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Studies on the Mechanisms Underlying Adaptive Changes in Rat Liver Phosphoenolpyruvate Carboxykinase*

David O. Foster, Paul D. Ray,† and Henry A. Lardy

ABSTRACT: The regulation of hepatic phosphoenolpyruvate carboxykinase activity *in vivo* was studied by measuring the activity of the enzyme in the soluble fraction of livers obtained from rats subjected to various alterations in their hormonal or metabolic status. Puromycin and actinomycin D were employed in some experiments to inhibit, respectively, protein and ribonucleic acid synthesis. The data indicate that the tissue activity of the enzyme is regulated not only by changes in the rate of biosynthesis of the enzyme, but also in one or more other ways which may involve activation processes or changes in the rate of degradation of the enzyme. Treatment of rats with hydrocortisone or D-mannoheptulose apparently results in an enhanced synthesis of carboxykinase. The administration of glucose to fasted rats depressed carboxykinase activity by 28% in 4 hr, whereas administration of puromycin caused a 9% decrease in activity in the same period. When both puromycin and glucose were given, activity fell by only 10%. These data were interpreted as signifying that protein synthesis is required for expression of the depressive action of glucose on the enzyme. Neither adrenal cortical hormone nor insulin appear to have a direct function in the regulation of carboxykinase.

The adaptation of many of the enzymic activities of mammalian tissues in response to alterations in the endocrine, nutritional, or environmental status of animals has been intensively studied in recent years (Freedland and Harper, 1957; Hechter and Halkerston, 1965; Potter and Ono, 1961; Smith and Haijer, 1962; Weber, 1963). In no case, however, has the precise mechanism of control been established. The problem is very complex, for changes in the tissue activity of an enzyme may occur in a number of ways. Segal *et al.* (1965) have listed several possibilities. They are: "changes in the rate of biosynthesis of the enzyme, changes in the degradation rate of the enzyme, changes in the level of activators or inhibitors, direct interaction of the hormone and the enzyme, interconversion of active and

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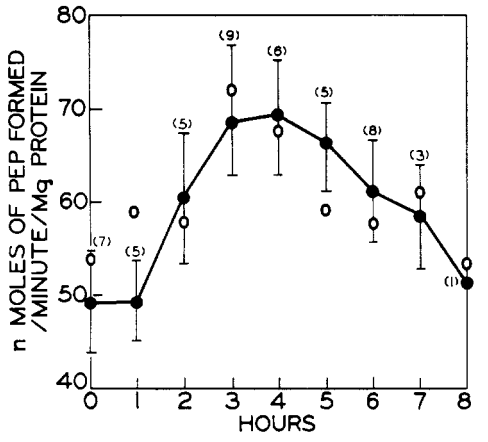

FIGURE 1: Hepatic PEP carboxykinase activity of fed adrenalectomized rats after a single 5 mg/kg dose of hydrocortisone (-●—●-) or triamcinolone acetonide (O). The number of animals contributing to each point for the data obtained with hydrocortisone is shown above the vertical bars which represent standard deviations.

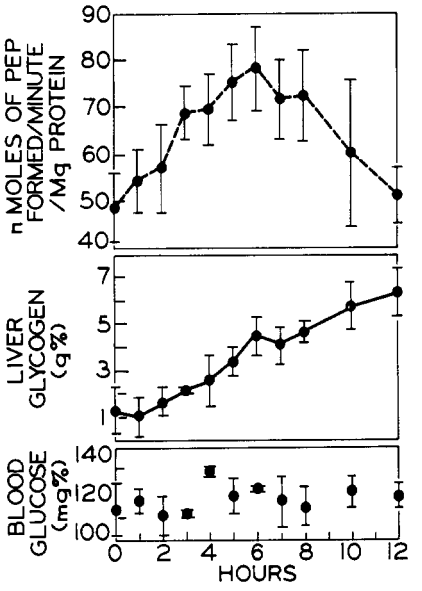

FIGURE 2: Changes in hepatic PEP carboxykinase activity, liver glycogen, and blood glucose after administration of a 25-mg/kg dose of hydrocortisone to fed adrenalectomized rats. Each point represents data from 3 to 5 animals and the vertical bars give the standard deviations.

inactive forms, formation of altered molecular types of intrinsically greater or lesser activity, and changes in the structural components of the cell in which the enzymes reside."

