ments in which fragments of R17 RNA are subjected to ribonuclease V action. Large fragments are customarily found in R17 RNA preparations. Such fragments were isolated by sucrose gradient centrifugation of a preparation of total R17 RNA (*7*) (peaks II and III in Fig. 2), and were found susceptible to degradation (Table 1). Furthermore, as had been observed for ribosomal RNA, heating increased the fraction of the RNA that could be rendered soluble in alcohol by ribonuclease V (Table 1).

Even more striking, intact chains of R17 RNA were sensitive to attack by ribonuclease V when they were repurified after heat treatment (Table 1). Fragmentation and heating probably increase the accessibility of the 5′ end to ribosomes. However, this view, if correct, raises an interesting question regarding multicistronic messenger RNA molecules that, like R17 RNA, contain an untranslated portion of informational RNA at the 5′ end. [As another example, the *o* site in the lac operon, which is adjacent to the structural genes of the operon (*8*) is believed to be transcribed (*9*) but not translated (*10*).] Either these initial sequences, when exposed, can somehow be traversed by ribosomes and thus degraded, or they are degraded by a mechanism that does not involve ribosome movement.

Whatever the reason for its resistance to degradation, the stability of intact, unheated R17 RNA suggests that many of the conditions required for protein synthesis are necessary for ribonuclease V action but are not sufficient for it. At least one further requirement seems to be that the mRNA be susceptible to ribonuclease V.

An obvious implication of these results is that individual species of mRNA, even when active in protein synthesis in vivo and in vitro, can probably still escape degradation. Perhaps addition of an appropriate nucleotide or polynucleotide structure at the 5′ end of the molecule can prevent degradation. In phage RNA, in which this might be the case, the resultant stability would help to insure successful infection, and might have offered the selective advantage for the development of the initial, untranslated segment.

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Chloramphenicol: Effect on DNA Synthesis during Phage Development in Escherichia coli

Abstract. *Chloramphenicol added at various times during intracellular development of various bacteriophages causes a reduction in the rate of synthesis of phage DNA. The reduction is faster the smaller the size of the DNA molecule in the mature phage.*

After inhibition of protein synthesis, for example with chloramphenicol (CAP), DNA synthesis in *Escherichia coli* proceeds until completion of the current cycle of duplication and then stops (*1*). When protein synthesis is resumed, DNA replication begins again, after a delay presumably representing the synthesis of the protein or proteins necessary for initiation. In *E. coli* cells infected with T5 or λ bacteriophage, DNA synthesis is sensitive to inhibition by CAP (*2*). Infection with T-even phages seemed to be an exception because inhibition of DNA synthesis was observed only if the drug was added to the infected complexes during the first period after infection (*3, 4*). After the addition of CAP to *E. coli* cells infected with phage λ, T4, T5, or T7, the rate of DNA synthesis declines exponentially and in a manner inversely proportional to the molecular weight of the DNA of the phage.

The phages were wild-type T4, T5, and T7 and *E. coli* B and its photoreactivation deficient mutant *pht*$^-$ and *E. coli* K12 C600 (λ *c*857). All experiments were performed in M9 medium (0.022*M* KH_2PO_4, 0.042*M* Na_2HPO_4, 0.018*M* NH_4Cl, 8.5×10^{-3}*M* NaCl, 0.001*M* $MgSO_4$, and 0.4 percent glucose) supplemented with 1 percent Difco vitamin-free casamino acids decolorized by filtration through activated charcoal.

For phage infection experiments, bacteria concentrated from growing cultures were mixed with phage. After 2 minutes at 37°C the mixture was diluted into warmed medium to a concentration of 5 to 10×10^7 cells per milliliter. Pulse labeling was performed by transferring 1-ml aliquots of the cells to warmed tubes containing 0.2 μc of [^{14}C]thymidine (New England Nuclear; 30 μc/μmole). After 1 minute 1 ml of cold 10 percent trichloroacetic acid (TCA) and 1 percent thymidine were added. Precipitates were collected on Millipore filters, washed twice with 5 ml of 5 percent TCA, and

Table 1. Direct and indirect measurement of total DNA synthesized in T4 infected cells.

DNA measured by	Time	No CAP	Time of addition of CAP (minutes after infection)				
			6	12	14	16	24
Pulse label*	30 min	100†	55	62	69	71	94
	60 min	254		111	120	131	165
Diphenylamine reaction‡	30 min	100†	52	62	69	69	89
	60 min	164§		83	110	121	149

* Measured by integrating the area determined by the curves of [^{14}C]thymidine label plotted on linear scale. † All values are normalized to this control taken as 100. ‡ See (*6*). § In the absence of CAP, partial lysis of bacteria during centrifugation results in the loss of diphenylamine reacting material. No loss of optical density was observed in CAP treated or untreated cultures in the course of the experiment.

counted in a gas-flow counter. Chloramphenicol (Lepetit S.p.A., Milan, Italy) was added at appropriate times to a final concentration of 250 μg/ml.

For induction of λ phage *E. coli* K12-C600 (λ *c*857) was grown to approximately 8×10^7 cell/ml at 32°C, and at time 0 the cells were transferred to 38.5°C, causing synchronized induction of the thermoinducible prophage (*5*).

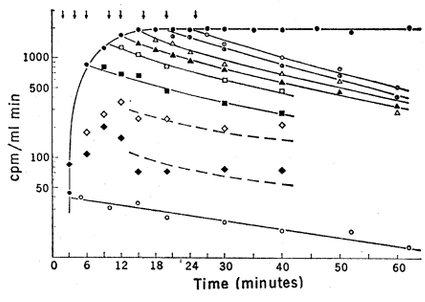

Fig. 1. Rate of DNA synthesis in *E. coli* B cells infected with T4 (multiplicity of infection = 4) treated at different times with CAP. ● - ●, no CAP (control); ○ - ○, CAP added 15 seconds before infection; ◆ - ◆, CAP at 2 minutes after infection; ◇ - ◇, CAP at 4 minutes; ■ - ■, CAP at 6 minutes; □ - □, CAP at 10 minutes; ▲ - ▲, CAP at 12 minutes; △ - △, CAP at 16 minutes; ◑ - ◑, CAP at 20 minutes; and ⊙ - ⊙, CAP at 25 minutes. The control cells were superinfected at 8 minutes (multiplicity of infection = 10). The production of phage per bacteria, measured by lysing at 80 minutes, was less than 0.01 when CAP was added before 10 minutes after infection, and increased from 1.2 to 200 for later CAP addition.

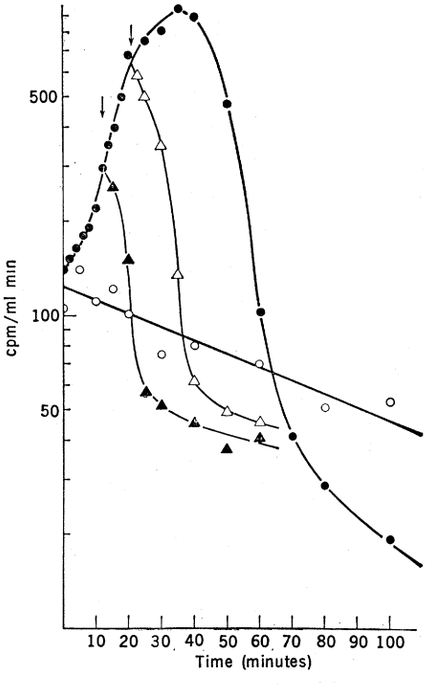

Fig. 2. Rate of DNA synthesis in *E. coli* K12 C600 (λ *c*857) after thermoinduction. ● - ●, no CAP; ○ - ○, CAP at 0 minutes; ▲ - ▲, CAP at 12 minutes; and △ - △, CAP at 22 minutes.

In experiments with T4, when lysis inhibition was desired, superinfection was performed at 8 minutes with a multiplicity of infection of 10. For experiments at a low multiplicity of infection, *E. coli* B *pht*⁻ was used after ultraviolet irradiation at a dose that gave 5×10^{-7} cell survivals.

Pulse-labeling experiments with cells infected with phage T4 at a multiplicity of infection of 4 showed that there was a decrease in the rate of DNA synthesis after addition of CAP at any time after infection (Fig. 1). The total amounts of DNA synthesized were assessed by integrating the areas under the curves of Fig. 1 and are in agreement with those reported previously (*3*). An experiment in which the rate of DNA synthesis was followed and the total amount of DNA synthesized was measured by the diphenylamine reaction is reported in Table 1. Similar experiments with bacteria killed by ultraviolet radiation infected at multiplicities between 0.1 and 10 showed that the rates of DNA synthesis after addition of CAP declined in the same manner as had been observed with normal host bacteria.

After addition of CAP, there is a significant decrease in the rate of DNA synthesis in bacteria infected with T5 or T7 or in induced λ lysogens (Figs. 2, 3, and 4). In the case of λ phage, after the initial steep decline in the rate of DNA synthesis, the decline becomes parallel to that observed in the control cells in which induction was prevented by the addition of CAP before heating. This suggests that λ phage induction does not shut off DNA synthesis.

Comparison of the results with λ, T4, T5, and T7 phages reveals a correlation between the molecular weight of the phage DNA and the rate at which DNA synthesis declines following CAP addition. Table 2 shows the relation between the rate of the decline of DNA synthesis (assumed to be exponential) and the molecular weight of the DNA of the respective mature phages. Phage P22 is included for comparison.

The initiation of a round of replication may require protein synthesis for phage DNA as well as for the bacterial DNA. Inhibition of T4 synthesis by CAP occurred at a rate similar to the

Table 2. The rate of decline of DNA synthesis after addition of CAP to *E. coli* infected cells; τ is the time required for the rate to fall to e^{-1} times initital rate measured in the linear portion of the curves. Numbers of independent experiments from which the mean values were calculated are in parentheses.

Phage	τ (min)	DNA molecular weight ($\times 10^6$ daltons)
T4	36.3 ± 3.1 (14)	120
T5	22 ± 3.4 (5)	77
λ	7 ± 0.5 (2)	33
T7	1.5 ± 0.3 (3)	24
P22*	5 ± 0.4 (4)	27

* From data of Favre *et al.* (*7*).

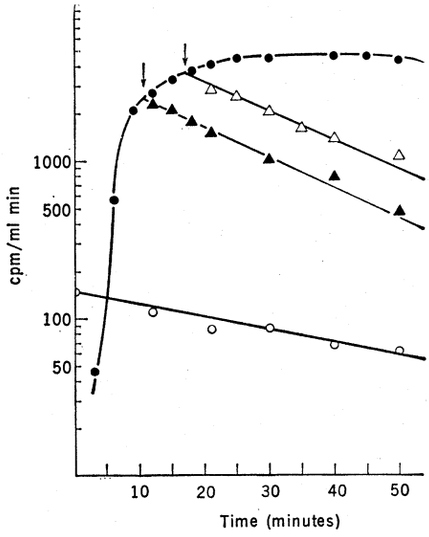

Fig. 3. Rate of DNA synthesis in *E. coli* B infected with T5 (multiplicity of infection = 8). ● - ●, no CAP; ○ - ○, CAP added at 0 minutes; ▲ - ▲, CAP at 10 minutes; and △ - △, CAP at 18 minutes.

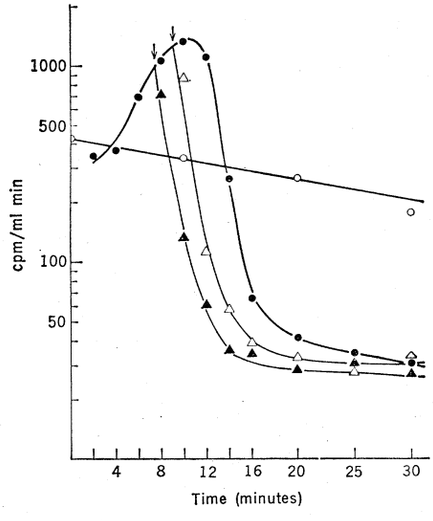

Fig. 4. Rate of DNA synthesis in *E. coli* B infected with T7 (multiplicity of infection = 10). ● - ●, no CAP; ○ - ○, CAP at 0 minutes to uninfected cells; ▲ - ▲, CAP at 7.5 minutes; and △ - △, CAP at 9 minutes.

inhibition of *E. coli* DNA (*1*) despite the fact that the *E. coli* chromosome is about 20 times longer than the T4 DNA molecule. Whether the similarity is the accidental result of two different mechanisms or is due to similarity between the molecular structure of the replicating phage DNA and the bacterial chromosome, as the synthesis of DNA molecules with a higher molecular weight of the mature phages, remains undecided.

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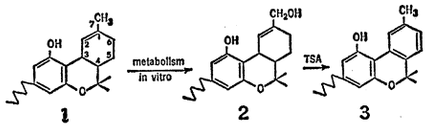
Δ^1-Tetrahydrocannabinol: Structure of a Major Metabolite

Abstract. *Δ^1-Tetrahydrocannabinol, the major psychotomimetically active compound of* Cannabis, *was metabolized in vitro by the 10,000g supernatant from rabbit liver. By mass and nuclear magnetic resonance spectrometry, the major metabolite was identified as 7-hydroxy-Δ^1-tetrahydrocannabinol. The latter compound of* Cannabis, *was metabolized in vitro by the 10,000g supernatant from*

Δ^1-Tetrahydrocannabinol (Δ^1-THC; **1**) is the major psychotomimetically active cannabinoid of *Cannabis*. Recently we described the rapid metabolism of Δ^1-THC in the rabbit (*1*) and found that at least three major metabolites are excreted in the urine. Using an in vitro technique we have now isolated and identified 7-hydroxy-Δ^1-THC (**2**) as a major metabolite of **1**.



1 **2** **3**

We used the in vitro system described by Tagg *et al.* (*2*) with some modifications. Four female rabbits given prior treatment with sodium phenobarbital were killed. The livers were homogenized with an equal volume of 1.15 percent KCl solution, and the homogenates were centrifuged for 10 minutes at 10,000*g*. The supernatant (500 ml) was incubated for 1 hour with Δ^1-[^{14}C]THC (*3*) (120 mg; 188 μc/mmole), nicotinamide (772 mg), nicotinamide-adenine dinucleotide phosphate (42 mg), glucose-6-phosphate (2864 mg), $MgCl_2 \cdot 6H_2O$ (1640 mg), and 0.06*M* phosphate buffer (200 ml, *p*H 7.0).

Extraction of the incubation mixtures with light petroleum removed most of the unchanged Δ^1-THC. The remaining radioactive material was extracted into diethylether. Thin-layer chromatography (*1*) of these extracts showed that 40 percent of the added Δ^1-THC had been converted to more polar compounds. In a control experiment with boiled supernatant Δ^1-[^{14}C]-THC was recovered unchanged.

The radioactive ether extract was chromatographed on a Florosil column (1.5 by 100 cm) and eluted first with mixtures of diethylether and light petroleum of increasing polarity and finally with pure ether. The remaining Δ^1-THC was found in the ether : light petroleum (1:5) fraction, and the metabolite was obtained from the pure ether fraction. Thin-layer chromatography (*4*) of the ether fraction showed a phenolic compound with an R_F of 0.5 containing 95 percent of the total radioactivity on the plate. This metabolite was further purified by preparative thin-layer chromatography (*4*) to yield 20 mg of a semicrystalline substance, which according to the specific activity was over 95 percent pure.

The nuclear magnetic resonance (NMR) spectrum (*5*) of the metabolite **2** was largely in agreement with that of Δ^1-THC with the following differences. The signal at $\delta = 1.69$ from the methyl group in position 1 of Δ^1-THC (*6*) was absent, and instead a two-proton signal at $\delta = 4.02$ ($-CH_2OH$) was recorded. Three one-proton signals of $\delta = 6.70$, 6.25, and 6.11 represented one vinylic and two aromatic protons.

The mass spectrum (*7*) showed a molecular ion at *m/e* (mass to charge) 330 (35 percent; Δ^1-THC + 16) and prominent peaks at *m/e* 315 (6 percent; $-CH_3$), *m/e* 312 (16 percent; $-H_2O$), *m/e* 300 (24 percent; $-CH_2O$), and *m/e* 299 (100 percent; $-CH_3O$). These data together with the information from the NMR spectrum indicated a hydroxylation only on the vinylic methyl group in position 1 of Δ^1-THC and no change in the aromatic moiety. This was further confirmed by the infrared (increased hydroxyl absorption) and ultraviolet spectra (maximum absorbance at 279 nm in ethanol) which were almost identical to the corresponding spectra of Δ^1-THC. These spectral data are consistent with the formulation of the metabolite as 7-hydroxy-Δ^1-THC (**2**).

Burstein *et al.* (*8*) reported the isolation in vivo and structure of a metabolite of the isomer $\Delta^{1(6)}$-THC in the rabbit. The metabolite was either 5-hydroxy, or more likely, 7-hydroxy-$\Delta^{1(6)}$-THC. In the latter case, the biochemical oxidation of Δ^1-THC and $\Delta^{1(6)}$-THC would be similar. Also this metabolite was dehydrated to cannabinol (**3**) with the use of *p*-toluenesulfonic acid (TSA) in refluxing benzene. With their procedure (*8*) we converted the in vitro metabolite **2** to the same compound **3**; the product was confirmed by thin-layer (*1*) and gas-liquid chromatography (5 percent XE-60; 5 percent SE-30 on Gas Chrom P).

Δ^1-Tetrahydrocannabinol seems to be excreted in completely metabolized form (*1*, *8*, *9*). The demonstration of 7-hydroxy-Δ^1-THC (**2**) as the first structurally known metabolite of Δ^1-THC (**1**) and the chemical conversion of the metabolite to the previously known cannabinol (**3**) could form the basis for an identification of *Cannabis* smokers. Experiments in mice have shown the metabolite to be pharmacologically strongly active.

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