sults with added DB cyclic AMP, isoproterenol—a potent stimulator of endogenous production of cyclic AMP in both S and R cells (*7*)—caused an increase in S cell phosphodiesterase activity (sixfold at 4 hours), but no change in R cells (Fig. 2A). The increased phosphodiesterase activity in S cells was preceded by an initial prompt rise in cyclic AMP content (Fig. 2B). Both the rise in cyclic AMP and the later increase in phosphodiesterase were prevented by propranolol, a β-adrenergic antagonist (Fig. 2). Cycloheximide (0.2 m*M*) also completely prevented the isoproterenol-induced increase in S cell phosphodiesterase, suggesting that endogenously generated cyclic AMP, like exogenous DB cyclic AMP, acts by inducing increased synthesis of the enzyme (Fig. 2A).

We have suggested (*7*) that the decreased accumulation of cyclic AMP produced by isoproterenol in S cells, as compared with the R population, is due to the greater capacity of S cells to degrade cyclic AMP via the phosphodiesterase. In our experiment, the time course of cyclic AMP accumulation after exposure to isoproterenol (Fig. 2B) suggests, in addition, that the induction of phosphodiesterase is in part responsible for a fall in S cell cyclic AMP, which followed an early peak. Thus, at 2.5 hours, when phosphodiesterase activity had increased more than fourfold, the cyclic AMP content had decreased to near baseline. Cycloheximide, which blocked the increase in phosphodiesterase, also prevented the fall in cyclic AMP in S cells, so that even at 4 hours, cyclic AMP was still elevated.

In contrast, cyclic AMP in R cells exposed to isoproterenol (Fig. 2B) rose to a much higher initial level, and fell more slowly. The subsequent fall of R cell cyclic AMP was not affected by cycloheximide, presumably because induction of phosphodiesterase synthesis did not occur and therefore could not be inhibited.

In summary, our experiments suggest that the protein kinase system, including its cyclic AMP–binding regulatory subunit, is indeed involved in cyclic nucleotide–mediated enzyme induction in lymphoma cells. If the only role of the regulatory subunit is to control protein kinase activity, then it appears unlikely that cyclic AMP promotes enzyme induction in mammalian cells in a way comparable to that in bacteria. In bacteria, a cyclic AMP–binding protein regulates gene transcription, apparently without the involvement of a protein kinase (*9*).

In mammalian cells, indirect experiments in other systems suggest that cyclic AMP promotes enzyme induction primarily by stimulating specific posttranscriptional events (*3*). What these events are, however, is unknown. Although the present experiments do not define the locus of regulation, they show that protein phosphorylation may be involved. Cyclic nucleotide stimulation of the phosphorylation of both nuclear histones (*10*) and certain ribosomal proteins (*11*) suggests that either of these structures might be involved. More work is certainly necessary to answer this question.

Finally, induction of phosphodiesterase by endogenously generated cyclic AMP provides a mechanism by which a cell could modulate its response to continued or repeated hormonal stimulation of adenyl cyclase. Although similar increases in phosphodiesterase activity after hormonal stimulation have been observed in other cultured cells (*4*), we do not know whether this is a general phenomenon. Possibly, induction of phosphodiesterase synthesis could serve as an effective protective mechanism for cells which, like those studied here, are killed by cyclic AMP.

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Zinc: A Trace Element Essential in Vitamin A Metabolism

Abstract. *Zinc is necessary to maintain normal concentrations of vitamin A in plasma. By using animals deficient in both zinc and vitamin A, it was demonstrated that zinc is necessary for normal mobilization of vitamin A from the liver. These results suggest that cases of depressed vitamin A in plasma, unresponsive to vitamin A therapy, may respond to zinc supplementation.*

Forty years ago it was reported that certain cirrhotic patients with impaired dark adaptation did not improve after vitamin A therapy, which suggests that another factor may be involved in vitamin A metabolism (*1*). It is probable that those patients had insufficient metabolizable zinc, since patients suffering from cirrhosis have been shown to have depressed concentrations of zinc in plasma (*2*). Zinc-deficient swine have been reported to have low concentrations of vitamin A in serum, and treatment with massive oral doses of vitamin A was of no apparent benefit (*3*). More recently, zinc supplementation was necessary for maximum efficacy of vitamin A therapy to lambs deficient in both zinc and vitamin A (*4*). We have also noted, after studying 61 Baltimore, Maryland, children, 5 years of age, that plasma zinc and vitamin A were positively correlated ($P < .05$). The results of the above investigations suggest an interrelationship between vitamin A and zinc.