Recent reports from this laboratory (Shrago *et al.*, 1963; Lardy *et al.*, 1964; Young *et al.*, 1964) have shown that the phosphoenolpyruvate (PEP)[1] carboxykinase activity of rat liver is altered by various hormonal and dietary treatments. This communication presents some additional observations on the mode of regulation of PEP carboxykinase. The data indicate that alterations in the activity of the enzyme may be brought about in several different ways.

Experimental Procedure

Normal and adrenalectomized rats weighing from 180 to 200 g were obtained from the Badger Research Corp., Madison, Wis. Unless otherwise stated, all animals were males. Bilaterally adrenalectomized rats were used 3–10 days after surgery. Except for periods of fasting, the animals were fed *ad libitum* with a commercial (Rockland) diet and water, or 1% sodium chloride if adrenalectomized. Alloxan diabetes was produced by intravenous injection of 10 mg of alloxan monohydrate (Distillation Products) per 200-g rat. Animals were used 8–10 days after injection of alloxan if their blood glucose concentrations exceeded 450 mg %.

The following compounds were generous gifts from the indicated donors: hydrocortisone, Drs. K. Folkers and M. Tishler of Merck and Co.; actinomycin D, Dr. N. Bohonos, Lederle Laboratories; D-mannoheptulose, Dr. Nelson Richtmeyer, National Institutes of Health, Bethesda, Md. Mannoheptulose was also prepared in our laboratory by Mr. Verner Paetkau.

PEP carboxykinase was assayed in the supernatant fraction of rat liver homogenized in cold 0.25 M sucrose and centrifuged at 105,000*g* for 1 hr. The assay is based on the measurement of the PEP formed during incubation of the enzyme with saturating levels of oxalacetate and inosine triphosphate (ITP). Conditions were as previously described by Nordlie and Lardy (1963) except that KCl and sucrose were omitted from the incubation medium. PEP was cleaved with mercuric ion (Lehmann and Meyerhof, 1934) and the liberated inorganic phosphate was estimated by the method of Sumner (1944).

Blood glucose was assayed by the glucose oxidase method (Glucostat, Worthington Biochemical Corp.). Blood samples were collected after decapitation of the rats. Glycogen, isolated by the method of Good *et al.* (1933), was measured by the anthrone procedure (Seifter *et al.*, 1950). The protein in the supernatant fraction of liver was determined by the biuret method (Layne, 1957). After the addition of biuret reagent, protein samples were extracted with ether to remove lipid materials which cause turbidity. All chemicals employed were reagent grade and solutions were prepared in deionized glass-distilled water.

Actinomycin D, 875 μg/kg, was injected intraperitoneally in 0.9% NaCl. Puromycin dihydrochloride (Nutritional Biochemicals Corp.) was dissolved in

[1] Abbreviations used in this work: PEP, phosphoenolypyruvate; ITP, inosine triphosphate.

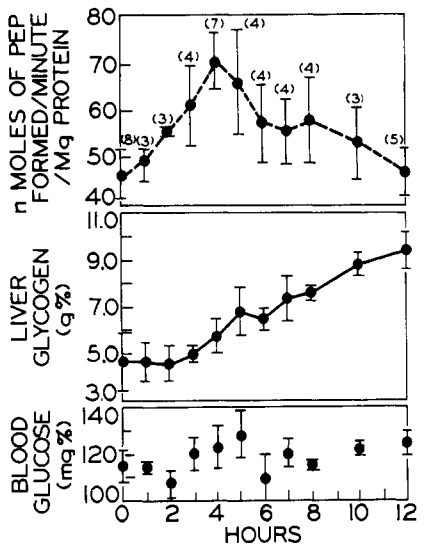

FIGURE 3: Changes in hepatic PEP carboxykinase activity, liver glycogen, and blood glucose of fed intact rats after treatment with hydrocortisone in doses of 25 mg/kg. The number of animals contributing to each point is given above the vertical bars which represent standard deviations.

