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Induction of Hepatic Enzyme Synthesis *in Vivo* by Adenosine 3′,5′-Monophosphate*

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SUMMARY

The N^6,$O^{2'}$-dibutyryl analogue of cyclic adenosine 3′,5′-monophosphate (dibutyryl cyclic AMP) elevates the level of hepatic tyrosine-α-ketoglutarate transaminase (L-tyrosine: 2-oxoglutarate aminotransferase, EC 2.6.1.5) some 3- to 6-fold in intact and adrenalectomized rats. The response of this enzyme is more rapid to dibutyryl cyclic AMP than to any other inducer of the transaminase. Hourly injection of dibutyryl cyclic AMP can maintain the elevated transaminase level for at least 5 hours. The rate of synthesis of tyrosine transaminase, as measured by an isotopic-immunochemical procedure, is enhanced by treatment with dibutyryl cyclic AMP. There is no detectable effect of dibutyryl cyclic AMP on the synthesis of total soluble proteins, however. Combinations of dibutyryl cyclic AMP and hydrocortisone produce additive or, in some cases, synergistic increases in transaminase activity, but the responses to combinations of dibutyryl cyclic AMP and either glucagon or insulin are not additive. Two other soluble liver enzymes, P-enolpyruvate carboxykinase (GTP:oxaloacetate carboxy-lyase transphosphorylating, EC 4.1.1.32) and serine dehydrase (L-serine hydro-lyase, EC 4.2.1.13) are also elevated by treatment of intact rats with either dibutyryl cyclic AMP or glucagon, but another, tryptophan pyrrolase (L-tryptophan:oxygen oxidoreductase, EC 1.13.1.12, tryptophan oxygenase), is not affected by either agent. Hydrocortisone induces both the transaminase and pyrrolase 3- to 5-fold but the steroid has no effect on either the carboxykinase or the dehydrase. The results support the view that cyclic AMP is the intracellular mediator of the action of glucagon on hepatic enzyme synthesis.

Glucagon promotes a transient induction[1] of tyrosine transaminase (L-tyrosine:2-oxoglutarate aminotransferase, EC 2.6.1.5) both *in vivo* (1) and in the perfused liver (2). Despite repeated administration of the hormone, the induced enzyme activity falls rapidly to the basal level within 2 to 4 hours, and the elevated rate of transaminase synthesis persists for only 30 to 120 min after the initial injection (3). These changes in enzyme level are, thus, completely understandable in terms of alteration in the rate of synthesis of enzyme protein. Glucagon elevates the intracellular level of cyclic AMP[2] in liver (4), and recently we have found that cyclic AMP, as well as glucagon, is capable of inducing tyrosine transaminase in fetal rat liver maintained in organ culture (5–7). We have now extended these earlier studies by examining the ability of cyclic AMP to modify enzyme levels *in vivo* in adult rat liver. Our results show that the N^6,$O^{2'}$-dibutyryl analogue of cyclic AMP is an effective inducer of tyrosine transaminase *in vivo*. Furthermore, the cyclic nucleotide enhances the activities of two other soluble hepatic enzymes, P-enolpyruvate carboxykinase (GTP:oxaloacetate carboxy-lyase transphosphorylating, EC 4.1.1.32) and serine dehydrase (L-serine hydro-lyase, EC 4.2.1.13), but does not affect tryptophan pyrrolase (L-tryptophan:oxygen oxidoreductase, EC 1.13.1.12, tryptophan oxygenase). Hydrocortisone induces both the transaminase and pyrrolase but has no effect on the other two enzymes in intact rats fed a protein-free diet.

EXPERIMENTAL PROCEDURE

Materials—Male rats from the Charles River Breeding Laboratories were used throughout these experiments. Glucagon and insulin were obtained from Sigma. Dibutyryl cyclic AMP was obtained from Calbiochem and from Schwarz BioResearch. Both preparations had essentially the same effectiveness on a weight basis. Theophylline was from Mann. The protein-free diet was obtained from General Biochemicals. The antiserum to tyrosine transaminase and the partially purified transaminase used as carrier in the immunochemical experiments were prepared as described previously (8).

