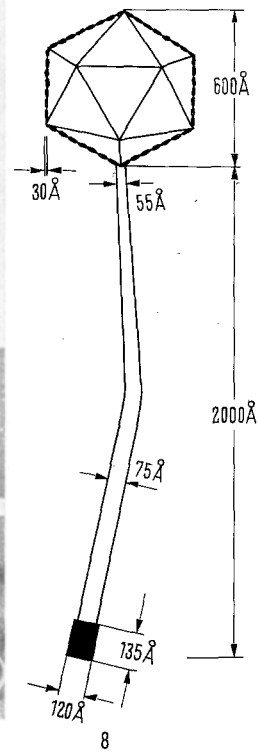


Fig. 1. Virions C2 negatively stained with PTA. × 95,000.

Fig. 2. Icosahedral head of phage C2 viewed along threefold axe, showing capsomere-like structures. Photographic reversal, PTA stain. × 600,000.

Fig. 3. Fluorescence (arrows) from a droplet band of phage suspension after acridine orange staining. × 600.

Fig. 4. Colonies of 3 lysogenic (b, c, d) and one (e) atypical-like mycobacterial culture obtained from ATCC 607 (a). × 0.5.

Fig. 5. Attached phages (arrow), intracellular bodies, and phageproduced holes in the bacterial wall are present. PTA stain. × 8500.

Fig. 6. Colonies from a, b and c (see Figure 4) plated on a culture of ATCC 607 grown on RVA agar medium. Clear zones indicating the presence of a bactericidal substance, are seen around colonies b and c. × 0.5.

Fig. 7. Cluster of ATCC 607 cells at different stages of infection: intracellular bodies (arrows), 500–1800 Å in diameter, are present in lysed (right) and non-lysed bacteria. PTA stain. × 8500.

Fig. 8. Schematic diagram of mycobacteriophage C2.

determined by Dr. I. TAKAHASHI following the procedure of SCHILDKRAUT et al.[9], using DNA of PBS15 as reference. Colonies from infected cultures of ATCC 607 grown continuously in YOUMANS's medium[3] were tested for lysogeny.

Observations. C2 virions consist of an icosahedral head (Figures 1 and 2), and an elongated non-contractile tail with striations and a base plate-like structure. The neck of the tail is slightly constricted; a ring structure was occasionally seen at the tip. Empty heads were rounded. Capsomere-like units were seen in the head (Figure 2), and 7–8 of these units were recognized along the side of the hexagonal profile. Similar structures have been described in phage B-1[10]. Phage structure and dimensions are summarized in Figure 8.

Phage-host cell attachment was first observed 15 min after mixing and at 30 min holes were present in the cell wall, suggesting the presence of a lytic enzyme in the tail tip. At this time, there was no evidence of DNA injection into the host. Many bacteria lysed (phage release) at 90 min (Figure 7), but on one occasion lysis was observed at 60 min (Figure 5). Infected bacteria became electron transparent and they showed intracellular bodies which seem to increase during the latent period (Figures 5 and 7); occasionally paired fibrous structures on the cell envelope, similar to those described for *M. jucho*[11], were seen.

Acridine orange staining of phage C2 preparations (optimal concentration: 10^{11} particles/ml) gave the green fluorescence, characteristic of double-stranded nucleic acid; DNAse pretreated samples did not show this fluorescence, which indicates the DNA nature of the nucleic acid. The guanine + cytosine content of C2 DNA was 61% as determined by its buoyant density. This DNA was infectious to ATCC 607 and BCG, but not to *C. hoffmani*, *E. coli*, or mouse L cells. More than 99% of the plaque-forming activity was lost during the phenol extraction.

From cultures of ATCC 607 three lysogenic colonies were obtained (Figure 4). Their cultural features and chromogenicity were different from ATCC 607. The UV-irradiation did not produce significant phage induction; similar results were reported for *M. butyricum*[12]. Two of these lysogenic cultures produced a bacteriocin-like substance (Figure 6), which was more stable at 37 °C than the phage and remained in supernatant after high-speed centrifugation.

A cytopathic effect was observed in mouse L cell monolayers infected with phage C2 (E. MANKIEWICZ, personal communication, 1966). Attempts to confirm this observation, using HeLa, L and monkey kidney cells, were unsuccessful.

Résumé. Le mycobactériophage C2 a une morphologie typique tête-queue, la longueur de la tête étant de 60 nm et celle de la queue de 200 nm. Le génome du phage est un acide désoxyribonucléique. Le phage C2 joue un rôle important dans la génétique mycobactérienne.

J. MENEZES and V. PAVILANIS

Département de Microbiologie et Immunologie et Institut de Microbiologie et d'Hygiène de l'Université de Montréal, Laval (Canada), 31 January 1969.

[1] Supported by grant No. MA-2718 from M.R.C., Canada. We thank Dr. E. MANKIEWICZ for her suggestions and samples, and Mr. L. BERTHIAUME for assistance in electron microscopy. One of us (J.M.), presently at Dept. Microbiology, University of Ottawa, is a M.R.C. Fellow.

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