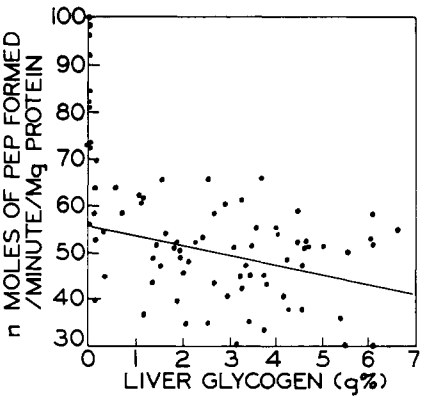

FIGURE 4: Regression of PEP carboxykinase activity on liver glycogen. The data were obtained from adrenalectomized rats killed at intervals from 0 to 12 hr after initiation of fasting. Glycogen values less than 0.01 g % were not used in calculating the regression line.

water, adjusted to pH 6.5 with sodium hydroxide, and injected intraperitoneally at hourly intervals in doses of 87 mg/kg. Glucose, 5g/kg, was administered in 3–4 ml of aqueous solution by stomach tube. Hydrocortisone, suspended in 0.9% NaCl, was given subcutaneously at a dose of 5 or 25 mg/kg. Aqueous solutions of D-mannoheptulose, 3 g/kg, were injected subcutaneously. Control animals were given injections of the appropriate vehicle.

Results

Table I summarizes the influence of fasting and of adrenal hormone or insulin deficiency on hepatic PEP carboxykinase activity. Fasting caused a 2-fold elevation of activity in both intact and adrenalectomized rats. The absence of the adrenal gland did not alter carboxykinase activity in the fed animal. In chronic diabetes produced by alloxan, the enzyme was elevated more than 4-fold over the level found in the normal rat. Adrenalectomy of the alloxan diabetic rat reduced the enzyme by 46% within 3 days after the surgery. D-Mannoheptulose produces an acute diabetic-like state in the rat by inhibiting the release of insulin by the pancreas (Coore *et al.*, 1963). PEP carboxykinase was elevated to the same extent in the intact as in the adrenalectomized rat by the administration of mannoheptulose.

The effect on PEP carboxykinase of the administration of a single dose of 5 mg of hydrocortisone/kg to adrenalectomized rats is illustrated in Figure 1. For comparison, data are also presented for single rats given triamcinolone acetonide. Enzyme activity increased rapidly, reached a maximum in 3–4 hr, and then declined to the normal level by about 8 hr. Since there was the possibility that the above dose of hydrocortisone was insufficient to cause maximal elevation of the enzyme, the experiment was repeated with a dose of 25 mg/kg. The results, which are shown in Figure 2, were not greatly dissimilar to those obtained with the smaller dose. PEP carboxykinase activity peaked at a slightly higher level and at about 5–6 hr rather than at 3–4 hr as

TABLE I: The Effects of Starvation and Hormonal Deficiencies on the Activity of PEP Carboxykinase in Rat Liver.

Treatment (no. of animals)	PEP Carboxykinase Activity ± Std Dev (nmoles of PEP formed/min per mg of protein)
Normal (25)	48.1 ± 6.6
+ Mannoheptulose[a] (10)	70.5 ± 6.7
Fasted for 12 hr (15)	96.4 ± 8.1
Fasted for 24 hr (11)	96.2 ± 6.1
Adrenalectomized (36)	48.8 ± 7.4
+ Mannoheptulose[a] (10)	74.6 ± 13.0
Adrenalectomized; fasted for 12 hr (11)	97.4 ± 6.3
Alloxan diabetic (5)	218 ± 34
Alloxan diabetic; adrenalectomized[b] (7)	118 ± 19

[a] D-Mannoheptulose was given 4 hr before death.
[b] Animals were killed 3 days after bilateral adrenalectomy.

