Alkaline Phosphatase. POSSIBLE INDUCTION BY CYCLIC AMP AFTER CHOLERA ENTEROTOXIN ADMINISTRATION

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Research Article

The present studies were undertaken to determine the role, if any, of cyclic 3′,5′-adenosine monophosphate (cyclic AMP) as a chemical inducer of rat liver alkaline phosphatase. Cholera enterotoxin, given intravenously to rats, led to a rapid rise in the activity of hepatic adenyl cyclase that was 7½ times greater than control values in 6 h. Cyclic AMP levels were also significantly increased above control values while the activity of cyclic nucleotide phosphodiesterase was unchanged. Hepatic alkaline phosphatase activity was increased 5½ times above control in 12 h, but its rise followed that of adenyl cyclase and cyclic AMP by several hours. Cycloheximide inhibited the rise of hepatic alkaline phosphatase but not that of adenyl cyclase. The administration of glucagon, a known stimulator of hepatic adenyl cyclase, and of dibutyryl cyclic AMP, led to similar striking increases in hepatic alkaline phosphatase activity. This alkaline phosphatase increase was blocked by the prior administration of cycloheximide. Bile duct ligation, a known stimulator of hepatic alkaline phosphatase activity, failed to produce any significant changes in adenyl cyclase or cyclic AMP. Concomitant treatment of rats with bile duct ligation and cholera enterotoxin or bile duct ligation and glucagon, had no additive effect on the increase in hepatic alkaline phosphatase activity, although the increase occurred earlier. These results suggest that: (*a*) cyclic AMP may act as […]

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CHOLERA ENTEROTOXIN ADMINISTRATION

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A b s t r a c t   The present studies were undertaken to determine the role, if any, of cyclic 3',5'-adenosine monophosphate (cyclic AMP) as a chemical inducer of rat liver alkaline phosphatase. Cholera enterotoxin, given intravenously to rats, led to a rapid rise in the activity of hepatic adenyl cyclase that was $7\frac{1}{2}$ times greater than control values in 6 h. Cyclic AMP levels were also significantly increased above control values while the activity of cyclic nucleotide phosphodiesterase was unchanged. Hepatic alkaline phosphatase activity was increased $5\frac{1}{2}$ times above control in 12 h, but its rise followed that of adenyl cyclase and cyclic AMP by several hours. Cycloheximide inhibited the rise of hepatic alkaline phosphatase but not that of adenyl cyclase. The administration of glucagon, a known stimulator of hepatic adenyl cyclase, and of dibutyryl cyclic AMP, led to similar striking increases in hepatic alkaline phosphatase activity. This alkaline phosphatase increase was blocked by the prior administration of cycloheximide. Bile duct ligation, a known stimulator of hepatic alkaline phosphatase activity, failed to produce any significant changes in adenyl cyclase or cyclic AMP. Concomitant treatment of rats with bile duct ligation and cholera enterotoxin or bile duct ligation and glucagon, had no additive effect on the increase in hepatic alkaline phosphatase activity, although the increase occurred earlier. These results suggest that: (*a*) cyclic AMP may act as an inducer of hepatic alkaline phosphatase; (*b*) the stimulation of hepatic alkaline phosphatase by cholera enterotoxin is mediated by cyclic AMP; (*c*) the rise in hepatic alkaline phosphatase following bile duct ligation is not mediated by cyclic AMP; (*d*) the same alkaline phosphatase in rat liver may be induced by two (or more) mechanisms, only one of which requires cyclic AMP.

INTRODUCTION

We have previously shown that elevation of the serum alkaline phosphatase following bile duct ligation in rats results from an increase in the activity of hepatic alkaline phosphatase with subsequent leakage of this enzyme into the blood (1). This increase in hepatic alkaline phosphatase activity is dependent upon *de novo* RNA and protein synthesis (1, 2). Although enzyme induction is a likely explanation for the increase in alkaline phosphatase activity in the obstructed liver, the precise mechanism by which bile duct ligation increases this enzyme activity remains uncertain.