Treatment—Rats were adrenalectomized 1 to 2 days before use and maintained on a normal diet and 1% NaCl as drinking water. The adrenalectomized rats were fasted overnight before experiments began. Intact rats were maintained on a protein-free diet for 5 days and fed *ad libitum* throughout this period. All injected materials were given intraperitoneally as solutions in 0.15 M NaCl.

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[1] The term induction is used to describe the selective stimulation of the rate of enzyme synthesis without suggesting any mechanism.

[2] The abbreviations used are: cyclic AMP, cyclic adenosine 3′,5′-monophosphate; dibutyryl cyclic AMP, N^6,$O^{2'}$-dibutyryl cyclic adenosine 3′,5′-monophosphate.

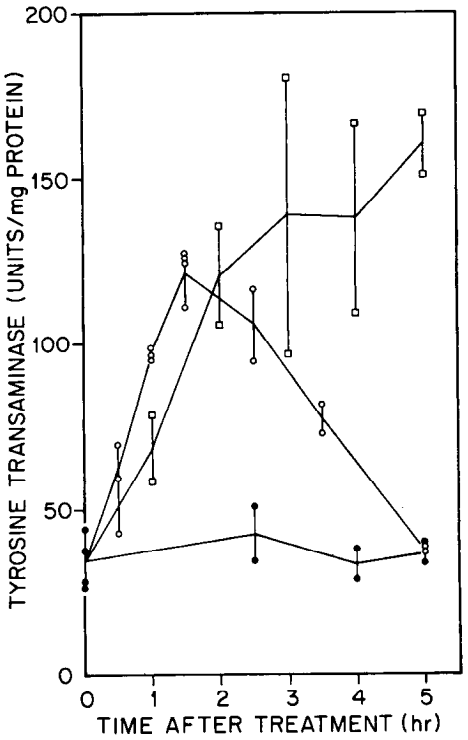

FIG. 1. Induction of tyrosine transaminase in rat liver by single or repeated treatments with dibutyryl cyclic AMP. Adrenalectomized rats weighing 200 to 300 g were fasted overnight before the experiments began. Each point represents a single determination. ●, untreated controls; ○, rats given 5 mg of dibutyryl cyclic AMP in a single treatment at zero time; □, rats given 2 mg of dibutyryl cyclic AMP at zero time and again at each hour for the remaining time.

Analytical Procedures—Enzymes were assayed in supernatant fractions of 20% (w/v) homogenates prepared in 0.15 M KCl-0.001 M EDTA (pH 8) and centrifuged for 40 min at 105,000 × *g*. Tyrosine transaminase was assayed as described previously (9). The unit of activity is the amount required to form 1 μg of *p*-hydroxyphenylpyruvate in a 10-min assay. P-enolpyruvate carboxykinase was assayed by a modification (17) of the procedure of Ballard and Hanson (10). Serine dehydrase activity was determined by the spectrophotometric method (11). Tryptophan pyrrolase was assayed by the method of Knox, and Piras, and Tokuyama (12) which involves preliminary incubation with tryptophan, heme, and ascorbate to obtain maximum activity. Protein was measured by the biuret procedure (13). The isotopic-immunochemical technique for determining the rate of transaminase synthesis has been described previously (1, 8, 14, 15). Incorporation of ^{14}C-leucine into soluble protein was determined by pipetting samples onto filter paper discs and washing away nonprotein radioactivity by the method of Mans and Novelli (16).