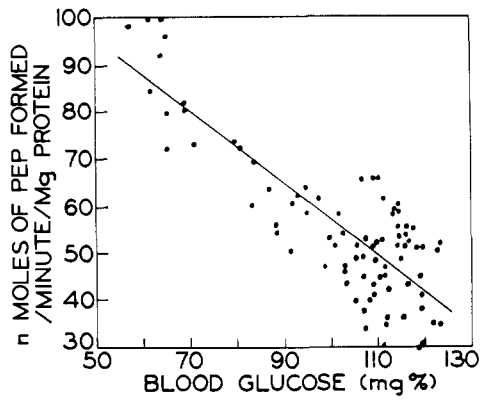

FIGURE 5: Regression of PEP carboxykinase on blood glucose concentration. The data were obtained from the same animals as the data of Figure 4.

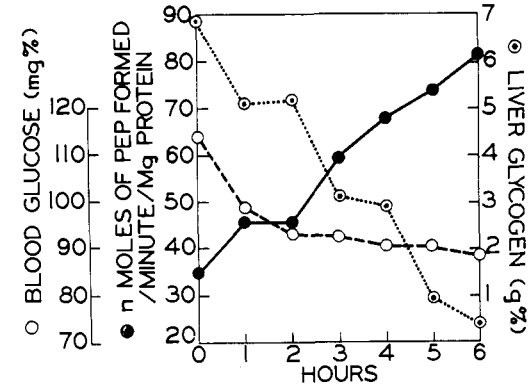

FIGURE 6: The effect of phloridzin at doses of 500 mg/kg (at zero time) on the carbohydrate levels and PEP carboxykinase activity of intact rats. Each point represents data obtained from 1 or 2 animals.

occurred with the lower dose of hydrocortisone. By about 12 hr activity had returned to normal. While the enzyme responded in a biphasic manner, hepatic glycogen increased at a nearly constant rate. Blood glucose concentration showed no significant changes. Postmortem, visual examination of the site of injection revealed the presence of considerable steroid remaining subcutaneously 12 hr after treatment. Figure 3 shows the changes in carboxykinase, glycogen, and blood glucose of intact rats given a single 25 mg/kg injection of hydrocortisone. With the exception that maximum enzyme activity was reached between 4 and 5 hr, the changes observed were essentially identical with those which occurred in the adrenalectomized animals.

The data presented in Figures 2 and 3 suggested that the failure of PEP carboxykinase to be maintained at the elevated level attained shortly after administration of the steroid might be due to the accumulation of large amounts of glycogen. An attempt was made to discover whether a relation existed between the concentration of hepatic glycogen and the activity of the enzyme. Adrenalectomized rats were fed Wayne Lab Blox[2] for 5 days before initiating the experiment. Food was then removed from the animals and groups of 4–6 rats were killed at hourly intervals from 0 to 12 hr. Hepatic glycogen concentration, PEP carboxykinase activity, and blood glucose concentration were determined for each animal. These data were used to construct the regression lines shown in Figures 4 and 5. No relation between carboxykinase activity and the concentration of hepatic glycogen was apparent from Figure 4 (correlation coefficient of -0.31). Figure 5 reveals, however, a distinct correlation (correlation coefficient of -0.81) between enzymic activity and blood glucose concentration.

To examine further the relation between blood glucose and PEP carboxykinase, renal loss of glucose was created in normal rats by administration of appropriate doses of phloridzin (Lotspeich and Woronkow, 1958). The results of a single experiment are shown in Figure 6. A linear rate of increase in carboxykinase resulted in a greater than 2-fold elevation of the enzyme in 6 hr. Blood glucose concentration fell rapidly during the first 2 hr after injection of phloridzin, but remained nearly constant for the next 4 hr, while the enzyme continued its increase. A rapid mobilization of hepatic glycogen apparently contributed to the maintenance of blood glucose concentrations of about 90 mg %.

The effect upon carboxykinase activity of glucose and hydrocortisone in combination was investigated (Table II). Glucose suppressed the enzyme in the fed, normal rat[3] as it does in the fasted animal (Young *et al.*, 1964). As expected, hydrocortisone elevated carboxykinase. The separate actions of glucose and steroid on enzymatic activity were antagonistic when both compounds were given. No antagonism of the formation of glycogen was apparent.

Shrago *et al.* (1963) found that PEP carboxykinase was elevated by the administration of lactate to normal rats. Table II also shows that glucose antagonizes this effect of lactate on the enzyme.