Graybill, Kaplan, and Pierce (3) demonstrated that the intravenous injection of purified cholera enterotoxin into dogs produced an elevation of serum levels of alkaline phosphatase of hepatic origin. Recently, the results of several studies have strongly suggested that cholera enterotoxin produces its effects in both intestinal and nonintestinal sites by altering tissue levels of cyclic 3',5'-adenosine monophosphate (cyclic AMP) (4–9). Thus, Kimberg, Field, Johnson, Henderson, and Gershon (4) and others (5, 6) have shown that the enterotoxin is capable of increasing intestinal mucosal adenyl cyclase activity, and it is now clear that this nucleotide is related to the striking enterotoxin-induced alterations in fluid and electrolyte secretion and the associated fulminant diarrhea (7). Studies concerned with cholera enterotoxin-related enhancement of lipolysis in fat cells (8) and of glycogenolysis in platelets and in liver (9)

have suggested that these extraintestinal effects of the toxin are likewise mediated by increased tissue levels of cyclic AMP.

Several workers have implicated cyclic AMP as an inducer of certain enzymes in mammalian liver as well as in bacterial systems (10–21). In view of the previously demonstrated effects of cholera enterotoxin on both cyclic AMP metabolism and hepatic alkaline phosphatase activity, the present studies were undertaken to explore the role, if any, of cyclic AMP as a chemical inducer of rat liver alkaline phosphatase. The results demonstrate that the intravenous administration of cholera enterotoxin in the rat is indeed followed by a striking rise in hepatic adenyl cyclase activity and a subsequent increase in the activity of rat liver alkaline phosphatase. Furthermore, the results suggest that while cyclic AMP *may* induce hepatic alkaline phosphatase under certain circumstances, this mechanism is probably not operative following bile duct ligation in the rat.

METHODS

Animals. Male Charles River CD strain rats, weighing approximately 100 g were used for all studies (Charles River Breeding Laboratories, Inc., Wilmington, Mass.). Animals were housed in hanging wire cages and received standard rat chow and tap water ad libitum for several days preceding the studies. Bile duct ligation and sham operations were performed under light ether anesthesia. When ligated, the common bile duct was tied as close to the liver as possible to avoid damage to the pancreatic ducts. Animals were sacrificed by concussion followed by spinal dislocation.

Chemicals and drugs. Purified cholera enterotoxin[1] dissolved in sterile water was given intravenously into the tail vein. Animals received either 15 μg of the enterotoxin in a volume of 0.5 ml or a similar volume of isotonic saline (controls). Crystalline glucagon was obtained from Eli Lilly & Co., Indianapolis, Ind., was dissolved in the manufacturer's diluent[2] to a concentration of 1.0 mg/ml, and was administered subcutaneously in order to maximize its effect on hepatic cyclic AMP levels (22). N^6-2′O-dibutyryl-cyclic 3′,5′-adenosine monophosphate (dibutyryl cyclic AMP) and cycloheximide were purchased from the Sigma Chemical Co., S. Louis, Mo.; the dibutyryl cyclic AMP was diluted in sterile distilled water to a concentration of 2.0 mg/ml, and was administered intraperitoneally. Cycloheximide was diluted in normal saline to a concentration of 1.0 mg/ml and was administered intramuscularly.

Determinations. In order to determine cyclic AMP levels, samples of liver with a wet weight of approximately 100 mg were removed from the animals immediately after sacrifice. The samples were immediately frozen with liquid nitrogen and pulverized. After the liquid nitrogen had evaporated, cyclic AMP was extracted with 5.0 ml of ice-cold 5% trichloroacetic acid containing ^{3}H-cyclic AMP as a

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[1] Purified cholera toxin, prepared according to the procedure described in *J. Infect. Dis.* 121 (Suppl.) : S63, 1970, and under contract for the National Institute of Allergy and Infectious Diseases by Dr. R. A. Finkelstein, The University of Texas Southwestern Medical School, Dallas, Tex.

[2] Glycerin 1.6% with 0.2% phenol.

recovery marker. After centrifugation, HCl was added to the supernates to a concentration of 0.1 N and the trichloroacetic acid was extracted with diethyl ether. Supernates were then evaporated to dryness at 50°C using an Evapo-Mix evaporator (Buchler Instruments Div., Nuclear Chicago Corp., Fort Lee, N. J.). The residues were redissolved in 50 mM acetate buffer, pH 4.0, and cyclic AMP levels were then measured by the protein kinase binding assay described by Gilman (23). Results were expressed as picomoles cyclic AMP per milligram protein. Protein was determined by the method of Lowry, Rosebrough, Farr, and Randall (24) on the trichloroacetic acid precipitates dissolved in 1 N NaOH. Triplicate samples were obtained from each liver, and duplicate determinations of cyclic AMP levels were performed on each sample.

