was less consistent and less extensive than with natural messenger. This finding suggests that spectinomycin may interfere with some function of natural messenger RNA not possessed by synthetic polynucleotides. Moreover, in striking contrast to streptomycin and other aminoglycoside antibiotics (*2*), spectinomycin caused no detectable misreading of synthetic polynucleotides.

Comparison with spectinomycin may throw light on the action of streptomycin. Both drugs selectively inhibit protein synthesis in cells and inhibit polypeptide synthesis in vitro by interacting with the 30*S* ribosomal subunit; high-level resistance to either drug can arise by one-step mutation. However, only streptomycin is bactericidal, selects for dependent resistance, and causes detectable misreading of the genetic code. The absence of killing by spectinomycin supports the suggestion (*2*) that misreading may be responsible for the bactericidal action of streptomycin.

The close linkage between the Spc^R and Sm^R sites further supports the suggestion (*11*) that this region of the genome controls the structure of the 30*S* ribosomal subunit. Since spectinomycin acts on the same unit as streptomycin, and yet inhibits protein synthesis without causing misreading, the possibility must be considered that these two effects of streptomycin depend on different mechanisms.

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Lability of Host-Cell DNA in Growing Cell Cultures Due to Mycoplasma

Abstract. *In HeLa-cell cultures chronically infected with* Mycoplasma, *(PPLO) host-cell DNA is unstable as detected by incorporation of H^3- or C^{14}-thymidine into DNA and subsequent release into the medium as acid-soluble radioactivity. This characteristic can be transmitted to PPLO-free cultures of strain L cells by inoculation with preparations of PPLO from the HeLa cells, although chronically infected cultures of L cells continue to multiply. In addition, virus preparations also may carry PPLO contamination through numerous passages.*

It has been reported (*1*) that during infection of strain L cells with tissue-culture-adapted equine abortion virus (EAV, equine herpes virus), host-cell DNA prelabeled with C^{14}- or H^3-thymidine was degraded, while in normal control cultures it remained stable. In further studies, 2′-fluorodeoxyuridine and amethopterin were effective inhibitors of DNA synthesis in control cultures of L cells, but, in infected cultures, viral synthesis continued in the presence of these inhibitors (*2*). Apparently, the thymine-deficient state was relieved by the formation of thymidine nucleotides resulting from nuclease activity.

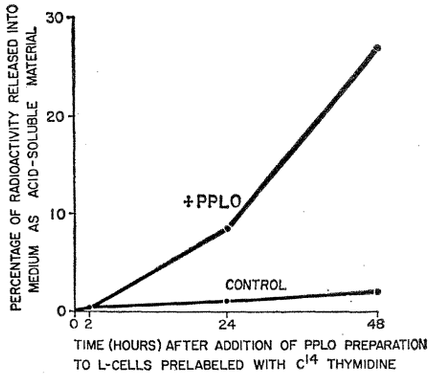

Fig. 1. The degradation of host-cell DNA of prelabeled L-cell suspension cultures inoculated with a preparation of PPLO. The cells were prelabeled with C^{14}-thymidine (specific activity, 30.5 mc/mmole; final concentration, 0.1 mc/ml) for 68 hours prior to addition of PPLO. The PPLO inoculum was prepared by differential centrifugation of preparations of HeLa cultures homogenized by high-frequency sound and resuspension of the pellet in a small volume of tissue-culture medium.

Additional studies (*3*) revealed that prelabeled DNA of apparently normal HeLa-cell cultures was not stable and was degraded in a manner remarkably similar to that found in viral-infected L-cell cultures. This instability is not unique; Chang and Vetrovs (*4*) recently found that in living, primary, human-amnion cultures, the DNA is degraded in significant quantity. These findings are of exceptional interest because DNA in living cells has been characterized as being metabolically stable (*5*).

To help explain the difference in DNA stability, the hypothesis was advanced that this peculiar property (instability) of DNA in our tissue cultures might be the result of covert contamination of HeLa cultures and viral inocula with a fastidious strain of pleuropneumonia-like organisms (PPLO, *Mycoplasma*). Although previous routine attempts in our laboratory to detect PPLO were negative, judged by attempted culture on commercial PPLO medium, arginase activity (*6*), and electron microscopy, it was decided to reinvestigate this problem. Accordingly, material from both HeLa- and strain L-cell cultures (maintained with 500 units of penicillin and 100 μg of streptomycin per milliliter) and from HeLa- and L-adapted equine abortion virus was cultured on a modified solid medium for PPLO devised in our laboratory. This medium contains Bacto-tryptose blood agar base, 3.5 g; Bacto-yeast extract, 1 g; and Bacto-PPLO serum fraction, 2 ml; prepared in 100 ml of double glass-distilled H_2O. It gives best results if used when freshly made. After inoculation, cultures were gassed with a mixture of carbon dioxide (5 percent) and nitrogen (95 percent), sealed, and incubated for 4 to 6 days at 37°C; duplicate cultures were grown aerobically. With the use of this medium (but not with commercial PPLO agar), typical colonies of pleuropneumonia-like organisms were obtained aerobically and anaerobically from HeLa cultures and with both the L-adapted and HeLa-adapted virus preparations, but the stock cultures of L cells were free of this agent. These findings were supported by the demonstration of PPLO in stained HeLa cells (*7*).

