

# The Effect of Steroids and Ammonium Chloride Acidosis on Phosphoenolpyruvate Carboxykinase in Rat Kidney Cortex

## II. THE KINETICS OF ENZYME INDUCTION

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**ABSTRACT** The kinetics of the induction of rat kidney phosphoenolpyruvate carboxykinase activity after triamcinolone and ammonium chloride administration have been investigated with a view to the further differentiation of the two processes.

The half-life of kidney phosphoenolpyruvate carboxykinase activity, as measured from the decay curve after a single doses of triamcinolone, is approximately 1.4 hr. This compares with a half-life for the enzyme from acidotic kidney of approximately 3.4 hr.

Analysis of the data indicates that the induction of phosphoenolpyruvate carboxykinase activity by triamcinolone may be attributed to an increase in *de novo* protein synthesis. Induction by acidosis is qualitatively distinct and is partly attributed to a reduction in the rate of decay of phosphoenolpyruvate carboxykinase activity.

The activities of the gluconeogenic enzymes glucose-6-phosphatase, fructose-1,6-diphosphatase, and phosphoenolpyruvate carboxykinase in both liver and kidney have been measured in animals separately treated with triamcinolone and ammonium chloride. Triamcinolone significantly increases the activities of liver phosphoenolpyruvate carboxykinase, kidney glucose-6-phosphatase, and kidney phosphoenolpyruvate carboxykinase only; ammonium chloride stimulates a 200% increase in kidney phosphoenolpyruvate carboxykinase, but has no effect on the other enzymes.

The induction processes whereby triamcinolone in-

creases phosphoenolpyruvate carboxykinase activities in liver and kidney differ quantitatively.

## INTRODUCTION

Phosphoenolpyruvate carboxykinase (PEPCK)<sup>1</sup> activity in rat kidney is increased during ammonium chloride acidosis and after administration of the synthetic glucocorticoid, triamcinolone (9 $\alpha$ -fluoro-16 $\alpha$ ,17 $\alpha$ -isopropylidenedioxy-1-dehydrocorticosterone) (1, 2). Although the increased PEPCK activities are reflected in increased antibody titers, the two processes of induction may be differentiated through their response to actinomycin D (1). Previous studies of PEPCK induction in kidney have involved measurement of enzyme activities at single time-points after treatment with inducer (1-3). It is of interest, therefore, to compare the time-courses of enzyme induction by ammonium chloride and triamcinolone with a view to their further differentiation and the eventual elucidation of the mechanisms involved. In this paper we present the results of a study of the kinetics of PEPCK induction and turnover in rat kidney under these two conditions and discuss the metabolic basis for these findings.

## METHODS

*Animals.* Male Wistar rats (120-200 g) were used throughout. Rats were fed diet 41B (4), and were allowed water or 0.18% NaCl ad lib. 0.18% NaCl replaced water in experiments involving NH<sub>4</sub>Cl-induced acidosis; in these experiments, NaCl was given to both controls and experimental animals.

Adrenal glands were removed by means of a midline dorsal incision under ether anaesthesia. Adrenalectomized

<sup>1</sup> *Abbreviation used in this paper:* PEPCK, phosphoenolpyruvate carboxykinase.

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animals were given 1% NaCl in place of water and were left for 5 days before further use.

Triamcinolone acetonide was administered by intramuscular or intraperitoneal injection of the suspension appropriately diluted in 0.9% NaCl. Acidosis was induced by the administration of 0.4 M  $\text{NH}_4\text{Cl}$  in water by stomach tube. Controls received either injections of 0.9% NaCl or 0.4 M NaCl by stomach tube as appropriate.

*Tissue extractions.* Animals were killed between 0700 and 0900 hr by decapitation. The kidneys, and where appropriate, the livers were rapidly removed, rinsed in ice-cold 0.9% NaCl, freed of capsules and connective tissue, blotted, and weighed. One kidney, or approximately 1 g liver, was then homogenised in 9 vol of 0.154 M KCl, 5 mM 2-mercaptoethanol at 0°C in a motor-driven all-glass tissue grinder (Kontes Glass Co., Vineland, N. J.). Portions of these homogenates were stored for assay of total protein. The remainder was centrifuged at approximately 1000 g for 2 min (MSE Minor, VWR United Corp., Crawley, U. K.) to remove large particles of cell debris. Samples of this first supernate were stored at 0°C for the subsequent assay of glucose-6-phosphatase. Approximately 1.5 ml of each supernate was further centrifuged at 12,000 g for 8 min at 4°C (Eppendorf Zentrifuge 3200, V. A. Howe Ltd., London). The resulting clear supernates were stored at 0°C and used for subsequent assay of fructose-1,6-diphosphatase, PEPCK, and soluble protein.

*Assay procedures.* Glucose-6-phosphatase activity was measured in a stopped-assay procedure as described by Nordlie and Arion (5). Glucose formation was measured with glucose oxidase (6).