For determinations of adenyl cyclase and alkaline phosphatase activities, the remaining liver was rapidly removed, chilled, and minced. 1 g of minced liver was homogenized with 50 vol of 0.25 M sucrose containing 0.02 M glycylglycine buffer, pH 7.8, and 0.001 M $MgSO_4$, in a Potter-Elvehjem homogenizer fitted with a Teflon pestle. The homogenate was sedimented at 2,000 g for 10 minutes at 0–4°C in a Sorvall RC2 centrifuge (Ivan Sorvall, Inc., Newtown, Conn.). The pellet was resuspended in 10 vol of 0.02 M glycylglycine buffer, pH 7.8, containing 0.001 M $MgSO_4$, and resedimented as described above. This washing procedure was performed a total of four times. The final pellet was resuspended with a glass rod in sufficient glycylglycine-$MgSO_4$ to yield a final protein concentration of between 6.0 and 15.0 mg/ml determined as described above. Assays for adenyl cyclase activity were performed by a previously described modification (25) of the method of Krishna, Weiss, and Brodie (26). The activity of cyclic nucleotide phosphodiesterase was measured by a method described previously (25).

The remaining liver, about 2 g, was used for measurement of alkaline phosphatase. It was stirred magnetically for 30 min in three changes of 0.25 M sucrose in a beaker and homogenized in 10 vol of the same solution in a Waring blender. Then 2% sodium deoxycholate was added to a final concentration of 0.5%, and the mixture was stirred for 20 min. Duplicate determinations of alkaline phosphatase activity were then made with *p*-nitrophenyl phosphate at 30°C and pH 10.2 in a Gilford thermostatted spectrophotometer (Gilford Instrument Laboratories, Inc., Oberlin, Ohio) (27). Assays mixtures contained in 1 ml : 600 μmol 2-amino-2-methyl-1 propanol, pH 10.2, 4 μmol *p*-nitrophenylphosphate, 0.2 μmol $MgCl_2$, and 0.001–0.01 International Units of alkaline phosphatase.

RESULTS

Temporal relation between administration of cholera enterotoxin and changes in hepatic enzyme activity. Intravenous administration of cholera enterotoxin, 15 μg/ 100 g body weight, led to a rise in hepatic adenyl cyclase activity and a more delayed increase in hepatic alkaline phosphatase activity (Fig. 1). Adenyl cyclase activity was significantly elevated 1 h after injection ($P < 0.01$) and by 6 h it had reached a peak at 7½ times control values. Alkaline phosphatase activity began to rise 3 h after enterotoxin administration, but did not become significantly elevated until 6 h ($P < 0.01$); at 12 h values reached a maximum of 5½ times control. Both adenyl
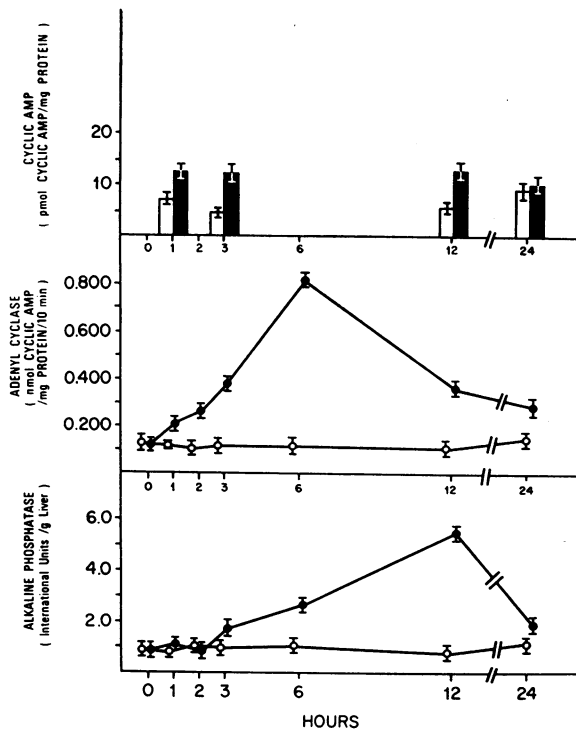
FIGURE 1 Temporal relationships between administration of cholera enterotoxin and elevations in hepatic adenyl cyclase, cyclic AMP, and alkaline phosphatase. Groups of four control and four test animals were sacrificed at 0, 2, 3, 6, 12, and 24 h after intravenous administration of cholera enterotoxin, 15 μg per animal, or of normal saline in a similar volume; 10 control and 10 test animals were sacrificed at 1 h. (● or ■ = test animals, ○ or □ = control animals, ⲓ = mean±1 SEM.)

