ciently small to justify the plan of our experiment. Twenty-four hours after the administration of radioactive orotic acid the specific activity of rRNA was about the same whether or not a "chase" of nonradioactive orotic acid had been given 4¼ hours after the original injection of labeled acid, showing that even without a "chase" the incorporation of labeled precursor into RNA had become insignificant. The use of a "chase", however, was retained in the experimental procedure as it was well tolerated by the rats and could only serve to decrease still further the specific activity of the precursor pools.

The average specific activity of the RNA isolated from ribosomes from each group of six animals as a function of time after the administration of C^{14}-orotic acid is plotted in Fig. 2*A*. An interpretation of the decline of specific activity, however, must take into account liver growth over the course of the experiment, which is also shown in Fig. 2*A*. For this reason the logarithm of the product of the average specific activity and liver weight is plotted as a function of time in Fig. 2*B*. The relation is very nearly linear, and justifies mathematical representation by

$$\frac{\mathrm{d} \ln (s \cdot w)}{\mathrm{d}t} = -k$$

where s is the observed specific activity and w the liver weight at time t. Since the product $s \cdot w$ is proportional to the amount of radioactive rRNA in a given liver, m, and since the above points were all obtained at times when the rate of *formation* of radioactive rRNA molecules was negligible (as mentioned above), the slope of the line in Fig. 2*B* gives the desired destruction constant directly: $k = -\mathrm{d} (\ln m)/\mathrm{d}t = 121$ hours, or approximately 5 days. The fact that the decrement is logarithmic shows that a molecule of rRNA has no fixed life span, but rather that it is destroyed randomly regardless of its "age."

Estimates of the turnover of messenger RNA in dividing bacteria (*11*) and in HeLa cells growing in tissue culture (*12*) indicate that it is short-lived, and it is thought that, in these cells, the rate of protein synthesis may be controlled exclusively by the rate of synthesis of messenger RNA. In contrast, there is evidence that in liver a significant fraction of messenger RNA has a lifetime of more than 3 days (*13*), and it would therefore seem possible that here the rate of synthesis of specific proteins is dependent upon other factors. In view of the present finding that rRNA in liver, once it is formed, turns over rapidly with respect to the generation time, it would seem possible that protein synthesis in these cells may be influenced at least in part by regulation of ribosomal turnover.

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References and Notes

1. C. I. Davern and M. Meselson, *J. Mol. Biol.* **2**, 153 (1960).
2. A. V. Rake and A. F. Graham, *J. Cell. Comp. Physiol.* **60**, 139 (1962).
3. Since parenchymal cells account for the largest part by far of the RNA in liver [C. Rouiller, Ed., *The Liver: Morphology, Biochemistry, Physiology* (Academic Press, New York, 1963), vol. 1], and since the specific activity of the total liver RNA of a rat fed a uniformly labeled diet reaches over 80 percent of that of the diet in only 10 days [D. L. Buchanan, *Arch. Biochem. Biophys.* **94**, 500 (1961)], the turnover observed under the conditions of our study reflects primarily the turnover of RNA in these parenchymal cells. That our data closely fit a single logarithmic function strongly suggests that the observed turnover is attributable to a single predominant population of cells.
4. T. Staehelin, F. O. Wettstein, H. Oura, H. Noll, *Nature* **201**, 264 (1964).
5. J. N. Loeb, R. R. Howell, G. M. Tomkins, in preparation.
6. T. E. Webb, G. Blobel, V. R. Potter, *Cancer Res.* **24**, 1229 (1964).
7. R. R. Howell, J. N. Loeb, G. M. Tomkins, *Proc. Natl. Acad. Sci. U.S.* **52**, 1241 (1964).
8. The radioactivity in a 0.5-ml sample of each supernatant treated with sodium dodecyl sulfate was determined in a Packard Tri-Carb liquid scintillation spectrometer after the addition of 10 ml of Bray's solution.
9. An error of 30 percent in the determination of the specific activity of the rRNA from one animal is not important in view of the variation of specific activity seen from one animal to another within a given group (see standard deviations plotted in Fig. 2*A*). More importantly, a discrepancy of this magnitude would not significantly affect the calculation of the turnover rate since, as can also be seen in Fig. 2*A*, there was, over the course of the experiment, more than a *25-fold* change in the observed specific activity.
10. R. B. Hurlbert and V. R. Potter, *J. Biol. Chem.* **195**, 257 (1952).
11. C. Levinthal, A. Keynan, A. Higa, *Proc. Natl. Acad. Sci. U.S.* **48**, 1631 (1962); A. Kepes, *Biochim. Biophys. Acta* **76**, 293 (1963).
12. S. Penman, K. Scherrer, Y. Becker, J. E. Darnell, *Proc. Natl. Acad. Sci. U.S.* **49**, 654 (1963).
13. M. Revel and H. H. Hiatt, *ibid.* **51**, 810 (1964).
14. R. J. Britten and R. B. Roberts, *Science* **131**, 32 (1960).
15. G. A. Bray, *Anal. Biochem.* **1**, 279 (1960).

