Bacteriophage 2C, when stored at 4 °C loses its biological activity rapidly. It has been observed that the DNA from such inactive phages is also degraded. The preliminary studies indicate that the "heavy" strand of the DNA gets degraded first followed by the "light" strand.

The infectivity of phage preparations of different ages was assessed by the soft-agar technique using Bacillus subtilis strains, although no reason for its occurrence has so far been found. In this communication, I report some accidental observations which show that the DNA from such inactive phages is degraded, and this may contribute to biological inactivation of phage 2C during storage.

The infectivity of phage preparations of different ages was assessed by the soft-agar technique using B. subtilis 168/2 and it's DNA has an abnormal base, hydroxy methyl uracil and it's DNA has an abnormal base, hydroxy methyl uracil, instead of thymine.

The sedimentation coefficients of the DNA samples were measured by boundary sedimentation method in a Beckman model E analytical ultracentrifuge. To avoid the breakage of DNA molecules, cells while partially assembled were filled with the DNA solution from the top with a broad, smooth-mouthed pipette. The centrifugation was carried out at a DNA concentration of 25 µg/ml in 1 M-NaCl. To obtain S20,w, a correction factor of 1.144 was used for the density and viscosity of 1 M-NaCl.

The MAK columns were prepared as described by MANDEL and HERSHEY. The denaturation of the phage DNA was brought about by heating at 95 °C for 10 min in standard saline citrate (0.15 M NaCl, 0.015 M sodium citrate, pH 7) at a concentration of 20 µg/ml followed by rapid cooling. The method of chromatography was essentially the same as described in ref. 10. The ultraviolet absorption of the column effluent was continuously recorded at 254 nm with a Gilson Electronics U.V. recorder. The flow rate was 35 ml/hr and the fractions collected had volumes of 4 ml. The salt concentration was checked by refractive index measurements. The equilibrium ultracentrifugations of DNA in neutral CaCl was carried out in a Spinco model E analytical ultracentrifuge for 20 hrs at 44,000 rpm at 25 °C.
using a 4° sector, 12 mm Kel F centerpiece. The density of the CsCl was 1.76 g/cm³. The ultraviolet photographs were traced with a Joyce Loebl microdensitometer.

The results presented in the Table indicate that the biological activity of the virus was reduced with the increase in storage period. Corresponding to the loss in the biological activity of the phage particles, the sedimentation coefficients of the DNA were decreased.

During my attempt to isolate the complementary strands, I also observed that the "heavy" strand of the phage DNA was preferentially degraded, while in the course of time the other strand was also slowly degraded.

The complementary strands of the DNA from fresh, 1-month-old and 12-month-old phages were denatured and chromatographed on MAK columns using a linear salt gradient of 0.3 – 1.0 M NaCl in 0.02 M phosphate buffer at pH 6.8. The DNA from fresh phage preparations yielded two U.V.-absorbing peaks at salt concentrations of 0.35 M NaCl and 0.62 M NaCl, respectively (Fig. 1.A). The first peak always had a "shoulder". The total recovery of the two peaks was only 62% (based on U.V. measurements). The portion of the DNA sticking to the column could not be eluted, even under a salt concentration of 3.0 M NaCl. Such difficulty had also been encountered by Trauffaut. However, when 1.5 M NaCl + 1.0 M NH₄OH, or 1.5 M NaCl + 0.02 M Na₃PO₄, was passed, a third fraction was eluted. The three fractions were analyzed in an analytical ultracentrifuge. It was found that the first fraction did not band in the CsCl density gradient while the second and the third fractions banded corresponding to the densities of 1.753 g/cm³ and 1.746 g/cm³, respectively (Fig. 2.E and F). The density of the second fraction corresponded to that of the "light" strand of the phage DNA whereas the density of the third fraction was between the native DNA and the "light" strand. Since the recovery of the second fraction alone was about 48%, I suspect that the first and the third fractions may be degradation products of the "heavy" strand. The denatured DNA, when centrifuged without chromatographic fractionation, showed two U.V.-absorbing bands (Fig. 2.B) corresponding to the densities of 1.753 g/cm³ and 1.765 g/cm³. These values are slightly higher than the

<table>
<thead>
<tr>
<th>Period of storage (in months)</th>
<th>Percent viable phage particles (over the initial titer)</th>
<th>Sᵥ,ₘ (in g/cm³)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fresh</td>
<td>100</td>
<td>48</td>
</tr>
<tr>
<td>1</td>
<td>3.71</td>
<td>33</td>
</tr>
<tr>
<td>6</td>
<td>1.85</td>
<td>17</td>
</tr>
<tr>
<td>12</td>
<td>0.06</td>
<td>15</td>
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</tbody>
</table>

Fig. 1. Fractionation of bacteriophage 2C DNA, extracted from phage stocks of different ages and heat denatured, by methylated albumin kieselguhr column. A. DNA from fresh phages; B. DNA from 1-month-old phages; and C. DNA from 12-month-old phages. ——— Fractions eluted with a linear salt gradient of 0.3 – 1.0 M NaCl in phosphate buffer at pH 6.8. ○○○ Fractions eluted with 1.5 M NaCl + 1.0 M NH₄OH, or 1.5 M NaCl + 0.02 M Na₃PO₄. Details of chromatography are given in the text.
values already reported for the "light" and "heavy" strands of phage 2C DNA. The behavior of the heavy strand may result from the presence of some "weak spots" which get broken during chromatography.