cyclase and alkaline phosphatase activities began to decline after reaching maximal elevations, but neither had returned to control values by 24 h after injection. Cyclic AMP levels, measured at 1, 3, 12, and 24 h after injection of cholera enterotoxin, showed significant elevation over control values by 3 h ($P < 0.01$) and had returned to control levels at 24 h (Fig. 1).

The amount of cyclic AMP present in the liver can be influenced not only by its rate of synthesis but also by its rate of destruction. Assays for cyclic nucleotide phosphodiesterase activity were conducted as described in Methods with liver cell membranes prepared from control animals as well as those injected with cholera enterotoxin 6 h prior to sacrifice. Control values were 0.038 and those from the treated animals were 0.030 μmol of cyclic AMP destroyed per milligram protein. Differences in the activity of phosphodiesterase were not responsible for the increased levels of cyclic AMP in the liver after the administration of cholera enterotoxin.

Relation between protein synthesis and cholera enterotoxin-related changes in adenyl cyclase and alkaline phosphatase activities. Previous studies have shown that

the rise in hepatic alkaline phosphatase activity following bile duct ligation depends on intact protein synthesis by the liver (1, 2). On the other hand, the rise in intestinal mucosal adenyl cyclase activity and cyclic AMP levels after exposure to cholera enterotoxin is independent of new protein formation (28). To determine whether the elevations in hepatic adenyl cyclase and alkaline phosphatase activities following enterotoxin administration depend on intact protein synthesis, enterotoxin-treated animals were studied with and without cycloheximide pretreatment. Fig. 2 shows that cycloheximide pretreatment, in doses that inhibit hepatic protein synthesis by 80% (29), inhibited the rise in alkaline phosphatase but did not affect the enterotoxin-related elevation of adenyl cyclase activity.

Relation between hepatic cyclic AMP concentration and elevation of hepatic alkaline phosphatase. The preceding results suggested that the increase in hepatic cyclic AMP concentration following the administration of cholera enterotoxin might be causally related to the subsequent elevation of hepatic alkaline phosphatase activity. In order to examine this relationship more closely, studies concerned with the effects of glucagon and dibutyryl cyclic AMP on hepatic alkaline phosphatase activity were undertaken. Glucagon in a dose of 0.5 mg was administered subcutaneously on two occasions 5 min apart and animals were sacrificed 10, 20, and 60, 360, and 720 min after the second injection. Alkaline phosphatase activity was unchanged at the early times, but as shown in Fig. 3, it was 2½ times control values at 6 h ($P < 0.01$). At 10 and 20 min after glucagon administration, there was a marked elevation of hepatic cyclic AMP levels ($P < 0.005$ vs. controls, Fig. 4). The cyclic AMP
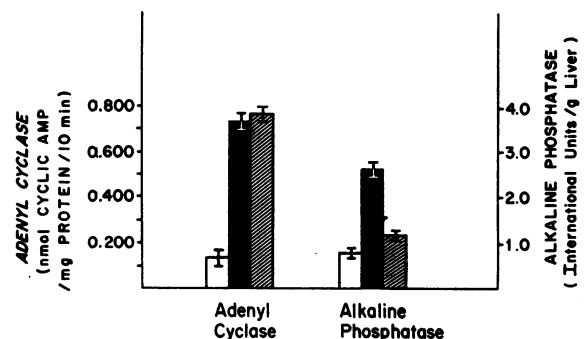

