yielded definitive evidence concerning the nature of the defect or the mechanism of action of B_6. The second point is important because of the possible benefits from therapy with this vitamin. Cystathionase activity in liver obtained by biopsy from patients B and D (*2, 4*) and its response to B_6 are reported here.

Liver specimens from patients with cystathioninuria converted cystathionine-H^3 to cystine-H^3 only in very small amounts (Table 1). The patient with the greater conversion had received high doses of B_6 up to a week before biopsy; this may account for some enzyme activity although the aminoaciduria had returned. When an excess of pyridoxal phosphate was added to the incubation mixture, cystine-H^3 production rose considerably. Nevertheless, the homogenate from the patients did not produce the amount achieved by the controls. In only one of the patients did the pyridoxal-treated specimen approach the activity of specimens, from control subjects, not so treated. Tissue obtained from the patients was insufficient to determine the effect of adding graded amounts of pyridoxal phosphate. The 180-minute incubation period was chosen as appropriate to test the system, although certain experiments had shown that lengthier incubation produced further conversion. Enzyme from human liver was considerably less active than that from the rat. No cystathionase activity could be demonstrated in normal human leukocytes. In the patients with cystathioninuria, the response to excess pyridoxal phosphate suggests that the enzyme is present, but in inadequate amounts. No clinical evidence of pyridoxine deficiency was apparent nor was there any other evident biochemical abnormality. Further, challenge with L-tryptophan yielded normal amounts of metabolites whether or not challenge was accompanied by added B_6. Thus a dietary deficiency of this vitamin or defective metabolism to the aldehyde, pyridoxal, is unlikely.

Table 1. Production of cystine-H^3 from cystathionine-H^3 by liver homogenate. Liver was obtained by open biopsy in patient D and all of the normal controls, and by Menghini needle in patient B. Specimens were immediately placed in a chilled vial and frozen at −24°C. The liver was homogenized at 0°C in 0.15*M* tris-HCl buffer, *p*H 8.0, and homogenate containing 1.7 to 6.7 mg of protein was incubated with amounts of pyridoxal phosphate as indicated for 180 minutes at 37.5°C. Protein was determined by the Lowry method (*11*). To each flask, 0.067 μmole of L-cystathionine-H^3 containing 10^6 dpm (disintegrations per minute) was added. The reaction was terminated by the addition of excess 1 percent picric acid (*12*) which was removed by passing the supernatant through Dowex 2 (Cl^-) (*13*). Cystine was separated by an ion-exchange method (*14*), and radioactivity was determined (*15*). The identity of the radioactive peak of cystine was confirmed by oxidation with performic acid and chromatography of radioactive cysteic acid on a basic resin (*16*).

Pyridoxal phosphate (mg)	Cystine (dpm × 10^3)	Cystine (dpm × 10^3) / (mg protein)
	Patient B	
0	2.3	0.6
0.1	8.8	2.4
	Patient D	
0	0.8	0.2
0.1	34.7	9.9
	Normal 1	
0	28.6	13.0
0.1	35.6	16.8
	Normal 2	
0	36.1	10.3
0.2	87.8	25.1
	Normal 3	
0	28.2	8.0
0.2	51.6	14.7
	Normal 4	
0	35.2	5.2
0.008	45.3	6.7
.02	38.0	5.6
.2	36.8	5.0
.4	59.0	7.5
	Normal 5	
0	97.3	14.5
0.2	120.0	18.0

That cystathioninuria has also been observed in patients with neural tumors (*9*) suggests the possibility of overproduction of the amino acid. The prolonged course of the disease in familial cystathioninuria, together with the results of the liver homogenate incubation, obviate tumor as the cause of the urinary excretion of this amino acid.

The various phenomena can best be explained by a structural alteration of the apoenzyme, resulting in failure to combine normally with the coenzyme. Accordingly, excessive coenzyme may result in an increase in the active combination. This has not been demonstrated in animal systems, although evidence of this possibility has been presented in bacterial systems (*10*). That adding pyridoxal phosphate does in fact lead to increased cystine production places therapy with high doses of B_6 on a sound basis. If additional persons with cystathioninuria also have associated physical or mental abnormalities, it is possible that B_6 therapy instituted at an early age, or even to the early fetus by the mother, may prevent or minimize the defects.

