and ligation in dilute buffer, and examined by the direct mounting and cytochrome c surface spreading techniques used here. The same distribution of molecules with 0, 1, 2, or more twists were counted by EM as were observed by gel electrophoresis.

Had these DNA circles been in the classic B structure in solution but shrunk in length such that their rise was almost exactly halfway between the classic B values (3.4-Å rise, 10.0 base pair screw) and A values (2.55-Å rise, 11 base pair screw), then we would expect this new structure to have a screw of 10.5 base pairs per turn. A change from 10.0 to 10.5 base pairs per turn for a DNA of 5200 base pairs would be accompanied by a gain of about 25 superhelical turns. That no such change was observed even though small changes due to salt effects can be detected is evidence that the rise of these ϕX174 circles in solution was not altered by the EM preparations. This conclusion was further buttressed by the finding that the lengths of the ϕX174 hybrid molecules were exactly that expected for the A helix when prepared by the same EM procedures, and that very different preparative techniques yielded consistent results.

The suggestion that DNA in dilute solution has, on the average, 10.5 base pairs per turn is in agreement with the recent calculations of Levitt (*3*), which yielded the same value. This agreement could be coincidental; we have yet to prove that the ϕX174 DNA retained its length throughout the EM preparations. Indeed, certain EM procedures can induce DNA length changes (*11*). Furthermore, because the A helix is more compact than the classic B helix and the A form is found at lower hydration, it might be more stable than normal duplex DNA. On the contrary, we have observed that the ϕX174 hybrid circles will change length when prepared with low (10 percent) concentrations of the denaturant formamide (*12*). Finally, it is not impossible that DNA would change in length without changing its overall twisting. Until these possible changes can be ruled out absolutely or evaluated, measurement of DNA lengths by EM can be taken as strong evidence for, but no proof of, the contention that the average rise of DNA in dilute solution is closed to 2.9 Å.

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8. A diffraction replica grating prepared by Ted Pella Company from a grating ruled to 54,800 lines per inch by Bausch & Lomb Company, was verified in the following manner. The square holes of the copper grid supporting the carbon grating replica, measured by light microscopy, were 38.75 ± 0.05 μm across. The grating lines were parallel to the sides of the square holes and counted by EM; 84 ± 1 lines crossed the 38.75-μm distance. One line, therefore, is 0.461 μm by this measurement and 0.464 μm by calculation from 54,800 lines per inch.
9. After every fifth micrograph taken of the sample, two were taken of the ruled grating, then five of the sample, and so on. Only those sets in which the variation in magnification was 1 percent or less were used. This required that the instrument (Hitachi H500) be left on (and unused) for 8 hours prior to taking the micrographs.
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11. In a recent study applying a detergent film technique [H. J. Vollenweider, A. James, W. Szybalski, *Proc. Natl. Acad. Sci. U.S.A.* **75**, 710 (1978)], a spectrum of rises for DNA including 2.9 Å were observed, depending on the details of dehydration. In the procedures used here, no such effects were observed: the molecules fell into single-sized distributions and exhibited the same length whether the grids were dehydrated with ethanol, or blotted dry after a brief washing with dilute buffer.
12. Both the ϕX174 DNA duplex and RNA-DNA hybrid molecules that were spread from 40 percent formamide solutions onto 10 percent solutions had lengths corresponding to rises of 3.0 to 3.2 Å. This is in agreement with the finding of Chow *et al.* [L. Chow, J. M. Roberts, J. B. Lewis, T. R. Broker, *Cell* **11**, 818 (1977)].
13. I thank P. Modrich, R. Chandross, and C. Carter for helpful discussion, C. A. Hutchinson III for the gift of the ϕX174 DNA's, and A. Tinker for development of the length measurement programs. Supported in part by a grant from the National Science Foundation.

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Histidine Transfer RNA Levels in Friend Leukemia Cells: Stimulation by Histidine Deprivation

Abstract. *Friend leukemia cells incubated with sublethal concentrations of histidinol for 5 to 6 days show up to twofold increases in their relative concentrations of histidine transfer RNA and no change in the relative concentrations of leucine transfer RNA. A similar effect is seen when cells are grown to stationary phase in the presence of 0.2 times the amount of histidine in Eagle's minimum essential medium. These observations support the theory that the concentrations of specific transfer RNA's are regulated by a mechanism that is sensitive to the extent of their aminoacylation.*

Intracellular concentrations of specific transfer RNA's (tRNA's) are closely correlated with the needs of the cell for their cognate amino acids in protein synthesis (*1*, *2*). However, the signals that regulate the synthesis and degradation of specific tRNA's and thereby control their concentrations are not understood. Although rates of degradation and synthesis of different tRNA species differ (*3*, *4*), it is not known how these rates are regulated to produce shifts in the tRNA population in cells undergoing differentiation.