FIGURE 2 Effect of cycloheximide on the cholera enterotoxin induced rise in adenyl cyclase and alkaline phosphatase. There were four animals in each experimental group. One group received normal saline (control), one group cholera enterotoxin, 15 μg i.v. (cholera) and one group cycloheximide 300 μg i.m. 40 min before the administration of cholera enterotoxin. All animals were sacrificed 6 h after receiving normal saline or cholera enterotoxin. (■ = cholera treated animals, □ = control animals, ▧ = cholera and cycloheximide-treated animals, ⲓ = mean±SEM.)
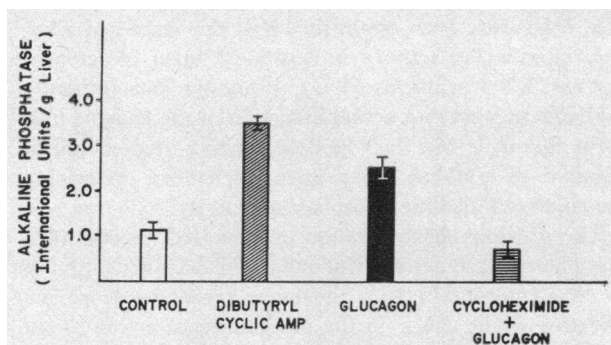
FIGURE 3 Effect of cycloheximide pretreatment on gluca-gon induced rise in alkaline phosphatase activity and effect of dibutyryl cyclic AMP administration on alkaline phos-phatase activity. Six test animals received glucagon twice (5 min apart) subcutaneously in a dosage of 0.5 mg (0.5 ml), and six control animals received normal saline 0.5 ml subcutaneously at identical times. Cycloheximide, 300 μg intramuscularly was given to another group of 6 rats 40 min before glucagon. All animals were sacrificed 6 h after the second glucagon injection. (I = mean±SEM.) Di-butyryl cyclic AMP in a dose of 2 mg (1.0 ml) was ad-ministered five times at hourly intervals intraperitoneally to five rats. Three control animals received 1.0 normal saline five times at hourly intervals by the same route. Ani-mals were sacrificed 6 h after the first dose. (I = mean ±1 SEM.)
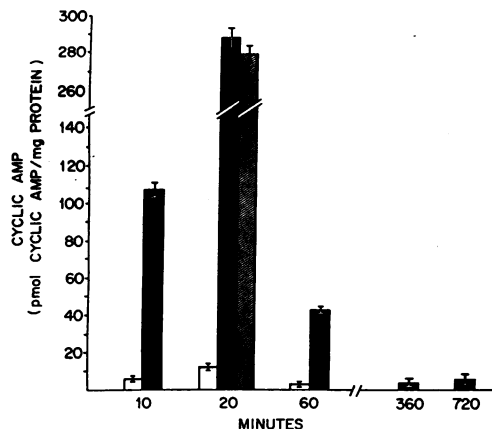

FIGURE 4 Effect of glucagon and cycloheximide plus glu-cagon on hepatic cyclic AMP. One group of nine animals were treated with normal saline, and another group of 24 animals with glucagon as described in Fig. 3. Three animals from each group were sacrificed at 10 min, 20 min, and 60 min after treatment, and 6 animals at 360 and 720 min. In addition three glucagon-treated animals each received cyclo-heximide 300 μg intramuscularly 40 min before glucagon administration and were sacrificed 20 min after glucagon administration. (□ = control animals, ■ = glucagon-treated animals, ▨ = glucagon and cycloheximide-treated animals, I =mean±1 SEM.)

elevation was less striking at 60 min and had returned to control values at 6 and 12 h after glucagon. As shown in Figs. 3 and 4, cycloheximide pretreatment prevented the rise in alkaline phosphatase activity, but did not inhibit the glucagon-induced elevation of cyclic AMP levels. Dibutyryl cyclic AMP administration produced a marked elevation in hepatic alkaline phosphatase activity ($P <$ 0.005, Fig. 3). These findings strengthened the postulated relationship between hepatic cyclic AMP concentration and the rise in hepatic alkaline phosphatase activity.

Effect of bile duct ligation on cyclic AMP levels and adenyl cyclase activity. The results already presented suggested that the cholera enterotoxin-induced elevation in hepatic alkaline phosphatase activity may be mediated by cyclic AMP. Studies were therefore undertaken to ex-plore the role, if any, of this nucleotide in the bile duct ligation-related increase in alkaline phosphatase activity. As shown in Table I, bile duct ligation failed to produce significant changes in either hepatic adenyl cyclase ac-tivity or cyclic AMP levels at 2, 60, 120, 180, or 480 min after operation. Despite the lack of change in adenyl cy-clase activity, bile duct ligation was followed by an ele-vation of alkaline phosphatase activity after an interval similar to that noted in the studies with cholera entero-toxin administration (1, 2).