The DNA of 1-month-old phages, when denatured and fractionated in a similar way, yielded two fractions (Fig. 1.B.). The first fraction, with a broad "shoulder", was eluted at about 0.36 M NaCl and the second fraction was eluted with 1.5 M NaCl + 1.0 M NH₄OH or 1.5 M NaCl + 0.02 M Na₃P0₄. In the CsCl density gradient analytical centrifugation, only the second fraction gave a U.V.-absorbing band, the density of which was 1.746 g/cm³ (Fig. 2.F.). When the denatured DNA of 1-month-old phages was centrifuged without chromatographic separation, a band corresponding to the density of the "light" strand of phage 2C DNA and a faint, minor band, having an intermittent density between the heavy and light strands, were observed. The latter did not give a distinct peak in the microdensitometer tracing. I suspect that this minor band may be a component of the "heavy" chain since there was no alteration in the density of the "light" strand.

The chromatographic analysis of the DNA extracted from 12-month-old phages gave essentially the same results as that obtained from the DNA of 1-month-old phages, except that there was a minor fraction eluted at 0.60 M NaCl. The peak eluted under alkaline pH was not as broad as that of the 1-month-old DNA. Also, in this case, only the fraction eluted with NaCl + NH₄OH, or NaCl + Na₃P0₄, banded in a CsCl density gradient analytical centrifugation (Fig. 2.F.). The minor peak may be a further degradation product of fraction 2, or may be a component of fraction 1 itself (comparison is made with reference to DNA from 1-month-old DNA). The unfractionated denatured DNA of 12-month-old phages yielded only one broad, diffused U.V.-absorbing band in the CsCl density gradient

Fig. 2. Microdensitometer tracings of photographs taken following analytical centrifugation of phage 2C DNA samples described in the text. The peak on the left in each tracing corresponds to Micrococcus lysodeikticus DNA (density, 1.731 g/cm³) which was added to the gradients as a density marker. A. Native DNA; B. DNA from fresh phage, heat-denatured; C. DNA from 1-month-old phages, heat-denatured; D. DNA from 12-month-old phages, heat-denatured; E. Fraction II of the heat-denatured DNA (from fresh phages) obtained from MAK column; F. Fraction eluted under alkaline pH from the MAK column. (For all 3 DNA samples, this fraction had the same density).
Die Wirkung von Hydroxyharnstoff auf die DNA-Synthese embryonaler Rattenzellen in Kultur

The Effect of Hydroxyurea on DNA Synthesis of Embryonic Rat Cells in Culture

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Hydroxyurea, DNA-synthesis, rat cells

\(^3\)H-thymidine incorporation by secondary cultures of embryonic rat cells and by cultures of a permanent rat cell line is strongly inhibited a few minutes after addition of hydroxyurea (HU) to the culture medium \((2 \times 10^{-3} \text{m} \text{ and } 5 \times 10^{-4} \text{m}, \text{ resp.})\); normal embryonic rat cells are considerably less sensitive to the drug than are permanent cells. About 76 percent of the population of a secondary culture can be synchronized at the beginning of the S-period, if the cells at first are accumulated in G\(_1\) phase by incubation in serum-free medium and subsequently are stimulated by the addition of ten percent calf serum in the presence of \(2 \times 10^{-4} \text{ m} \text{ HU}\).

The size of DNA replicated in the presence of HU has been analysed by centrifugation in alkaline sucrose gradients. The results indicate that under these conditions DNA subunits are preserved with sedimentation coefficients between 21S and 30S. These intermediates cannot be joined together, probably because a ligase is inhibited by HU.

Secondary culture incubated in medium-containing \(1 \times 10^{-4} \text{ m} \text{ HU}\) incorporate twice as much \(^3\)H-thymidine as do control cells in HU-free medium. This is due to an increased number of cells in the S phase, as was shown by autoradiography. The kinetics of thymidine incorporation indicate that the duration of G\(_1\) phase is shortened by small doses of HU.

Für die Untersuchung des Initierungsmechanismus der DNA-Synthese in Säugetierzellen ist es unerlässlich, die Population am Übergang von der G\(_1\)-zur S-Phase zu synchronisieren. Hierfür gibt es mehrere Methoden, deren allgemeines Prinzip die selektive Hemmung der Biosynthese von essentiellen Bausteinen für die DNA-Replikation ist.

Die Synchronisation mit Hydroxyharnstoff (HH) schien für unsere Zwecke besonders geeignet zu sein. Dieses Harnstoffderivat hemmt in Säugerzellkulturen sehr rasch und spezifisch die DNA-Synthese, wobei die RNA- und Proteinbiosynthese anscheinend unbeeinflußt bleiben. Bei länger dauernder Einwirkung von HH kommt es jedoch zu einem verstärkten Absterben von Zellen, wobei besonders die in der S-Phase arretierten betroffen sind. Die

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