In an effort to establish whether changes in the rate of synthesis of PEP carboxykinase were involved in the alterations in tissue activity of the enzyme, two bioantagonists of protein synthesis, puromycin and actinomycin D, were used. Puromycin, in doses slightly smaller than used in the present work, has been shown by Gorski *et al.* (1961) to inhibit 85% of the *in vivo* incorporation of labeled glycine into rat liver proteins. Actinomycin D in doses of 50 and 100 μg/100-g mouse

[2] In order to study carboxykinase activity over a wide range of glycogen concentrations, a high initial concentration of glycogen was desired. Past experience indicated that our rats maintained higher concentrations of hepatic glycogen when fed Wayne Lab Blox rather than Rockland chow.

[3] A previous report that administered glucose did not affect PEP carboxykinase levels (Table I, Shrago *et al.*, 1963) was due to a calculating error. The original data show a suppression of 20% 4 hr after glucose administration.

TABLE II: Antagonistic Actions of (A) Glucose and Hydrocortisone or (B) Glucose and Lactate on the Hepatic PEP Carboxykinase of Fed Rats Treated for 4 Hours.

Expt	Treatment (no. of animals)	PEP Carboxykinase Activity ± Std Dev[a] (nmoles of PEP formed/min per mg of protein)	Blood Glucose (mg %)	Hepatic Glycogen (g %)
A	Control (3)	47.7 ± 5.0	116 ± 11	5.48 ± 1.09
	+ Hydrocortisone + glucose (3)	50.3 ± 5.8	142 ± 10	9.14 ± 2.04
	+ Hydrocortisone (3)	72.4 ± 5.5	126 ± 10	8.33 ± 2.41
	+ Glucose (3)	31.1 ± 1.6	136 ± 19	8.66 ± 0.65
B	Control (3)	47.3 ± 4.4	115 ± 3	4.73 ± 0.79
	+ Glucose + lactate[b] (3)	45.2 ± 4.0	145 ± 5	6.92 ± 0.77
	+ Glucose (3)	29.6 ± 6.4	126 ± 9	7.16 ± 0.62
	+ Lactate (3)	62.0 ± 3.2	137 ± 14	4.26 ± 1.19

[a] Values joined by a vertical line are not significantly different ($P > 0.05$); values not so joined are significantly different ($P < 0.01$). Significance of differences between sample means was tested by Duncan's New Multiple Range Test (Steel and Torrie, 1960). [b] Sodium lactate, 5 g/kg, was given by stomach tube.

blocked 85 and 100%, respectively, the incorporation of labeled orotic acid into messenger ribonucleic acid (m-RNA) and ribosomal RNA of the liver (Trakatellis *et al.*, 1964). Table III shows that the administration of actinomycin D to normal animals not only prevented the increase in activity caused by mannoheptulose, but also decreased the enzyme to a value significantly lower than that of the control rats.

TABLE III: Inhibition by Actinomycin D of the Elevation of Hepatic PEP Carboxykinase Measured 4 Hours after the Administration of D-Mannoheptulose to Fed Rats.

Treatment (no. of animals)	PEP Carboxykinase Activity ± Std Dev[a] (nmoles of PEP formed/min per mg of protein)
Control (9)	49.6 ± 7.3
+ Mannoheptulose (7)	72.0 ± 5.6
+ Actinomycin D (7)	39.9 ± 4.4
+ Actinomycin D + mannoheptulose (4)	37.2 ± 2.6

[a] See footnote *a*, Table II.

When actinomycin D was administered to adrenalectomized rats prior to and during a period of starvation for 12 hr, the usual 2-fold increase in carboxykinase due to fasting was prevented (Table IV), but activity did not fall below its initial level, *viz.*, that of fed animals. The inability of actinomycin D to depress carboxykinase during response of the animals to the stress of fasting contrasts with the rapid lowering of activity when actinomycin is administered to the fed animal (Table III). The effect of fasting on carboxykinase was partly suppressed by hydrocortisone. The ability of the latter to enhance gluconeogenesis even in animals given actinomycin D (Ray *et al.*, 1964) likely diminished the effects of starvation on blood sugar and liver glycogen. No decrease in PEP carboxykinase below that of fed animals occurred when both hydrocortisone and actinomycin D were given.

