publication, we learned that Kim *et al.* have observed x-ray diffraction patterns of yeast $tRNA^{Phe}$ and have interpreted their patterns to be also consistent with the presence of double helical segments, of approximately half a turn, in the molecule (*9*).

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Adenosine 3′,5′-Monophosphate Increases Capacity for RNA Synthesis in Rat Liver Nuclei

Abstract. *Liver nuclei isolated from rats injected with adenosine 3′,5′-monophosphate exhibit an increased capacity for RNA synthesis compared with nuclei from control animals. This effect, which is highly specific for the cyclic nucleotide, can be observed within 1 hour after injection in both unoperated and adrenalectomized rats. These findings suggest that induction of enzyme synthesis mediated by way of adenosine 3′,5′-monophosphate may be controlled, at least in part, at the level of gene transcription.*

A wide variety of tissue responses to specific hormones are known to be mediated at the molecular level by adenosine 3′,5′-monophosphate (cyclic AMP), and increased cellular concentrations of cyclic AMP can cause both activation of existing enzymes and induction of the synthesis of new enzymes (*1*). This latter effect could be controlled by actions of cyclic AMP during either translation or transcription, or both.

A possible explanation for effects of cyclic AMP on transcription has recently been reported by Langan (*2, 3*), who has observed that the phosphorylation of histones is stimulated by cyclic AMP. Because histones are thought to usually inhibit gene transcription, it has been postulated that the cyclic AMP–mediated phosphorylation of histones causes them to be displaced from the DNA and thereby allows genes to be transcribed into RNA. Indeed, glucagon, which is known to induce the synthesis of a number of specific enzymes in rat liver, has been found to cause both increased liver concentrations of cyclic AMP and an increase in the phosphorylation of a specific serine residue in F1 histone (*3*). This hypothesis is further supported by the finding that the induction of at least one liver enzyme by glucagon is inhibited by actinomycin D (*4*).

If Langan's hypothesis is correct, then cyclic AMP should cause an increase in the rate of RNA synthesis in rat liver, although the overall effect might not be very great due to the relatively small number of enzymes whose synthesis is induced by this nucleotide. Because it has been shown that the synthesis of specific enzymes can be induced in rat liver by direct administration of cyclic AMP to normal rats (*4, 5*), we decided to determine whether such treatment has any detectable effect on nuclear RNA synthesis. Our experiments show that within 1 hour after administration of cyclic AMP, a dramatic increase occurs in the ability of liver nuclei to synthesize RNA.

Male Sprague-Dawley rats between 150 and 250 g in weight were injected with cyclic AMP (10 mg per 100 g of body weight) or an equivalent volume of saline (0.9 percent NaCl) 1 hour before the animals were killed. Liver nuclei were isolated as a pellet in 2.4*M* sucrose (1 m*M* $MgCl_2$) (*6*), and were incubated in the presence of ^{14}C-labeled adenosine triphosphate (ATP) and unlabeled nucleoside triphosphates to monitor their capacity for RNA synthesis (Table 1). The DNA concentration was determined by the indole method (*7*), and RNA synthesis was expressed as radioactivity incorporated into acid-insoluble material per milligram of DNA.

Although some variability was encountered in different groups of rats, animals injected with cyclic AMP consistently yielded liver nuclei with an in-

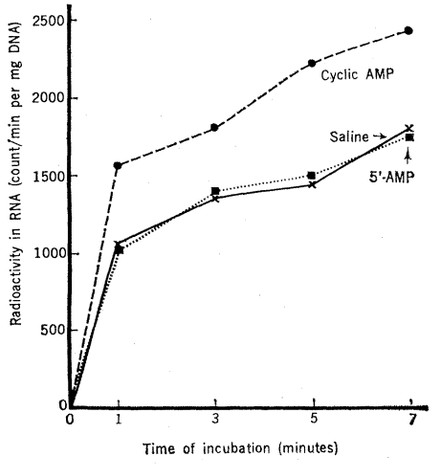

Fig. 1. Time course of RNA synthesis in liver nuclei from rats injected with cyclic AMP, 5′-AMP, or saline (0.9 percent NaCl) 1 hour before the animals were killed. Conditions were those described in Table 1. The stimulation in the rate of RNA synthesis only occurs with cyclic AMP, and the effect is noticeable within the first minute of incubation.

Table 1. RNA synthesis in liver nuclei from rats injected with cyclic AMP or saline (controls). Nuclei were incubated for 5 minutes at 37°C in a medium containing 100 μM each of cytidine triphosphate; guanosine triphosphate, and uridine triphosphate; 12 μM [8-^{14}C]ATP (30 mc/mmole); 0.2M sucrose; 3.2 mM $MgCl_2$; and 8 mM tris-HCl (pH 8.3). Portions were placed on Whatman 3MM filter paper disks, washed three times in 5 percent trichloroacetic acid–1 percent sodium pyrophosphate, two times in ether, and dried; the radioactivity was determined by liquid scintillation counting.