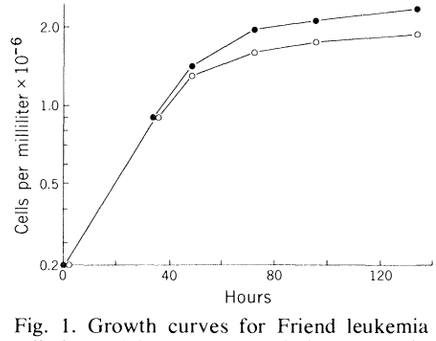

Fig. 1. Growth curves for Friend leukemia cells in Eagle's MEM (closed circles) and in MEM containing less (0.2 times) histidine (open circles). Each point is the average of two determinations on duplicate cultures.

The extent of aminoacylation might play a role in controlling tRNA concentrations. If an amino acid is not being used for protein synthesis, its cognate tRNA's will tend to become 100 percent aminoacylated. If, on the other hand, an amino acid is in great demand, the steady-state level of aminoacylation of its cognate tRNA's will tend to decrease (*5*). We postulated that the synthesis of a particular tRNA species would be stimulated or that its degradation would be inhibited when the extent of deacylation of that particular tRNA species increased. Our experiments indicate that, at least for the case of histidine tRNA ($tRNA^{His}$) in Friend leukemia cells, this postulate is correct.

L-Histidinol, a potent inhibitor of protein synthesis in cultured mammalian cells, acts by competetively inhibiting

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histidyl-tRNA synthetase (E.C. 6.1.1.21) and thereby increasing the steady-state concentration of deacylated $tRNA^{His}$ (*6*). Treatment of Friend leukemia cells with appropriate concentrations of this drug inhibits the synthesis of protein, ribosomal RNA (rRNA), and total tRNA (*7, 8*), with relatively little effect on the synthesis of nucleoplasmic RNA. Similar effects of L-histidinol have been observed in L cells (*9*) and in Ehrlich ascites tumor cells and 3T6 cells (*10*). It has been proposed that histidinol, by inhibiting aminoacylation of $tRNA^{His}$, induces a negative pleiotypic response in animal cells which is similar to the response produced in cells entering the G_0 stage of the cell cycle as a result of serum starvation (*9*).

Friend leukemia cells [cell line 745 (*11*), clone 18] were grown in Eagle's minimum essential medium (MEM) supplemented with 10 percent fetal calf serum (Gibco) at 37°C in an atmosphere of 5 percent CO_2. Cells to be used for experiments were centrifuged for 3 minutes at half speed in a clinical centrifuge (International model CL) and were resuspended at a density of 3 to 4 × 10^5 cells per milliliter in MEM containing 10 percent fetal calf serum and 0.2 times the concentration of histidine in MEM. The reduced histidine concentration was 20 μM; this includes the histidine contributed by the fetal calf serum present in the medium. Cells were incubated in this medium in the presence or absence of L-histidinol (Sigma) at various concentrations. The concentrations of L-histidinol used were in the range 0.3 to 0.5 mM. At these concentrations, cell division, although severely inhibited, was not completely prevented; cells suspended at an initial density of 4 × 10^5 per milliliter underwent one to two divisions over a 5- to 6-day period before growth ceased.

After 5 to 6 days of incubation in the presence of histidinol or 3 days in its absence, cells were harvested by centrifugation and total tRNA was prepared by extraction with sodium dodecyl sulfate and phenol centrifugation in a sucrose density gradient as described (*8*). After deacylation (*2*), the tRNA preparations were assayed for histidine acceptance and also separately for acceptance with a mixture of 14 equimolar, equal activity ^{14}C-labeled amino acids (Amersham). Acceptor assays were performed in duplicate in the presence of an excess of a mixed aminoacyl tRNA synthetase preparation obtained from rat liver as described by Yang and Novelli (*12*). In addition, incubation mixtures contained, in a total volume of 25 μl: tRNA, 0.20 to 0.35 A_{260} unit (absorbance at 260 nm); adenosine triphosphate (ATP), 8.0 mM for histidine assays, 2.5 mM for leucine assays, or 40.9 mM for assays with the amino acid mixture; tris-HCl, pH 7.5, 40 mM; $MgCl_2$, 16 mM for assays with histidine or the amino acid mixture, or 3.0 mM for leucine assays: plus [^{14}C]histidine at a concentration of 14 μM or leucine at 100 μM or ^{14}C-labeled amino acids at 18 μM (for each of the 14 amino acids in the mixture). After 30 minutes of incubation at 37°C, cold trichloroacetic acid insoluble radioactivity was trapped on glass-fiber filter papers and counted in a liquid scintillation spectrometer. In preliminary experiments with rabbit liver tRNA, it was determined that these conditions lead to maximal steady-state levels of aminoacylation; that is, the level of aminoacylation observed was not increased by increasing the quantity of aminoacyl tRNA synthetase preparation added to the reaction mixture. The acceptor activities for histidine and for the amino acid mixture in picomoles per A_{260} unit were calculated. The relative acceptor activity for histidine or leucine was defined as the ratio of histidine or leucine acceptor activity, respectively, to acceptor activity measured with the amino acid mixture. The values that we determined in three independent experiments are shown in Table 1. The absolute values of acceptor activity determined with the mixture of ^{14}C-labeled amino acids are also given in Table 1.