TABLE IV: Effects of Actinomycin D and Hydrocortisone on the Elevation of Hepatic PEP Carboxykinase Induced by Fasting Adrenalectomized Female Rats for 12 Hours.

Treatment (no. of animals)	PEP Carboxykinase Activity ± Std Dev[a] (nmoles of PEP formed/min per mg of protein)
12-hr fast (6)	92.4 ± 2.5
+ Hydrocortisone (6)	78.4 ± 3.6
+ Actinomycin D[b] (6)	55.5 ± 4.2
+ Hydrocortisone + actinomycin D[b] (6)	53.8 ± 3.7
Not fasted (11)	53.9 ± 11.4

[a] See footnote *a*, Table II. [b] Actinomycin D doses: 440 μg/kg at beginning of experiment and 875 μg/kg at 6 hr.

The comparative abilities of glucose, puromycin, and actinomycin D to lower the elevated carboxykinase of fasted, normal rats are given in Table V. Glucose was significantly more effective than actinomycin D and

TABLE V: The Effects of Glucose, Puromycin, and Actinomycin D on Hepatic PEP Carboxykinase Activity Measured 4 Hours after Treatment of 24-Hour Fasted Rats.

Treatment (no. of animals)	PEP Carboxykinase Activity ± Std Dev[a] (nmoles of PEP formed/min per mg of protein)	Blood Glucose (mg %)
Control (6)	93.4 ± 5.0	74.4 ± 7.3
+ Puromycin (6)	84.6 ± 7.1	181 ± 25
+ Glucose + puromycin[b] (6)	83.9 ± 3.9	374 ± 89
+ Actinomycin D (6)	76.8 ± 4.3	91.8 ± 12.7
+ Glucose + actinomycin D[b] (6)	75.7 ± 5.5	127 ± 13
+ Glucose (6)	67.4 ± 7.2	119 ± 8

[a] See footnote *a*, Table II. [b] Puromycin and actinomycin D were given 0.5 hr before glucose.

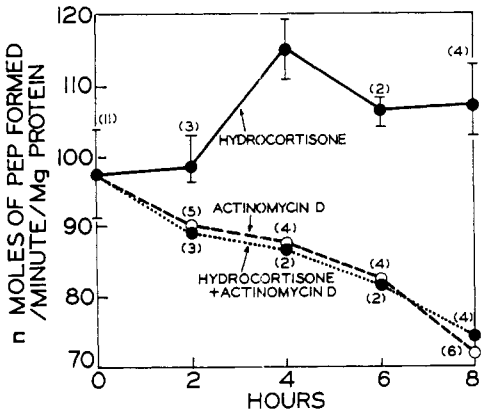

FIGURE 7: The effects of actinomycin D and hydrocortisone on hepatic PEP carboxykinase activity of adrenalectomized female rats fasted for 12 hr prior to treatment. The vertical bars represent standard deviations. The numbers in brackets above the bars or beside the points give the number of animals.

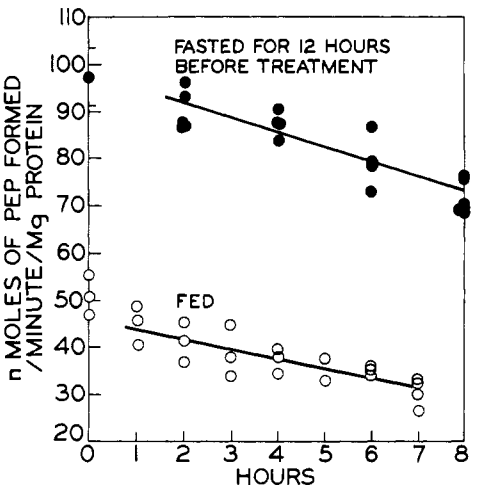

FIGURE 8: The regressions of PEP carboxykinase activity on hours of treatment with actinomycin D for fed and 12-hr fasted adrenalectomized rats. Each point represents a determination on a single rat.

the latter significantly more effective than puromycin in lowering the enzyme. The combinations of glucose and inhibitor were, however, only as effective as the respective inhibitor alone.