In animal studies designed to elucidate such a possible interrelationship, two groups of 15 conventional weanling rats each were employed. One

group was fed a diet inadequate in zinc [4 parts per million (ppm)], whereas the other group was fed the same diet supplemented with adequate zinc (20 ppm). Both diets contained the identical concentration of vitamin A—690 μg of vitamin A acetate per 100 g of diet. The animals were fed these diets for 104 days, and the results of these studies are shown in Table 1. The data demonstrate that although the concentration of vitamin A in the liver was similar in animals fed either adequate or inadequate quantities of dietary zinc, the plasma vitamin A concentration was significantly lower in the zinc-deficient animals. These findings suggest that in the animals deficient in zinc, vitamin A was not being adequately mobilized from the liver into the plasma. Therefore, experiments were designed to investigate the effects of zinc upon the mobilization of liver vitamin A. Germ-free rats were used because the axenic state allows zinc- and vitamin A–deficient animals to live longer than conventional animals maintained in a microbial-laden environment (*5, 6*). The animals became deficient in both zinc and vitamin A after being fed a double deficient diet for 28 days (*7*). During this period, the concentration of liver vitamin A dropped from 1.65 ± 0.32 [mean $\pm$ standard deviation (S.D.)] to 0.86 ± 0.08 μg/g (wet) and the plasma from 21 ± 2.3 to 5.5 ± 0.3 μg/100 ml. The animals exhibited signs and symptoms of both zinc and vitamin A deficiency. They were then divided into two groups of eight animals each. One group was given an oral dose of vitamin A acetate (80 μg three times per week) for 14 days, while the second group received the vitamin A plus zinc for the same period. Zinc was administered intraperitoneally as zinc glycinate, 200 μg of zinc given three times per week. Plasma, liver, and dietary vitamin A were determined fluorometrically by a method similar to that of Thompson (*8*). The results are depicted in Fig. 1. After 14 days, the liver vitamin A was significantly higher (52 ± 9.5 μg/g) in the group fed vitamin A without zinc in comparison with those receiving both vitamin A and zinc [9.0 ± 3.7 μg/g ($P < .001$)]. In contrast, the concentration of plasma vitamin A was lower (15.0 ± 1.50 μg/100 ml) for the rats receiving vitamin A without zinc in comparison with those receiving intraperitoneal injections of zinc in addition to the oral vitamin A [33.0 ± 2.40 μg/100 ml ($P < .001$)]

Table 1. The effect of dietary zinc on the concentration of liver and plasma vitamin A in rats. Plasma vitamin A was determined on eight animals from each dietary group; liver vitamin A was determined on ten animals from each group. N.S., not significant.

Diet	Vitamin A	
	Liver (μg/g)	Plasma (μg/100 ml)
Zinc sufficient (20 ppm of Zn)	130 ± 14*	39 ± 10*
Zinc inadequate (4 ppm of Zn)	110 ± 47	20 ± 2.8
Level of significance	N.S.	$P < .001$

* Mean $\pm$ S.D.

(*9*). These results demonstrate that without sufficient dietary zinc, vitamin A was accumulating in the liver and was not being mobilized sufficiently to maintain normal concentrations of plasma vitamin A.

To further investigate the essentiality of zinc for normal mobilization of liver vitamin A, 12 weanling germ-free rats were fed the diet deficient in both zinc and vitamin A for a total of 56 days. During the first 28 days no supplementations of either zinc or vitamin A were given. Then from day 28 to day 42 of the experiment oral vitamin A, but no zinc, was given, allowing vitamin A to accumulate in the liver. (Liver vitamin A accumulated to 35 ± 0.75 μg/g but plasma vitamin A remained low, 17 ± 0.58 μg/100 ml; six animals were used for these baseline values.) Following this period of accumulation of vitamin A in the liver, the oral vitamin A supplementation was discontinued and zinc therapy was initiated and continued for 14 days (day 42 to day 56 of the experiment). At the end of the zinc therapy, the remaining six rats were killed, and it was determined that liver vitamin A had decreased significantly ($P < .001$) to 3.16 ± 0.12 μg/g. However, plasma vitamin A had concomitantly increased to 35 ± 1.5 μg/100 ml. These findings further demonstrate that zinc therapy resulted in vitamin A being mobilized from the liver into plasma and suggests that zinc is necessary for maintaining normal concentrations of plasma vitamin A.

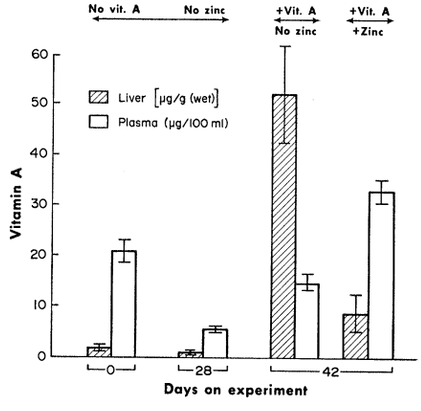

Fig. 1. The graph shows that zinc supplementation resulted in mobilization of liver vitamin A which was accompanied by an increase of plasma vitamin A to within the normal range. The animals were prepared by depleting vitamin A and zinc for 28 days. They were then killed on day 42 of the experiment, which was 14 days after treatment with either vitamin A only or vitamin A plus zinc.

Further studies are needed to elucidate the role of zinc in vitamin A metabolism. There is also the possibility that zinc may be involved in the synthesis or function of retinol–binding protein (*10*).

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