RESULTS

The first indication that cyclic AMP might induce tyrosine transaminase *in vivo* came from experiments in which theophylline (2 mg at 0 and 3 hours) was injected along with repeated doses of glucagon (50 μg per hour). Under these conditions, the transaminase activity was maintained for 6 hours at the induced level (156 units per mg of protein compared to a basal level of 42 units per mg of protein). This shows that the previously described failure of repeated glucagon injections to maintain the elevated transaminase level (1) is not due to a temporary stimulation of some process involved in transaminase synthesis, but rather to a refractory earlier step in the response of the liver to glucagon. Since theophylline has been shown to inhibit the breakdown of cyclic AMP (17), we concluded that cyclic AMP was mediating the effects of glucagon on the transaminase.

Effect of Dibutyryl Cyclic AMP on Transaminase in Vivo—To test this possible function of cyclic AMP, we administered dibutyryl cyclic AMP to adrenalectomized rats. The results of two such experiments are shown in Fig. 1. The transaminase activity was markedly elevated by treatment with dibutyryl cyclic AMP. Following a single injection of dibutyryl cyclic AMP, the enzyme level was increased in as little as 30 min in some experiments and the response was maximal by 90 to 120 min. Beyond 2½ hours, the enzyme level fell at a rate approximating the known rate of degradation of this enzyme ($t_{\frac{1}{2}} \cong 90$ min) (18), indicating that induction had ceased completely by this time. The time course of induction with glucagon (1) is essentially identical with that shown in Fig. 1. The marked increase in transaminase activity at 30 to 60 min following treatment with dibutyryl cyclic AMP stands in marked contrast to the 90-min lag in the induction of the transaminase by hydrocortisone (19).

If a smaller dose of dibutyryl cyclic AMP is given at hourly intervals, induction of the transaminase persists for at least 5 hours with no evidence of a refractory period (Fig. 1). These results suggest that hourly treatment with 2 mg of dibutyryl cyclic AMP is sufficient to maintain an intrahepatic concentration of the cyclic nucleotide capable of sustaining an elevated steady state level of transaminase for at least 5 hours. About 4 to 6 mg/100 g, body weight, is the dose of dibutyryl cyclic AMP required for a maximum response (~6-fold) of the transaminase 3 hours following a single injection (Fig. 2).

The maximum extent of induction with dibutyryl cyclic AMP is as high as that achieved with glucagon or insulin but less than that which follows induction by hydrocortisone (1–3, 7) (Table II). Theophylline alone prompts a 2- to 3-fold rise in the transaminase level in adrenalectomized rats which is consistent with the idea that intracellular cyclic AMP is a proximal inducer of the transaminase. This phenomenon has also been observed in isolated systems (5–7).

Immunochemical Analysis of Rate of Transaminase Synthesis following Dibutyryl Cyclic AMP Injection—The results in Table I illustrate the effects of dibutyryl cyclic AMP on the rate of synthesis of tyrosine transaminase as measured by an isotopic immunochemical procedure (1, 8, 14, 15). In the first experiment there was a 3-fold elevation in the activity of the transaminase and a comparable increase in the rate of enzyme synthesis as measured between 90 and 110 min after a single injection of dibutyryl cyclic AMP. This is in accord with the previous finding that glucagon enhances the rate of transaminase synthesis *in vivo* (1). That dibutyryl cyclic AMP did not affect the labeling of total soluble protein demonstrates the selectivity of the response. In a second experiment, it was found that the rate of transaminase synthesis was elevated about 3-fold as early as 20 to 40 min, *i.e.* at a time when little if any change in enzyme activity could be detected. Essentially the same effect is seen at 140 to 160 min after dibutyryl cyclic AMP treatment when the activity of the transaminase was markedly elevated. These results are in agreement with those obtained with the other inducers of the transaminase, which show collectively that the