Table 1. Relative acceptor activity for histidine and leucine of tRNA preparations isolated from histidinol-treated and control cultures of Friend leukemia cells. Results shown are the average of duplicate determinations ± one-half the range.

Histidinol concentration (mM)	Relative acceptance for		Acceptance for amino acid mixture (pmole/A_{260} unit)
	Histidine	Leucine	
	Experiment 1		
0	0.034 ± .004		885 ± 5
0.4	0.049 ± .003		750 ± 40
	Experiment 2		
0	0.023 ± .001	0.087 ± .005	730 ± 4
0.3	0.042 ± .003	0.087 ± .010	650 ± 18
0.5	0.053 ± .002	0.086 ± .012	570 ± 11
	Experiment 3		
0	0.023 ± .001	0.111 ± .002	934 ± 14
0.3	0.038 ± .002	0.111 ± .010	600 ± 70
0.5	0.048 ± .002	0.113 ± .016	637 ± 80

Table 2. Relative acceptor activity for histidine and leucine of tRNA preparations isolated from growing and resting cells in Eagle's MEM or in MEM containing less (0.2 times) histidine. Results shown are the average of duplicate determinations ± one-half the range.

Growth state	Histidine concentration in MEM	Relative acceptance for	
		Histidine	Leucine
		Experiment 1	
Growing	×1.0	0.023 ± .001	0.111 ± .002
Resting	×0.2	0.043 ± .002	0.111 ± .003
		Experiment 2	
Resting	×1.0	0.026 ± .001	0.105 ± .002
Resting	×0.2	0.050 ± .002	0.098 ± .005

Histidinol treatment caused a dose-dependent increase in the proportion of total active tRNA which accepted histidine and caused no change in the relative leucine acceptance. The observed, up to twofold increase in relative histidine acceptor activity is especially interesting in view of the inhibition of total tRNA synthesis caused by histidinol treatment (*7, 8*).

The enrichment of $tRNA^{His}$ in vivo might be a specific result of the functional histidine deprivation caused by histidinol. However, since the experimental and control tRNA samples were isolated from growing and resting cells, respectively, the enrichment of $tRNA^{His}$ could also be a consequence of the differing growth states of the cultures. To check this, we isolated and assayed tRNA from cells growing exponentially in Eagle's MEM and also from resting cells after 134 hours of incubation in MEM medium and in medium with 0.2 times the concentration of histidine in MEM. From the growth curves for the last two cultures (Fig. 1) it can be seen that reduction of histidine to 0.2 times the concen-

tration in MEM had no effect on the initial growth rate but did cause a significant lowering of the final cell density. The relative acceptance of the three preparations for histidine and leucine are shown in Table 2. Clearly, incubation in Eagle's MEM has no effect on the relative histidine acceptance of tRNA, whether the cells are in the growing or the resting state. However, in cells that have been grown up to and maintained in the resting state in medium with 0.2 times the concentration of histidine in MEM, the relative concentrations of $tRNA^{His}$ doubled, whereas the relative concentrations of leucine tRNA ($tRNA^{Leu}$) were unchanged. These results show that the increase in relative histidine acceptance requires histidine deprivation and is not simply a nonspecific result of inhibition of cell growth.

Previous results from this laboratory (*2*) showed that isoleucine tRNA and noninitiator methionine tRNA concentrations in reticulocytes from different breeds of anemic sheep are correlated with the isoleucine and methionine contents, respectively, of the hemoglobin being synthesized. Those results suggested that specific tRNA levels are not directly programmed as part of the process of cell differentiation, but rather that they are controlled by a physiological adaptation to the pattern of amino acid utilization by the protein synthesizing apparatus. In this report, we have presented evidence that such a physiological adaptation may involve the extent of aminoacylation as a signal to control the relative levels of specific tRNA's. Whether specific tRNA levels are controlled by varying their synthetic rates (*13*) or by varying their rates of degradation or the rates of activation of inactive precursor tRNA's (*14*) cannot be determined from our data.