Effect of bile duct ligation plus cholera enterotoxin or glucagon administration on alkaline phosphatase ac-tivity. One possible explanation for the apparent in-

volvement of cyclic AMP in the cholera enterotoxin-re-lated increase in alkaline phosphatase and the lack of in-volvement of this nucleotide in the elevation of enzyme activity following bile duct ligation could be that two different alkaline phosphatase systems in the liver are involved, one induced by cyclic AMP and the other by some different mechanism following bile duct ligation. If indeed two different alkaline phosphatases are in-volved, one might expect that the combination of cholera

TABLE I

Mean Adenyl Cyclase Activities and Mean Cyclic AMP Levels after Bile Duct Ligation of Sham Operation

	Adenyl cyclase		Cyclic AMP	
Time	Sham operated	Bile duct ligated	Sham operated	Bile duct ligated
min	*nmol cyclic AMP/mg protein/10 min*		*pmol cyclic AMP/mg protein*	
20	0.125±0.033	0.103±0.023	6.4±1.1	5.9±0.8
60	0.105±0.024	0.122±0.008	7.0±1.6	6.3±1.0
120	0.107±0.018	0.105±0.013	6.5±0.5	6.7±0.6
180	—	0.116±0.007	—	—
480	0.115*	0.125±0.010	—	7.4±0.5

Six bile duct-ligated animals and 6 sham-operated controls were sacrificed at each time point. Values represent mean ±SEM.
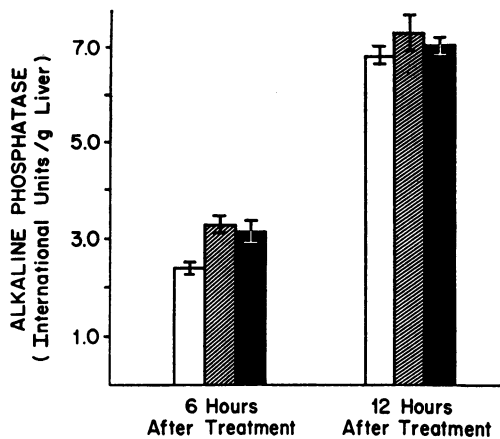* Mean for two animals.

Figure 5 Absence of additive effect of bile duct ligation and cholera enterotoxin or glucagon administration on alkaline phosphatase after bile duct ligation. 30 animals underwent bile duct ligation. 10 received simultaneous cholera enterotoxin, 10 received glucagon, and 10 served as controls. Five animals were sacrificed from each group at 6 and 12 h. ($\square$ = bile duct ligation alone, ▨ = bile duct ligation plus cholera toxin administration, ■ = bile duct ligation plus glucagon administration, ⊺ = mean±SEM.)

enterotoxin or glucagon administration and bile duct ligation in the same animal would lead to additive effects on hepatic alkaline phosphatase activity. In order to test this possibility, cholera enterotoxin and glucagon were administered to two groups of 10 rats just prior to bile duct ligation. 10 animals were subjected to bile duct ligation alone in order to serve as controls. Five animals in each group were sacrificed 6 and 12 h after ligation. Fig. 5 shows that cholera enterotoxin and glucagon failed to produce an additive effect with bile duct ligation at 12 h ($P < 0.05$), a time at which the peak elevation of alkaline phosphatase usually occurs. There was, however, a statistically significant increase in alkaline phosphatase activity at 6 h in the bile duct-ligated animals who also received either cholera enterotoxin or glucagon. These data suggest that cholera enterotoxin and glucagon might increase the rate of rise in hepatic alkaline phosphatase activity following bile duct ligation, but they do not provide evidence in support of the concept that there might be two different hepatic alkaline phosphatases. Alternatively, one must consider the possibility that the same hepatic alkaline phosphatase activity may be induced by two (or more) mechanisms, only one of which requires hepatic cyclic AMP.

DISCUSSION

Previous studies established that both bile duct ligation (1, 2) and the administration of cholera enterotoxin (3) are capable of increasing the activity of hepatic alkaline phosphatase activity. In the case of bile duct liga-

tion, it has also been established that the increased alkaline phosphatase activity is dependent upon intact protein and RNA synthesis (1, 2). While the data presently available suggest that actual induction of the enzyme may occur following bile duct ligation, further studies will be required to establish the precise mechanism underlying the enhanced alkaline phosphatase activity.

The previous demonstration of enhanced hepatic alkaline phosphatase activity following the administration of cholera enterotoxin (3), the more recent evidence implicating cyclic AMP in the mechanism of action of the enterotoxin (4–9), and the known effects of hepatic cyclic AMP as a chemical inducer of other hepatic (10–17) and bacterial (18–21) enzymes, suggested that this nucleotide may be a mediator involved in increasing hepatic alkaline phosphatase activity. Indeed, the present studies have demonstrated directly that the administration of cholera enterotoxin to rats is followed by an increase in hepatic adenyl cyclase activity with a rise in hepatic cyclic AMP levels, confirming the recent report by Gorman and Bitensky (30). Furthermore, the present investigations have established that in addition to cholera enterotoxin, the administration of glucagon in doses sufficient to lead to a striking elevation in hepatic cyclic AMP levels also causes a marked increase in rat liver alkaline phosphatase activity. The administration of dibutyryl cyclic AMP is likewise accompanied by a subsequent rise in hepatic alkaline phosphatase. The studies with both glucagon and cholera enterotoxin demonstrate that increased intracellular levels of cyclic AMP precede the rise in alkaline phosphatase activity. In addition, with both agents the prior administration of cycloheximide, an inhibitor of protein synthesis, prevents the rise in alkaline phosphatase activity without influencing the effects of either the enterotoxin or glucagon on cyclic AMP metabolism. Cycloheximide administered in doses sufficient to cause substantial inhibition of protein synthesis failed to influence the effect of the enterotoxin on the adenyl cyclase system in the present studies as well as in others involving small intestinal mucosa (28). If indeed enhanced protein synthesis is required for the effects of the enterotoxin to become manifest, there is now good reason to believe that increased synthesis of adenyl cyclase per se is not involved.

While the results of the present studies suggest that cyclic AMP may mediate a rise in alkaline phosphatase activity, extensive efforts to define a possible role for this nucleotide as part of the mechanism responsible for the enzyme rise following bile duct ligation led to negative results. The present investigations do not suggest that cyclic AMP and bile duct ligation influence different alkaline phosphatases in the liver, but rather they do suggest that there may be multiple mechanisms responsible for modulating this enzyme activity.

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One other aspect of the present study deserves additional comment: namely that the results presented do not prove that cyclic AMP levels in the liver cell itself are directly related to the observed effects on hepatic alkaline phosphatase. One must consider the possibility that the hepatic alkaline phosphatase rise may be secondary to the effect of elevated cyclic AMP levels in peripheral tissues. Thus, for example, one could speculate that hepatic alkaline phosphatase levels may respond to an increased rate of delivery of fatty acids to the liver following enhanced cyclic AMP-related peripheral lipolysis. In addition to their effects on hepatic adenyl cyclase, cholera enterotoxin, glucagon, and dibutyryl cyclic AMP have been shown to influence lipolysis in fat cells. Indeed, recent studies by Pekarthy, Short, Lansing, and Lieberman (31) demonstrated that the administration of a diverse group of agents, some of which are capable of mobilizing peripheral fatty acids and others which lead to an increase in choline and phosphatidylcholine excretion in bile, is associated with a rise in hepatic alkaline phosphatase activity. Pekarthy and his associates (31) suggested that the function of alkaline phosphatase was to hydrolyze phosphorylcholine so that choline could be transported across the bile canalicular membrane. They furthermore suggested that the level of enzyme activity was controlled by the quantity of phosphorylcholine available for biliary excretion. At the present time one must remain somewhat reserved about this hypothesis, for recent studies in this laboratory[3] have demonstrated that an increase in hepatic alkaline phosphatase activity is not always associated with an increased excretion of phosphatidyl choline in bile. We have found that there is a marked fall in total bile phospholipid excretion at a time when alkaline phosphatase activity is increased following the administration of cholera enterotoxin. Whether the effects of agents modifying hepatic alkaline phosphatase activity noted in this study as well as in that of Pekarthy et al. (31) are exerted directly on the cyclic AMP metabolic scheme in the hepatocyte or are secondary to peripheral influences of these agents must remain unresolved at the present time. Studies with isolated perfused liver or an organ culture system would be helpful in this regard. Finally, our data, suggestive as they may be, do not establish that the observed rises in alkaline phosphatase activity are necessarily due to enzyme inductions rather than to activation of a preformed enzyme.

ACKNOWLEDGMENTS

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