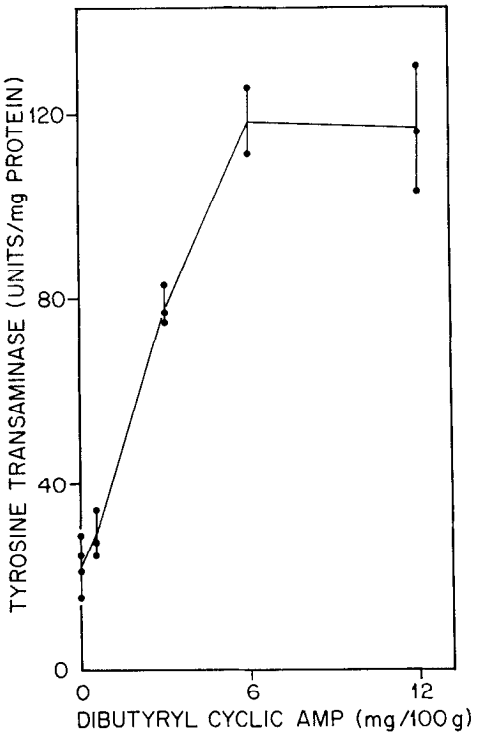

FIG. 2. Induction by various doses of dibutyryl cyclic AMP. Adrenalectomized rats weighing 130 to 145 g were fasted as described in the legend to Fig. 1. Each point represents a single determination made 3 hours after a single treatment with the amount of dibutyryl cyclic AMP indicated.

TABLE I

Immunochemical analysis of induction of tyrosine transaminase by dibutyryl cyclic AMP

In both experiments rats weighing about 200 g and adrenalectomized 1 to 2 days before were given dibutyryl cyclic AMP (6 mg/100 g) at zero time and ^{14}C-leucine (40 μCi, 273 mCi per mmole) 20 min before killing at the times listed following dibutyryl cyclic AMP. All analyses were on the 105,000 × *g* supernatant fractions. Radioactivity data are for 6-ml samples that were heated to 60° with α-ketoglutarate and pyridoxal phosphate before transaminase precipitation with antiserum. Carrier enzyme (5,188 units per mg of protein) was added to bring each fraction to 30,000 units for each of two precipitations. In Experiment 1, radioactivity of the second (control) precipitate averaged 33 cpm and was essentially constant. In Experiment 2, the carrier enzyme specific activity was 9,601 units per mg of protein and the radioactivity of the second precipitate averaged 16 cpm.

Duration of exposure to dibutyryl cyclic AMP[a]	Transaminase activity[b]	Radioactivity in		Relative radioactivity
		Transaminase (A)	Soluble proteins (B)	
	units/mg protein	*cpm*	*cpm* × 10^{-3}	$A/B \times 10^3$
Experiment 1				
None (2)...	41 ± 6	913 ± 184	288 ± 12	3.2 ± 0.6
110 min (3).	119 ± 14	2747 ± 594	297 ± 85	9.4 ± 1.6
Experiment 2				
None (3)...	45 ± 6	687 ± 48	365 ± 31	1.9 ± 0.1
40 min (3)..	49 ± 3	1496 ± 124	282 ± 13	5.3 ± 0.3
160 min (3).	186 ± 7	2129 ± 224	399 ± 28	5.5 ± 1.0

[a] The number of animals is given in parentheses.

[b] Data are the mean ±S.E.

TABLE II

Response of tyrosine transaminase to combinations of dibutyryl cyclic AMP and other inducing hormones

Rats weighing about 200 g were adrenalectomized and fasted as described in the legend to Fig. 1. Treatments were per 100 g of body weight: insulin, 1 unit (43 μg); hydrocortisone, 2.5 mg; dibutyryl cyclic AMP, 4 or 5 mg; glucagon, 150 μg. In Experiments 2 and 3, those given dibutyryl cyclic AMP were also given 2 mg of theophylline and those given insulin were treated with 1 ml of 10% glucose as needed to prevent hypoglycemic shock. Assays were made 2½ hours after treatment in Experiments 1 and 2; in Experiment 3, assays were made 3.3 hours after hydrocortisone and 1.8 hours after all other treatments.