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Virus-Induced Diabetes Mellitus: Reovirus Infection of Pancreatic β Cells in Mice

Abstract. *Reovirus type 3, passaged in pancreatic β-cell cultures, produced an insulitis when inoculated into 1- to 2-week-old mice. By means of a double-label antibody technique, in which we used fluorescein-labeled antibody to reovirus and rhodamine-labeled antibody to insulin, reovirus antigens were found in β cells. By electron microscopy, viral particles in different stages of morphogenesis were observed in insulin-containing β cells but not glucagon-containing α cells. The infection resulted in destruction of β cells, reduction in the insulin content of the pancreas, and alteration in the host's capacity to respond normally to a glucose tolerance test.*

The possibility that viruses might be one of the causes of juvenile-onset diabetes mellitus by infecting and destroying pancreatic β cells has received considerable attention. Mumps and members of the coxsackie B group have been the viruses most often suggested as possible causes of juvenile diabetes, but proof that these viruses can actually infect β cells and produce diabetes has not been obtained (*1*). Recently, however, it was shown that at least in vitro, human β cells can be infected and destroyed by mumps virus (*2*) and coxsackie virus B3 (*3*).

Support for the hypothesis that viruses

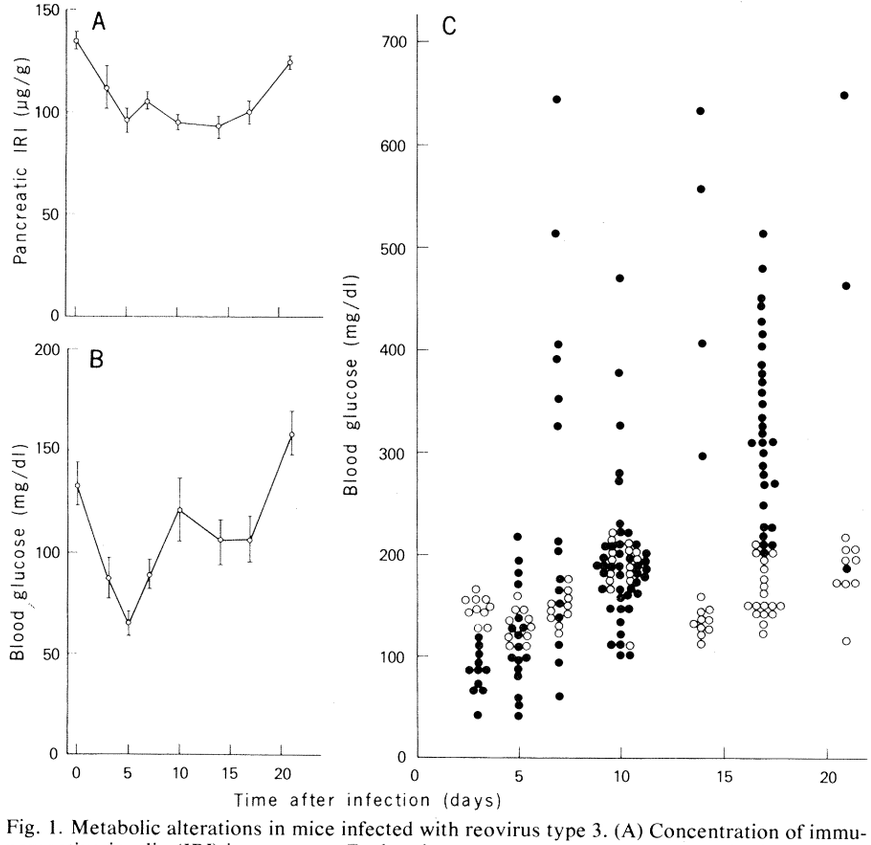

Fig. 1. Metabolic alterations in mice infected with reovirus type 3. (A) Concentration of immunoreactive insulin (*IRI*) in pancreas. Each point represents the mean and standard error of at least six mice. (B) Concentration of glucose in the blood. Each point represents the mean and standard error of at least six mice. (C) Glucose tolerance tests in infected (●) and uninfected (○) mice. The concentration of glucose in the blood was determined 60 minutes after the intraperitoneal administration of 2 mg of glucose per gram of body weight.

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