Condition	Radioactivity in RNA (count/min per mg DNA)	Stimulation (%)
Control	2097	
Cyclic AMP	3077	47
Control	1768	
Cyclic AMP	2444	38
Control	1857	
Cyclic AMP	2288	23
Control*	698	
Cyclic AMP*	1128	62
Control*	807	
Cyclic AMP*	1362	69
Control*	1467	
Cyclic AMP*	2183	49
Control†	2912	
Cyclic AMP†	3950	36
Control†	1937	
Cyclic AMP†	2606	35
Control*†	2270	
Cyclic AMP*†	2870	26

* Adrenalectomized rats. † Incubation was performed in the presence of a saturating amount of *E. coli* RNA polymerase.

creased capacity for RNA synthesis as compared with controls. The results from nine experiments are summarized in Table 1, where it can be seen that the degree of stimulation ranged from 23 to 69 percent in different experiments. Similar results are obtained when the experiments are performed in adrenalectomized rats, an indication that we are not dealing with a secondary effect caused by the release of adrenal steroids. We have also considered the possibility that the observed stimulation is due to a release of insulin induced by cyclic AMP (*1*), which in turn causes the increase in RNA synthesis (*8*). However, unlike the results in our system, the effect of insulin on RNA synthesis cannot be observed in the absence of added *Escherichia coli* RNA polymerase (*8*); thus it appears unlikely that we are dealing with a secondary effect of insulin.

Comparison of the kinetics of RNA synthesis in nuclei from control animals and animals treated with cyclic AMP indicates that the stimulation is apparent within the first minute of incubation (Fig. 1); thus the differences we observe are probably not artifacts generated during prolonged incubation of the nuclei. The effect on RNA synthesis is highly specific for cyclic AMP, because treatment of animals with the closely related nucleotide, adenosine 5′-monophosphate (5′-AMP), causes no detectable change in RNA synthesis (Fig. 1).

In order to determine whether the observed stimulation was due primarily to an increase in the activity of RNA polymerase or to an increase in the template activity of DNA, experiments were performed in which the nuclei were incubated in the presence of saturating amounts of exogenous *E. coli* RNA polymerase (Table 1). Nuclei from cyclic AMP–treated animals retained their increased capacity for RNA synthesis, so that we are dealing with a possible increase in template acitvity. However, in view of the obvious differences in mammalian and bacterial polymerases, one must be cautious in interpreting such findings.

Our results indicate that under conditions where cyclic AMP is known to induce formation of specific hormone-inducible enzymes in rat liver a dramatic increase in the capacity for nuclear RNA synthesis occurs. Although this finding is consistent with the proposed role of histone phosphorylation induced by cyclic AMP in altering gene transcription, one must be cautious in interpreting such findings. First of all, the observed increase of roughly 25 to 50 percent in RNA synthesis is much greater than would be needed or expected during the induction of a small number of enzymes. Furthermore, there is reason to believe that many of the effects of cyclic AMP on the induction of enzyme synthesis occur at levels other than transcription (*1, 4*).

Nonetheless, our experiments do demonstrate that cyclic AMP administration has dramatic effects on the capacity for RNA synthesis in rat liver nuclei, and our results are consistent with the findings that cyclic AMP can alter gene transcription in bacterial systems (*1*). Thus the effect of cyclic AMP on transcriptional activity in higher organisms needs to be considered and its significance must be determined before we have a complete understanding of the role of this nucleotide in the induction of enzyme synthesis.

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Thyrocalcitonin: Stimulation of Secretion by Pentagastrin

Abstract. *Administration of a small dose of pentagastrin, a synthetic pentapeptide containing the biologically active portion of the native hormone gastrin, results in a marked, rapid, transitory increase in thyrocalcitonin secretion in the pig. Gastrin or a related gastrointestinal peptide may be important in the physiological secretion of thyrocalcitonin, such as that which occurs when calcium salts are introduced into the gastrointestinal tract.*

It is well established that secretion of the hypocalcemic, hypophosphatemic hormone, thyrocalcitonin, can be regulated directly by the blood calcium concentration and that secretion of this hormone by the mammalian thyroid gland in response to a calcium load may afford protection against hypercalcemia (*1*). The original evidence for these conclusions involved nonphysiological procedures unrepresentative of situations encountered in normal life, for example, injections of high doses of calcium salts, parathyroid hormone, or vitamin D. However, Gray and Munson (*2*) have demonstrated a protective action of the rat thyroid gland against hypercalcemia after oral administration of small doses of calcium salts; thyroid-intact rats remained normocalcemic because of increased secretion of thyrocalcitonin, while thyroidectomized rats rapidly developed hypercalcemia. Subsequently, direct measure-