Figure 7 illustrates the effects of hydrocortisone and actinomycin D on PEP carboxykinase for a period of 8 hr after treatment of fasted adrenalectomized rats. The enzyme was elevated by the steroid and reached a peak of activity at 4 hr. In animals given hydrocortisone plus actinomycin D, the latter completely eliminated elevation of the enzyme by the steroid and caused a linear decrease in activity which occurred at the same rate as the decrease caused by actinomycin D alone.

In an attempt to determine whether nutritional status influences the turnover of hepatic PEP carboxykinase, fed and fasted adrenalectomized rats were treated with actinomycin D and the rates of decrease in carboxykinase activity were compared by plotting regression lines (Figure 8). The rate of disappearance of activity is presumably the sum of enzyme destruction minus the synthesis of enzyme mediated by preexisting m-RNA. Since the data fit both linear (Figure 8) and semilogarithmic (not shown) regression lines, it was not possible to determine the kinetic order of the decrease in activity. The apparent rate of decrease in the fasted rat was 1.5 times that in the fed animal and the difference was significant; however, in equal periods of time, the loss of activity expressed as per cent of initial activity was less in the fasted rats.

Discussion

The great difficulty encountered in *in vivo* experimentation designed to answer the question of what regulates

the activity of a mammalian enzyme is that treatment of the animal usually results in secondary metabolic alterations which, even if recognized, are not easily controlled. Changes in the enzyme may occur as a result of these secondary effects. In an attempt to obtain a comprehensive picture of the mechanism of regulation of PEP carboxykinase, a variety of experimental conditions which affect the activity of the enzyme have been studied in our laboratory.

Hepatic PEP carboxykinase activity is elevated when rats are fasted, made acutely diabetic with mannoheptulose, or made chronically diabetic with alloxan (Table I; Shrago *et al.*, 1963). Administration of glucocorticoids, glucagon, or lactate also elevates the enzyme (Shrago *et al.*, 1963). Feeding a diet low in carbohydrate increases carboxykinase and refeeding fasted rats with a diet containing sufficient carbohydrate decreases activity (Young *et al.*, 1964). The administration of a glucose load to fed or fasted animals (Tables II and V) and treatment of the alloxan diabetic rat with insulin (Shrago *et al.*, 1963) or removal of its adrenal glands (Table I) also depress PEP carboxykinase. In each of the experimental conditions there was a change either in the animal's need for blood sugar or in the availability of precursors for gluconeogenesis. The regulation of PEP carboxykinase is therefore closely associated with carbohydrate metabolism.

Since carboxykinase responds to the stress of fasting or to mannoheptulose administration equally as well in the adrenalectomized rat as in the intact animal (Table I), it is apparent that adrenal cortical hormone is not an essential element in the mechanism that controls the tissue activity of this enzyme. The increase in carboxykinase activity upon glucocorticoid administration is likely a consequence of alterations in carbohydrate or protein metabolism. An indirect action of cortical hormone is also indicated by the biphasic nature of the response of the enzyme to hydrocortisone (Figure 2). Further evidence that the effect of glucocorticoids on gluconeogenesis is not mediated primarily by their effect on gluconeogenic enzymes is the fact that triamcinolone gave about the same increase in PEP carboxykinase as did hydrocortisone despite the fact that it is far more effective in elevating liver glycogen in fasted adrenalectomized rats (Bernstein, 1958).

The influence of insulin on PEP carboxykinase also seems to be indirect. Insulin given to the diabetic rat causes a rapid decline in enzyme activity (Shrago *et al.*, 1963), but in the fasted rat insulin causes an increase in activity (Young *et al.*, 1964). The latter authors concluded from data obtained with fasted rats given various combinations of glucose, mannoheptulose, and insulin that "both insulin and carbohydrate are required to depress elevated levels of phosphoenolpyruvate carboxykinase." The decline in activity which follows adrenalectomy of the diabetic rat indicates that the mutual antagonism of insulin and glucocorticoids in the regulation of carbohydrate metabolism may be reflected in the controlled levels of PEP carboxykinase activity.