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Cystathioninuria: Nature of the Defect

Abstract. *A homogenate of liver obtained by biopsy from two patients with cystathioninuria, an inborn error of metabolism, cleaved radioactive cystathionine only slightly until an excess of pyridoxal phosphate was added. The apoenzyme failed to bind the coenzyme normally. Pyridoxine therapy of familial cystathioninuria thus has a sound basis.*

A grossly increased urinary excretion of the amino acid, cystathionine, has been observed in four patients (*1–4*). Patient A was a female, aged 64 years, imbecile with bilateral talipes calcaneo valgus (*1*). Patient B, now aged 47, is a man with developmental defects about the ears and mental aberrations (*2*). Patient C is a 12-year-old boy with mental deficiency and grand mal convulsions (*3*). Patient D is a 2-year-old boy with thrombocytopenia (*4*). His mental status is probably normal, but difficult to evaluate. The aminoaciduria results from an increase of the amino acid in the blood. Cystathionine is known to be involved in but one biochemical reaction, the transfer of sulfur from methionine to cysteine. The demonstration of probable heterozygotes in the families of three of the individuals suggests an inborn error of metabolism. Cystathioninuria has been attributed to a defect in the cystathionine-cleaving enzyme, cystathionase. The enzyme has been characterized; pyridoxal phosphate is the coenzyme (*5*).

One arresting fact has been the marked reduction in concentrations of cystathionine in urine and blood during administration of high doses of pyridoxine hydrochloride (B_6) orally or intramuscularly to three patients (B, C, and D). The B_6 appeared to increase the activity of cystathionase, and this effect may be explained by other possibilities. For example, cystathionine may be destroyed by deamination decarboxylation, or a "pre-cystathionine" deviation of sulfur may occur under the influence of B_6. In bacterial systems degradation of methionine requires pyridoxal phosphate (*6*). A homocysteine desulfhydrase system in *Proteus morganii* requires pyridoxal phosphate (*7*). A homocysteine desulfurase has been described in liver, kidney, and pancreas (*8*).

Studies in the patients have not

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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References and Notes

1. H. Harris, L. S. Penrose, D. H. H. Thomas, *Ann. Human Genet.* **23**, 442 (1959).
2. G. W. Frimpter, A. Haymovitz, M. Horwith, *New Eng. J. Med.* **268**, 333 (1963).
3. S. Berlow and M. Efron, paper presented at the meeting of the Midwest Society for Pediatric Research, 13 October 1964, Winnipeg, Canada.
4. J. G. Mongeau, G. W. Frimpter, M. W. Hilgartner, H. G. Worthen, in preparation.
5. Y. Matsuo and D. M. Greenberg, *J. Biol. Chem.* **230**, 561 (1958).
6. R. E. Kallio and A. D. Larson, in *A Symposium on Amino Acid Metabolism*, W. D. McElroy and H. B. Glass, Eds. (Johns Hopkins Press, Baltimore, 1955).
7. R. E. Kallio, *J. Biol. Chem.* **192**, 371 (1951).
8. C. Fromageot and P. Desnuelle, *Compt. Rend.* **214**, 647 (1942).
9. L. R. Gjessing, *Scand. J. Clin. Lab. Invest.* **15**, 474 (1963).
10. D. M. Bonner, Y. Suyama, J. A. Demoss, *Federation Proc.* **19**, 926 (1960).
11. O. H. Lowry, N. J. Rosebrough, A. L. Farr, R. J. Randall, *J. Biol. Chem.* **193**, 265 (1951).
12. P. B. Hamilton and D. D. Van Slyke, *ibid.* **150**, 231 (1943).
13. W. H. Stein and S. Moore, *ibid.* **211**, 815 (1954).
14. S. Moore, D. H. Spackman, W. H. Stein, *Anal. Chem.* **30**, 1185 (1958).
15. G. W. Frimpter, *J. Clin. Invest.* **42**, 1956 (1963).
16. E. Schram, S. Moore, E. J. Bigwood, *Biochem. J.* **57**, 33 (1957).
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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