Treatment	Tyrosine transaminase[a]		
	Experiment 1	Experiment 2	Experiment 3
	units/mg protein		
None.....	43 ± 3 (4)	20 ± 2 (6)	37 ± 5 (3)
Glucagon.....	163 ± 10 (4)		
Insulin.....		115 ± 9 (6)	74 ± 14 (3)
Hydrocortisone.		131 ± 1 (2)	197 ± 12 (3)
Dibutyryl cyclic AMP.....	154 ± 7 (4)	95 ± 9 (6)	119 ± 7 (3)
Glucagon + dibutyryl cyclic AMP.....	153 ± 10 (4)		
Insulin + dibutyryl cyclic AMP.....		125 ± 12 (6)	88 ± 7 (3)
Hydrocortisone + dibutyryl cyclic AMP...		194 ± 17 (3)	265 ± 3 (3)

[a] Data are mean ±S.E.; number of animals is given in parentheses.

rate of enzyme synthesis is elevated prior to any demonstrable increase in enzyme activity and without any significant enhancement of total soluble protein synthesis (1, 3). Thus, it appears that all the changes in enzyme level can be attributed to the effects of dibutyryl cyclic AMP on the rate of transaminase synthesis.

Response of Transaminase to Combinations of Inducers—We examined the question of whether or not the cyclic nucleotide yielded additive increments in the level of the transaminase when tested in combination with the various hormonal inducers of this enzyme (Table II). In the first two experiments, enzyme activity was measured 2½ hours after injection of each inducer. Glucagon and dibutyryl cyclic AMP elicited the same degree of elevation of transaminase activity, and the combination of the two produced no greater response than either one alone. In the second experiment, the response to dibutyryl cyclic AMP and hydrocortisone was additive, suggesting that they affect different steps in the synthesis of the transaminase (3, 5–7). In some cases, a considerably greater than additive response to these two inducers can be seen (5–7) (Table III). The combination of insulin and dibutyryl cyclic AMP was no more effective than either one alone. Similar results, obtained in the organ culture system, suggest that insulin induces the transaminase independently of cyclic AMP and, at the same time, blocks the action of the cyclic nucleotide (7). This interpretation is strengthened by the observation that insulin markedly inhibits the elevation

TABLE III

Effects of dibutyryl cyclic AMP and hormones on various soluble hepatic enzymes

Intact rats weighing about 200 g were placed on a protein-free diet for 5 days. Treatments were: hydrocortisone, 2.5 mg/100 g; dibutyryl cyclic AMP, 6 mg/100 g; 5′-AMP, 6 mg/100 g; glucagon, 150 μg/100 g; theophylline, 2 mg/100 g. Assays were performed 4 hours after single injections of the various agents on 105,000 × *g* supernatant fractions.

Treatment	No. of animals	Tyrosine transaminase[a]	P-enolpyruvate carboxykinase	Serine dehydrase	Tryptophan pyrrolase
		units/mg protein			
None	14	31 ± 3[a]	11 ± 1	4.2 ± 0.2	12 ± 1
Hydrocortisone	8	97 ± 22[b]	17 ± 3	5.0 ± 0.5	69 ± 7[b]
Dibutyryl cyclic AMP	9	181 ± 30[b]	58 ± 6[b]	12.3 ± 1.4[b]	17 ± 2
5′-AMP	3	29 ± 3	11 ± 2	4.0 ± 0.5	17 ± 1
Glucagon + theophylline	9	217 ± 29[b]	61 ± 6[b]	12.3 ± 1.5[b]	17 ± 3
Hydrocortisone + dibutyryl cyclic AMP	6	344 ± 52[b]	69 ± 8[b]	14.0 ± 2.6[b]	57 ± 9[b]
Hydrocortisone + glucagon + theophylline	6	284 ± 35[b]	65 ± 7[b]	11.5 ± 1.9[b]	46 ± 6[b]

[a] Data are the mean ±S.E.