Fructose-1,6-diphosphatase was assayed substantially as described by Underwood and Newsholme (7). The medium consisted of 50 mM Tris Cl, 5 mM  $\text{MgSO}_4$ , 20 mM 2-mercaptoethanol, 50  $\mu\text{M}$  fructose-1,6-diphosphate, 0.2 mM  $\text{NADP}^+$ , and 30  $\mu\text{g}$  each of phosphoglucose isomerase, glucose-6-phosphate dehydrogenase, and 6-phosphogluconate dehydrogenase in a final volume of 3.0 ml, pH 7.5. Reactions were initiated by addition of fructose-1,6-diphosphate.

PEPCK was assayed as described previously (1). Protein concentrations (in 2 N NaOH) were measured by the biuret method (8). Blood pH determinations were made on a Radiometer Astrup pH meter (V. A. Howe Ltd., London). A unit of enzyme activity is defined as that amount which catalyzes the formation of 1  $\mu\text{mole}$  of product per min at 30°C (glucose-6-phosphatase and fructose-1,6-diphosphatase) or 37°C (PEPCK).

*Calculation of kinetic data.* Decay curves were fitted to equation 1, where  $E_t = \text{PEPCK}$  at time "t,"

$$E_t - E_0 = (E' - E_0)e^{-k't} \quad (1)$$

$E_0 = [\text{PEPCK}]$  final value,  $E' = [\text{PEPCK}]$  at  $t = 0$ , i.e., the initial (highest) value, and  $k'$  = the first order rate constant for the decay of enzyme activity (9). This procedure was carried out with the aid of an ICL475 computer (International Computers Ltd., Reading, U. K.).

Subsequent calculations were made using a Hewlett-Packard 9100B bench top calculator (Hewlett-Packard Co., Palo Alto, Calif.).

*Materials.* Biochemicals and enzymes were from the Boehringer Corp. (London) Ltd. Tris, as Trizma base, and 2-mercaptoethanol were from Sigma Chemical Co., St. Louis, Mo. Triamcinolone acetonide (Adcortyl) was a kind gift from E. R. Squibb & Sons Ltd., Speke, Liverpool, U. K.

## RESULTS

*Expression of results.* In most experiments, results were expressed in four different ways, viz., (a) enzymic activity per gram wet tissue, (b) activity per milligram total protein, (c) activity per milligram soluble protein, and (d) activity per 100 g rat body weight. The arguments supporting such an approach have been fully discussed by Freedland and Harper (10). Since there were no significant changes in tissue mass or protein content during the time-courses of our experiments, variations in enzyme activities, if any, were reflected similarly in all four modes of expression, irrespective of the parameter used.

The masses of rat liver and kidneys, expressed as percentages of the total animal body weight, were  $5.3 \pm 0.1\%$  and  $0.82 \pm 0.01\%$ , respectively (mean  $\pm$  SEM,  $n = 51$  in both cases).

*Effects of triamcinolone and  $\text{NH}_4\text{Cl}$  on glucose-6-phosphatase, fructose 1,6-diphosphatase, and PEPCK in rat liver and kidney.* Of the gluconeogenic enzymes assayed in liver extracts, only PEPCK showed significantly increased activity (approximately 100%) after 48 hr of triamcinolone administration to normal fed 150-g rats (Table I). This observation is in agreement with previous reports (11–13). Glucose-6-phosphatase and fructose-1,6-diphosphatase activities were similar to those in control animals. Measurements at 72 and 96 hr confirmed this insensitivity to induction by triamcinolone. This lack of responsiveness of glucose-6-phosphatase contrasts with an earlier report of the efficacy of hydrocortisone as an inducer of this enzyme (14); this discrepancy may be attributable to dietary differences between the two experiments.

In kidney, in contrast, glucose-6-phosphatase concentrations were more than doubled by triamcinolone. At the same time, PEPCK activity was increased by more than 300%, while fructose-1,6-diphosphatase alone was unresponsive (Table I). These results are in accord with those reported earlier (3).

Table II shows the results of a similar experiment in which  $\text{NH}_4\text{Cl}$  administration by stomach tube replaced triamcinolone injection. The dosage employed was sufficient to elicit maximal responses. The liver gluconeogenic enzymes were unresponsive to acidosis over the 48 hr period. In kidney, PEPCK activities were increased more than 200%, while those of glucose-6-phosphatase and fructose-1,6-diphosphatase were similar to control values.

The differences between the control values for kidney PEPCK activities in Tables I and II is attributed to variation between batches of animals, rather than to the administration of 0.4 M NaCl to the acidotic-control group; this treatment has been shown in other experiments to be without effect on PEPCK activity.