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Inhibition of Protein Synthesis by Spectinomycin

Abstract. *Spectinomycin selectively inhibits protein synthesis in cells and in extracts of* Escherichia coli. *Mutations to high-level resistance to this antibiotic map close to the streptomycin locus, and the site of action of spectinomycin, like that of streptomycin, is the 30S ribosomal subunit, as shown by experiments with reconstituted 70S ribosomes containing subunits from sensitive and from resistant ribosomes. In contrast to streptomycin, however, spectinomycin is not bactericidal and causes no detectable misreading of polyribonucleotides.*

Advances in understanding the mechanism of action of streptomycin (*1, 2*) have prompted us to examine the effects of other aminoglycosides on bacterial cells and on polypeptide synthesis in extracts. This report concerns a recently isolated antibiotic, spectinomycin (actinospectacin, Trobicin), whose structure (Fig. 1) includes an amino-sugar moiety (actinamine) stereoisomeric with streptamine (*3*). Spectinomycin has been reported (*4, 5*) to inhibit the growth of a variety of gram-positive and gram-negative organisms. The bacteriostatic end point for some strains increased markedly upon

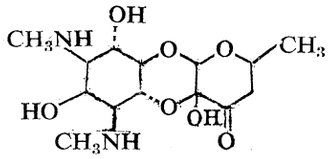

Fig. 1

subculturing after exposure to the drug —presumably owing to rapid outgrowth of resistant substrains (*5*). On further studying the action of spectinomycin, we have found that it shares certain properties with streptomycin and others with chloramphenicol.

When added to an exponentially growing culture, spectinomycin (50 μg/ml) rapidly and reversibly inhibited growth of *Escherichia coli* B (measured by either turbidity or viable count). Amino acid incorporation was slowed immediately but RNA synthesis continued (Fig. 2). Exposure for several hours to as much as 1000 μg/ml produced very little killing. Spectinomycin thus resembles in its action such reversible inhibitors of protein synthesis as chloramphenicol and tetracycline (*6*). Spectinomycin, like chloramphenicol (*7*), protected against killing by streptomycin. Unlike chloramphenicol and tetracycline, however, and like streptomycin, spectinomycin selected for single-step mutations to high-level (1000 μg/ml) resistance in *E. coli* B and K 12; the spontaneous frequency of occurrence was 10^{-9} to 10^{-8}. Drug dependence, though found among streptomycin-resistant mutants, has not been observed with spectinomycin. Resistance to the latter conferred no cross resistance to either streptomycin or chloramphenicol, nor did streptomycin resistance affect sensitivity to spectinomycin.

Table 1. Effect of spectinomycin on ^{14}C-alanine incorporation (in presence of all other amino acids) in response to MS-2 bacteriophage RNA, with reconstituted ribosomes. Ribosomal subunits were isolated from sucrose gradients (*9*); only fractions inactive alone in response to added RNA were used. Incorporation mixtures (0.25 ml) contained 35 μg of 30*S* subunit, 70 μg of 50*S* subunit, and 90 μg of MS-2 bacteriophage RNA. Incubation was for 45 minutes at 37°C. Spectinomycin (0.8 μg/ml) was added as indicated. R, resistant; S, sensitive.

Source of reconstituted ribosomes		Spectinomycin	^{14}C-alanine incorporation (count/min)	Inhibition (%)
30*S* subunit	50*S* subunit			
S	S	−	185	
S	S	+	61	67
S	R	−	251	
S	R	+	78	69
R	R	−	332	
R	R	+	323	3
R	S	−	245	
R	S	+	231	6

Spectinomycin resistance could be transferred to sensitive strains by conjugation in *E. coli* K12, or by transduction with P1 phage in strains K12 and B. The loci for spectinomycin (*Spc*) and for streptomycin (*Sm*) resistance are closely linked: in interrupted mating Spc^R was transferred at about the same time as Sm^R; and Sm^R and Spc^R could be co-transduced from a doubly resistant donor. In a cross of Hfr: $Spc^R Sm^S Met^-$ × F$^-$:$Spc^S Sm^R Met^+$, 1 percent of the $Spc^R Met^+$ recombinants were $Spc^R Sm^R$ (that is, recombinant between *Spc* and *Sm*). A similar cross between a Sm^R male and a Spc^R female yielded 1 percent $Sm^R Spc^R$ recombination.