Actinomycin D, by inhibiting the formation of m-RNA (Goldberg *et al.*, 1962), tends to depress the tissue activity of any enzyme whose rate of synthesis is proportional to that of its messenger. In some cases, actinomycin D may also inhibit protein synthesis by means other than its influence on messenger formation (Honig and Rabinowitz, 1965). A decline in enzyme activity, mediated by either a direct or indirect action of actinomycin on protein biosynthesis, would be antagonized by an elevation of enzyme activity by activation processes. The increase in PEP carboxykinase after administration of mannoheptulose (Table III) or hydrocortisone (Figure 7) was completely inhibited by actinomycin D. More significant, however, is the finding that neither mannoheptulose nor hydrocortisone antagonized the decrease in activity caused by actinomycin D. This strongly suggests that mannoheptulose and the steroid enhance carboxykinase activity by stimulating biosynthesis of the enzyme; however, it cannot be assumed that they *directly* influence either messenger or enzyme synthesis.

It is not clear why actinomycin blocked the increment in PEP carboxykinase due to fasting, but failed to depress activity below its normal level (Table IV). This is especially difficult to interpret since actinomycin D caused a continual decrease in the enzyme in both fed animals and animals fasted for 12 hr before they received the inhibitor (Figure 8). Segal *et al.* (1965) suggest that the cell may not have the same sensitivity to an inhibitor of protein synthesis during the period when an animal is responding to the stress of an altered environment. Alternatively, the tendency for PEP carboxykinase to be depressed by administered actinomycin D may, during the response of the animal to fasting, be counteracted by processes such as activation, enhanced translation of available messenger, and a decrease in the rate of enzyme destruction. The data of Figure 8 are compatible with the hypothesis that the rate of enzyme destruction and the rate of transcription of messenger are altered in the fasted rat, since the per cent of initial carboxykinase activity lost during several hours after administration of actinomycin D was less in fasted than in fed rats.

It was rather remarkable that glucose was more effective than actinomycin D and the latter more effective than puromycin in depressing the elevated level of carboxykinase of the fasted rat, but that, when either inhibitor was given with glucose, the latter had no apparent effect (Table V). Since the effect of glucose alone was greater than that of puromycin, inhibition of carboxykinase synthesis cannot account entirely for the action of glucose. An additional effect of glucose, perhaps on the rate of enzyme destruction, must be sought. Whatever this effect of glucose, it apparently requires protein synthesis for its expression for it is diminished by actinomycin D and abolished by puromycin. According to Sabesin and Isselbacher (1965) puromycin does not affect intestinal absorption of glucose. It is therefore unlikely that the inhibitors diminish the effect of glucose on carboxykinase by interfering with absorption of administered glucose.

The chemical nature of the true regulators of PEP

carboxykinase are as yet unknown. Glucose *per se* is not likely a regulator. The diabetic rat has a high hepatic carboxykinase activity even though it has a high concentration of blood glucose to which the liver cells are freely permeable (Cahill *et al.*, 1958). Furthermore, the correlation between enzyme activity and blood glucose concentration seen in Figure 5 does not occur under all experimental conditions (*cf.* Figures 2 and 6).

One might suppose that the inability of glucose to depress PEP carboxykinase in the diabetic rat is due to a low hepatic glucokinase activity (DiPietro and Weinhouse, 1960) and consequent impairment of the liver's ability to phosphorylate glucose, this then leading to a decreased hepatic concentration of some glycolytic intermediate which acts as a repressor. However, fructose is phosphorylated and oxidized at normal rates by liver slices from diabetic rats (Chernick and Chaikoff, 1951), but when given to diabetic rats it fails to depress carboxykinase significantly (Young *et al.*, 1964). One possible interpretation of these data is that suppression of liver PEP carboxykinase by glucose is mediated *via* a factor from peripheral tissue. The failure of glucose to suppress in the diabetic rat might be due to decreased peripheral utilization of glucose. As an extension of this hypothesis it would be predicted that metabolism of glucose in peripheral tissues influences the concentration of a humoral agent which in turn regulates hepatic PEP carboxykinase.

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