[b] Significance of the difference from untreated controls, $p = <0.001$. Where not so indicated, the p values for other differences were 0.05 or greater.

by cyclic AMP of P-enolpyruvate carboxykinase activity in organ culture (7). Furthermore, Park (20) and Chambaut *et al.* (21) have also reported antagonism between insulin and cyclic AMP, and insulin is known to lower the level of cyclic AMP in liver (22).

In a third experiment (Table II), hydrocortisone was injected 90 min before the other inducers to compensate for the pronounced lag observed in response to the steroid (19). The results are essentially identical with those in the previous experiment.

Effect of Dibutyryl Cyclic AMP on Other Soluble Hepatic Enzymes—The lack of effect of dibutyryl cyclic AMP on total protein synthesis suggested that the response of liver protein synthesis must be quite selective (7). To see if any other hepatic enzymes, especially those reported to be elevated by glucagon or diabetes, responded to the cyclic nucleotide, we studied the following soluble liver enzymes: P-enolpyruvate carboxykinase, serine dehydrase, and tryptophan pyrrolase. P-enolpyruvate carboxykinase and serine dehydrase were chosen because both are known to be elevated by glucagon, diabetes, and fasting, and depressed by feeding on a protein-free diet (23, 24). In addition, P-enolpyruvate carboxykinase has recently been found to be inducible in fetal liver by cyclic AMP *in utero* (25) and in organ culture (7). Tryptophan pyrrolase was studied because this enzyme does not appear to respond to glucagon (26) or to doses of insulin that are fully capable of inducing tyrosine transaminase.[3] Intact rats fed a protein-free diet for 5 days were used in these experiments to depress the activities of the carboxykinase and serine dehydrase and, thereby, to enhance the response to glucagon (23, 24). Tryptophan pyrrolase served as an internal control in that any effects of glucagon or dibutyryl cyclic AMP which might prompt glucocorticoid release could be monitored by increases in the level of this enzyme.

Hydrocortisone led to 3- to 5-fold increases in the activities of tyrosine transaminase and tryptophan pyrrolase, but had little effect on the carboxykinase or serine dehydrase (Table III). In contrast, dibutyryl cyclic AMP prompted 6-fold increases in the activities of the transaminase and carboxykinase and a 3-fold increase in serine dehydrase but had no significant effect on tryptophan pyrrolase. Glucagon and theophylline produced essentially the same results as dibutyryl cyclic AMP with all four enzymes. These results show that the effects of glucagon on three hepatic enymes can now be ascribed to cyclic AMP, and they are in agreement with recent work *in utero* (25), in the organ culture system (7), and, the work of Jost, Hsie, and Rickenberg (27) on serine dehydrase *in vivo*.

AMP injected in amounts comparable to dibutyryl cyclic AMP had no significant effect on any of these four enzymes (Table III). Combinations of the steroid with either dibutyryl cyclic AMP or glucagon (plus theophylline) revealed an additive or synergistic effect on the transaminase, but only the response of the principal inducer was seen in the case of the other three enzymes. Indeed, dibutyryl cyclic AMP and glucagon had slight inhibitory effects on the steroid induction of tryptophan pyrrolase.

Although the results were quite variable, in some cases, the synthetic catecholamine isoproterenol, in the presence of theophylline, also induced the transaminase, carboxykinase, and dehydrase as effectively as dibutyryl cyclic AMP without having any significant effect on tryptophan pyrrolase. Isoproterenol, which stimulates hepatic adenyl cyclase, also induces the transaminase and carboxykinase in the organ culture system (7).

DISCUSSION

The results presented in this paper support the conclusion that cyclic AMP is the intrahepatic mediator of the action of glucagon on enzyme synthesis *in vivo*. Thus, three enzymes known to be similarly influenced either by glucagon or by conditions (diabetes, fasting) which elevate the concentration of cyclic AMP in liver (28) have been shown to respond to the direct administration of the cyclic nucleotide. Under these conditions, no change in tryptophan pyrrolase occurred, demonstrating that glucocorticoid secretion was not involved in the observed responses.