TABLE I  
Effect of Triamcinolone Administration on Gluconeogenic Enzyme Activities of Rat Liver and Kidney

			U/g wet weight	mU/mg total protein	mU/mg soluble protein	U/100 g body weight	Significance	
Liver	G6Pase	Control	7.5±0.3	68±5	95±5	40±2	NS	
		Steroid	8.1±0.4	72±5	109±7	56±3		
	FDPase	Control	9.9±0.9	90±11	125±11	53±5	NS	
		Steroid	10.4±0.3	93±3	140±1	72±2		
Kidney	PEPCK	Control	2.1±0.2	19±1	26±3	11±1	P < 0.01	
		Steroid	4.4±0.5	39±3	59±5	30±3		
	G6Pase	Control	3.6±0.4	32±4	52±8	3.0±0.4		P < 0.005
		Steroid	8.2±0.3	69±3	120±8	7.8±0.3		
Kidney	FDPase	Control	11.3±0.1	100±4	161±7	9.5±0.2	NS	
		Steroid	12.4±0.8	104±4	181±7	11.9±0.7		
	PEPCK	Control	2.6±0.2	23±2	38±4	2.2±0.2	P < 0.001	
		Steroid	11.3±0.4	95±1	165±7	10.9±0.3		

150- to 200-g rats were injected intraperitoneally with 2.5 mg/100 g body weight of triamcinolone acetoneide ("steroid") or a similar volume of 0.9% NaCl ("control"). Four injections were given at 12-hr intervals; animals were killed at 48 hr, and the enzymes were subsequently assayed as described in the Methods section. Results are expressed as means ±SEM of three observations in all cases.

FDPase, fructose-1,6-diphosphatase; G6Pase, glucose-6-phosphatase.

*Time-course of PEPCK induction by triamcinolone.* Fig. 1 shows the increase in kidney PEPCK activity after intramuscular injection of triamcinolone. Similar results were obtained in an experiment in which the steroid was administered by intraperitoneal injection. The increase in PEPCK activity was in all cases preceded by a lag period of approximately 2 hr.

*Time-course of the decay of PEPCK activity after triamcinolone.* Normal fed rats given a single injection of triamcinolone (2.5 mg/130 g rat) still showed

high values for kidney PEPCK activities at 5 days after treatment. Most of the rats so treated died at 6 days. Postmortem inspection revealed that fat reserves were severely depleted. To avoid any problems of "adrenal rebound," animals were subsequently totally adrenalectomized 5 days before administration of steroid. The time-course of PEPCK induction in adrenalectomized animals was checked and was found to be similar to that observed in normal rats. Fig. 2 shows the decay in kidney PEPCK activity after injection of a

TABLE II  
Effects of NH<sub>4</sub>Cl Administration on Gluconeogenic Enzyme Activities of Rat Liver and Kidney

			U/g wet weight	mU/mg total protein	mU/mg soluble protein	U/100 g body weight	Significance
Liver	G6Pase	Control	10.5±0.8	75±7	121±12	34±2	NS
		Acidotic	9.0±0.4	60±3	99±1	29±1	
	FDPase	Control	12.7±0.3	91±2	146±3	42±2	NS
		Acidotic	12.2±0.3	81±3	134±3	39±1	
	PEPCK	Control	6.1±0.3	46±4	73±6	20±0.3	NS
		Acidotic	7.4±0.7	49±3	82±7	24±3	
Kidney	G6Pase	Control	4.2±0.1	39±5	64±6	3.2±0.1	NS
		Acidotic	4.3±0.2	46±4	63±3	3.5±0.3	
	FDPase	Control	11.9±0.3	106±11	179±10	9.0±0.4	NS
		Acidotic	10.2±0.7	110±9	151±8	8.2±0.4	
	PEPCK	Control	7.4±0.5	66±6	112±8	5.6±0.3	P < 0.001
		Acidotic	24.9±1.0	269±6	373±18	20.4±0.8	

150- to 200-g rats were given four doses of 5 ml 0.4 M NH<sub>4</sub>Cl ("acidotic") or 5 ml 0.4 M NaCl ("control") per 200 g body weight at 12-hr intervals. Animals were killed at 48 hr, and the enzymes were subsequently assayed as described in the Methods section. Results are expressed as means ±SEM of four observations in all cases.

FDPase, fructose-1,6-diphosphatase; G6Pase, glucose-6-phosphatase.

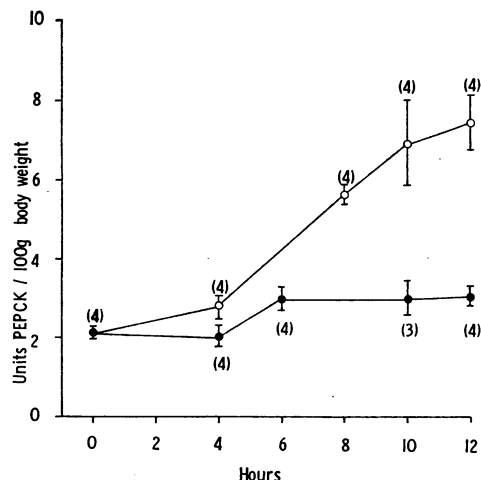


FIGURE 1 Time-course of the induction of kidney PEPCK by triamcinolone. Rats (approximately 200 g) were injected intramuscularly with 2.5 mg/100 g body weight of triamcinolone acetate (○) or a similar volume of 0.9% NaCl (controls, ●) at zero time (0700 hr). Tissue extractions and assays were performed as described in the Methods section. