Table 4. Comparison of the antipolio effects of dichloropyrimidines

Pyrimidine in AFE* medium	MNCTD** (μg/ml)	ID 95*** (μg/ml)	ID 95/MNCTD
2,4-Dichloropyrimidines	5	2.1	0.42
4,6-Dichloropyrimidine	20	4.6	0.23
6-Methyl-2,4-dichloro-pyrimidine	20	9.2	0.46
2-Amino-4,6-dichloro-pyrimidine	150	31.5	0.21

*Amino acid free Eagle's MEM. **Maximum non-cytotoxic dose. ***Minimum dose producing 95% inhibition on virus growth.

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Moreover, as already observed for 2-amino-4,6-dichloropyrimidine, the antiviral effect of the dichloropyrimidines is antagonized, in amino-acid-free-medium, by glutamine and cysteine, but not by pyrimidine precursors of nucleic acids. In complete media, containing glutamine and cysteine (or cystine) in the amino acid supplement, the antiviral effect is potentiated by 2-mercaptoethanol (table 3).

The limited number of compounds tested so far does not permit any conclusion on structure activity relationship inside the dichloropyrimidine group. As shown in table 4, it can only be said, at present, that the 4,6 positions of Chlorine atoms in the pyrimidine ring are to be preferred to the 2,4 (2,6) positions in that the former enhance the therapeutic index of the molecule.

Bacteriophage T4 mutants which propagate on E. coli K12 but not on E. coli B

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Summary. We have isolated and characterized 2 mutants of coliphage T4 which are able to propagate on E. coli K12 but not on E. coli B. We have assigned the mutations to genes 8 and 53, both structural genes. The products of genes 8 and 53 are found in the baseplate.

In an effort to understand the bacterial functions which are necessary for proper phage development, we have isolated and characterized many bacterial mutants unable to propagate bacteriophage λ. We have found 2 classes of such mutants which block λ DNA replication[2,3], another 2 classes which block λ RNA transcription[4,5] and a fifth class which affects the morphogenesis of several phages including λ, T4 and T5[6]. In the present report, we have extended our studies of host-phage interactions and show that one can isolate T4 mutants which discriminate between 2 naturally occurring hosts, E. coli K12 and E. coli B.

Materials and methods. The E. coli B^E sup$^-$ (called B) and E. coli K12 W3101 sup$^-$ (called K12) were the bacterial hosts. In order to isolate T4 mutants which propagate on K12 but not on B, a nitrosoguanidine mutagenized stock of bacteriophage T4rI was absorbed to K12 cells and the infected cells plated on a mixed bacterial lawn of K12 and B. We anticipated that derivatives of T4rI which grow on K12 but not on B would form small, turbid plaques on this mixed bacterial lawn as opposed to the large, clear plaques made by the parent strain. Plating efficiency of transfer and phage yield were as previously described[2]. Complementation tests were done in liquid by infecting the non-permissive Bsup$^-$ bacteria with 5 phage of each type per bacterium, and allowing the culture to lyse at 37 °C.

Results and discussion. 2 T4rI derivatives, called No. 4 and No. 20, were isolated as being able to propagate on K12 bacteria but not on B bacteria. The frequency of occurrence after nitrosoguanidine mutagenesis was approximately 5×10^{-4}. Table 1 shows that the growth of the 2 mutants is slightly depressed on the K12 host, but is severely inhibited on B (regardless of whether they are sup$^-$ or sup$^+$).

Preliminary experiments showed that both T4 No. 4 or T4 No. 20 infected bacteria lysed after 20 min of growth at 37 °C, indicating that the early events of infection as well as cell lysis functions occur normally during the abortive infection, and that the failure to yield phage results from a block at the level of phage morphogenesis. Subsequently we tested by spot complementation on Bsup$^-$ T4 No. 4 and T4 No. 20 against amber mutations in all T4 late genes. We found that T4 No. 4 complemented phage mutants in all genes except gene 8 and that T4 No. 20 did not complement phage mutants in gene 53 (table 2). From recombination data obtained on the K12 sup$^+$ host, it appears that the T4 No. 4 mutation is very

Table 1

Phage	e.o.p.* on B	e.o.p.* on K12	e.o.t.** on B	e.o.t.** on K12	Phage yield*** on B	Phage yield*** on K12
T4rI	1.0	1.0	1.0	1.0	65	95
T4rI No. 4	2.0×10^{-6}	1.0	0.4	1.0	1.1	29
T4rI No. 20	3.0×10^{-6}	1.0	0.3	1.0	1.2	27

*e.o.p., the efficiency of plating, denotes the number of plaques produced by a phage strain on a given bacterial host relative to the number on K12; **e.o.t., the efficiency of transmission, denotes the probability that an infected bacterial host will produce at least one viable phage progeny; ***phage yield denotes the average number of viable phage progeny released per infectious center.

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Table 2

Phage	Yield of phage in complementation test**	Yield of Wt phage in recombination test***
T4 No. 4	5×10^7	–
T4 No. 20	6×10^7	–
T4 am H626*	4×10^6	–
T4 am H28*	2×10^6	–
T4 No. 4+T4 am H626	4×10^7	1.2
T4 No. 4+T4 am H28	5×10^9	2.8
T4 No. 4+T4 No. 20	3×10^9	3.4
T4 No. 20+T4 am H626	2×10^9	2.5
T4 No. 20+T4 am H28	7×10^7	0.005
T4 am H626+T4 am H28	9×10^9	4.1

*am H626 is located in gene 8; am H28 is located in gene 53; **the crosses were performed on the non-permissive strain B sup⁻. The progeny phage yield was assayed on the permissive strain K12 sup⁺; ***the crosses were performed on the permissive strain K12 sup⁺. Total phage progeny was assayed or K12 sup⁺ and wild type recombinant phage on B sup⁻. The value given is the percentage of wild type phage present in the burst.

closely linked to amH462 (0.005% recombination) and that the T4 No. 20 mutation is very closely linked to amH28 (0.005% recombination). The 8 and 53 gene products of phage T4 are known to be components of the outer wedges of the bacteriophage baseplate structure[7]. We have considered 2 types of explanation to account for the existence of the T4 No. 4 and T4 No. 20 mutations. The first type suggests that the structural gene products of genes 8 and 53, induced by T4 No. 4 and T4 No. 20 respectively, are different in the 2 hosts. This could be due to differences in the translational apparatus of K12 and B cells as, for example, in the specificity of the tRNA. The second type of explanation derives from previous observations of host involvement in T4 phage morphogenesis[8–10], and in T4 tail fibre function[11,12]. It suggests that the 8 and 53 altered gene products are able to interact effectively with a K12 host component involved in baseplate assembly, but cannot do so with the corresponding B component. The elegant electron micrographs of Simon[13], which show that T4 baseplate formation takes place at or near the bacterial membrane, support such an explanation. It is interesting to note that the T4 mutant HL626, originally thought to be an amber mutation in gene 60, has been shown[14] to be analogous to T4 No. 4 and T4 No. 20 in that it grows on all K12 strains tested but not on all B sup⁻ or B sup⁺ strains tested. The gene 60 product is thought to associate with the bacterial membrane to promote proper phage DNA replication[15].

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Heterogeneity of HeLa cell DNA as evidenced by CsCl density gradient centrifugation

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Summary. HeLa cell DNA was analyzed through CsCl density gradient centrifugation and thermal denaturation. Neutral centrifugation without ionic treatment allowed the resolution of a main peak with density 1.699 g/ml and of 3 satellites DNAs with densities 1.683, 1.710 and 1.720. First derivatives of melting curves showed the presence of 4 DNA families, whose G+C content calculated from T_m values corresponded almost exactly to the G+C content expected from the previously densities. The extraction method seems particularly suitable for quantitative separation of DNA classes.

The existence of the heterogeneity of human DNA was demonstrated by Corneo et al.[2–4], who showed the presence of satellite DNAs after fractionation in Ag^+–Cs_2SO_4 density gradients. In order to obtain quantitative yields of all human DNA components, a method has been developed whose application in plant DNA extraction has showed remarkable results[5,6]. This paper presents the results obtained in the resolution and fractionation of HeLa DNA components using neutral CsCl density gradients.

Materials and methods. DNA was obtained from whole cells suspended in a solution containing $5\cdot10^{-2}$ M Tris buffer, pH 7.8, $8\cdot10^{-2}$ M EDTA, 4% Na-dodecylsarcosinate and lysed by gentle stirring for 1 h at room temperature. NaCl was added to a 2 M final concentration and the solution was stirred for 1–2 h. After dialysis at 4 °C against 1×SSC (0.15 M NaCl and 0.015 M trisodium citrate), RNase (100 μg/ml preheated at 90 °C for 10 min) and α-amylase (150 μg/ml) were added directly in the dialysis tube. After incubation at 37 °C for 1 h, pronase (500 μg/ml, self digested for 2 h at 37 °C) was added and allowed to act for 2 h at 37 °C. The solution was then centrifuged at 18,000 rpm for 15 min at 4 °C: to the supernatant CsCl was added to a refractive index of 1.4000. The CsCl solution containing the DNA was centrifuged to equilibrium in a 30 rotor of a Spinco L 2-65 B ultracentrifuge at 20 °C and 25,000 rpm or in a 40 rotor at

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