In extracts of *E. coli* B, spectinomycin inhibited polypeptide synthesis directed either by endogenous messenger RNA (Fig. 3) or by MS-2 bacteriophage RNA (*8*). Maximum inhibition (70 to 80 percent) was achieved at 1 μg/ml (3 × $10^{-6}M$). In extracts of Spc^R mutants, however, the drug exerted little or no inhibition, even at much higher concentrations. The ribosomes are the site of action of spectinomycin: when ribosomes and supernatant fractions from sensitive and resistant strains were interchanged, only those mixtures containing sensitive ribosomes were appreciably inhibited by the drug. Futhermore, when reconstituted and hybrid ribosomes were made by mixing 30*S* and 50*S* subunits from sensitive and from resistant ribosomes, the sensitivity or resistance to spectinomycin—just as to streptomycin (*9, 10*)—was found to reside in the 30*S* subunit (Table 1).

With synthetic polynucleotides as messenger RNA the inhibition of amino acid incorporation by spectinomycin

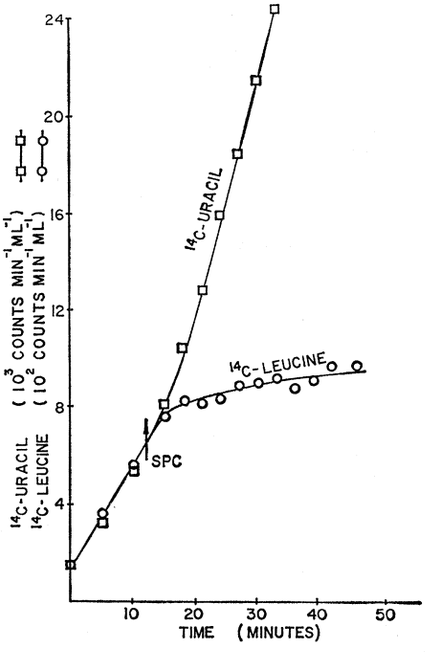

Fig. 2. Effect of spectinomycin (50 μg/ml) on ^{14}C-leucine and ^{14}C-uracil incorporation. Each compound was added to one portion of a culture of *Escherichia coli* B in early exponential growth with aeration at 37°C. Samples (0.5 ml) were removed for precipitation by trichloroacetic acid and filtration at the times indicated.

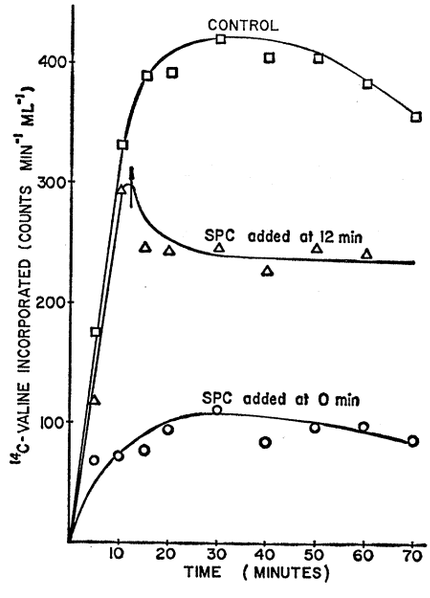

Fig. 3. Effect of spectinomycin on ^{14}C-valine incorporation by deoxyribonuclease treated S-30 extract (*12*) without added messenger RNA. Incorporation was carried out according to Nirenberg (*12*), except that NH_4Cl was used in place of KCl. Samples (0.2 ml) were removed from 2.5-ml incubation mixtures. Spectinomycin (1 μg/ml or 3 × $10^{-6}M$) was added as indicated.

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.

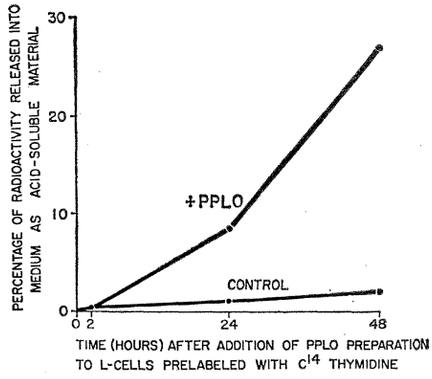

Fig. 1. The degradation of host-cell DNA of prelabeled L-cell suspension cultures inoculated with a preparation of PPLO. The cells were prelabeled with C^{14}-thymidine (specific activity, 30.5 mc/mmole; final concentration, 0.1 mc/ml) for 68 hours prior to addition of PPLO. The PPLO inoculum was prepared 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