At this point there was good correlation between contamination with PPLO and instability of host-cell DNA, but further evidence was required to prove

Table 1. Cell multiplication and stability of DNA in L-cell suspension cultures following addition of PPLO material.

Time* (hr)	DNA specific activity† (count/min per μg)		Cell density‡ (10^6 cells/ml)		Radioactivity in medium§ (count/min per 10 ml)	
	Control	Infected	Control	Infected	Control	Infected
2	4010	3960	0.97	1.00	850	350
24	2200	2490	1.67	1.54	3550	41,000
48	1410	1730	3.08	2.56	8100	137,000

* Time after washing and resuspension of labeled cells in nonradioactive medium, and addition of PPLO material. Experimental conditions described in legend for Fig. 1. † Calculated as previously described (*1*). Continued decrease of specific activity indicates DNA synthesis and cell growth. ‡ Cell viability determined by exclusion of erythrosin B was not significantly different between control and infected cultures, ordinarily remaining between 95 and 99 percent in both. § Percentages of total radioactivity are plotted in Fig. 1. The intracellular acid-soluble fraction did not contain significant radioactivity.

Table 2. Cell multiplication and stability of DNA in L-cell suspension cultures chronically infected with PPLO.

Time* (hr)	DNA specific activity† (count/min per μg)		Cell density‡ (10^6 cells/ml)		Radioactivity in medium§ (count/min per 10 ml)	
	Control	Infected	Control	Infected	Control	Infected
2	2880	2580	1.07	0.98	1700	5,100
24	1790	1820	1.59	1.45	7150	35,900
48	1100	1480	2.24	1.53	6350	60,900

* Time after washing and resuspension of labeled cells in nonradioactive medium. Experimental conditions described in legend for Fig. 2. † Same as in Table 1. ‡ Same as in Table 1. § Percentages of total radioactivity are plotted in Fig. 2. The intracellular acid-soluble fraction did not contain significant radioactivity.

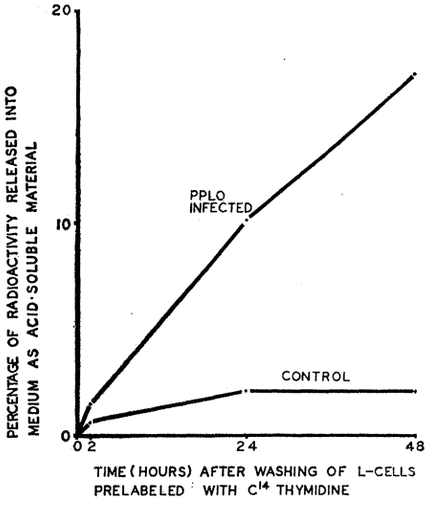

Fig. 2. The degradation of DNA of L-cell suspension cultures chronically infected with PPLO. Cells from the sixth subculture after infection with PPLO were labeled with C^{14}-thymidine (specific activity, 30.5 mc/mmole; final concentration, 0.1 mc/ml) for 68 hours prior to washing and resuspension in nonradioactive medium. The contribution of DNA from PPLO was not measured separately, but is not considered to be significant for the following reasons: (i) The difference between initial radioactivity of DNA in control and infected cultures of similar cell densities is small, and (ii) the data of Fig. 1, which are quite comparable, were obtained under conditions which excluded labeling of DNA of PPLO.

that association of PPLO with tissue-culture cells was causing the breakdown of host-cell DNA. Transmission of this characteristic to strain L cells by PPLO would supply this evidence. Accordingly, prelabeled L cells, free of PPLO, were inoculated with material from the PPLO-infected but virus-free HeLa cultures (for details see legend for Fig. 1), and the medium was assayed for acid-soluble radioactivity as described (*1*). The results are presented in Fig. 1 and Table 1 and show that the DNA from L cells is unstable under these conditions, breaking down to acid-soluble components which are released into the medium. In this regard it may be significant that some *Mycoplasma* show nuclease activity (*8*). The PPLO-infected L cells have retained this characteristic as well as chronic PPLO infection through six subcultures (Fig. 2 and Table 2). These cultures do not differ from the controls, when judged by dye-exclusion studies and efficiency of plating. The cells (initially or chronically infected with PPLO) do not exhibit significant cytopathology or changes in cell density; this is consistent with other studies (*9*).

The data indicate that a nonlytic strain of PPLO may infect tissue cultures and may be transmitted through several subcultures without causing overt cell damage or death, and in these living and multiplying cells a partial degradation of host-cell DNA occurs. In this regard, a recent report (*10*) of variations in the basic chromosome constitution of cells infected with PPLO may be important. Such agents may also be carried in virus-passage material after storage at −40°C for several months. These findings emphasize the importance of insuring that, in tissue-culture systems, both cell (*9*) and virus are not covertly infected.

It has been brought to our attention that related studies by Nardone *et al.* are included in this issue of *Science*. These workers found that contamination with *Mycoplasma* prevented the incorporation of tritiated nucleosides by cultures of L cells. Because of marked differences in experimental conditions, it is not surprising that equivalent phenomena were not observed. It would be surprising, however, if no effect of *Mycoplasma* contamination were noted, and in this general respect our findings and those of Nardone *et al.* are mutually supportive. Finally, we would agree emphatically that caution must be used in evaluating metabolic studies of tissue cultures in which contamination by *Mycoplasma* has not been considered.

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