The time course of induction of the transaminase with dibutyryl cyclic AMP is analogous with that with glucagon (1) and more rapid than with the glucocorticoids (19). The maximum response to the cyclic nucleotide occurred at the time when

[3] D. Holten and F. T. Kenney, unpublished observations.

the response to corticoids is just beginning. These results and the synergistic response of the transaminase to a combination of steroid and dibutyryl cyclic AMP suggest that the site of action of cyclic AMP may be closer to the site of protein synthesis than that of the steroid. Recent work in our laboratory indicates that glucocorticoids act by augmenting the level of functional messenger RNA, whereas cyclic AMP exerts its effect on a post-transcriptional process.[4] The synergistic response of the transaminase to both inducers can, thus, be readily understood in terms of a sequential modification of enzyme synthesis by these two agents.

The observation that dibutyryl cyclic AMP causes an elevation in the rate of transaminase synthesis is in agreement with studies in the organ culture system (7) and confirms the previous findings that glucagon also enhances the rate of transaminase synthesis *in vivo* (1). In virtually all other respects, the results *in vivo* are also analogous with those in the isolated system (7). These results demonstrate that cyclic AMP can act to promote enzyme synthesis in addition to its ability to activate pre-existing enzyme protein (29). Immunochemical analyses will be required to determine whether or not the response of the carboxykinase and dehydrase involve synthesis *de novo*.

The fact that tryptophan pyrrolase does not respond to dibutyryl cyclic AMP treatment demonstrates the selectivity of the hepatic response to the cyclic nucleotide. The lack of significant elevation of total soluble protein synthesis by dibutyryl cyclic AMP is also consistent with a selective response. These results are not surprising in view of the fact that only a limited number of hepatic enzymes is known to be influenced by glucagon (30).

The effects of fasting, insulin, glucagon, and diabetes on the carboxykinase (23) and serine dehydrase (24) can now be reasonably explained by changes in the intrahepatic level of cyclic AMP. Presumably the synthesis of other enzymes which respond in a similar or opposite manner to these environmental manipulations is also regulated by cyclic AMP in a positive or negative manner; *e.g.* the increase in fumarase (31) and ATP·AMP phosphotransferase (32) caused by fasting and diabetes probably reflects positive elevation of enzyme synthesis by cyclic AMP. In contrast, the inhibitory effects of glucagon on insulin-mediated elevation of hepatic glucokinase apparently result from a negative influence of the cyclic nucleotide (33). The precise basis for the insulin-cyclic AMP antagonism remains obscure for the present, but there is evidence that it may represent actual competition and not just an effect of insulin on cyclic AMP levels (7, 20, 21, 34).

The lack of effect of hydrocortisone on serine dehydrase in rats on a protein-free diet has been observed previously (35). The marked elevation in transaminase and tryptophan pyrrolase levels *in vivo* following the same treatment and the lack of effect of glucocorticoids on the carboxykinase in the organ culture system suggest that adrenal steroids are not direct inducers of the carboxykinase and serine dehydrase. Under certain conditions, these two enzymes do respond to corticoids, but the response is not as great as with glucagon which does elevate the carboxykinase in an isolated system (7). Thus, cyclic AMP appears to be the primary inducer of the carboxykinase and serine dehydrase. The suggestion has been made that glucocorticoids "sensitize" the systems involved in the response of these enzymes to the principal regulator (36). Although this suggestion could explain much of the data, no glucocorticoids are required to obtain a response in the isolated systems (7). The exact nature of the role played by glucocorticoids in the regulation of enzymes such as the carboxykinase and serine dehydrase remains to be established by further work.

[4] K.-L. Lee and F. T. Kenney, manuscript in preparation.

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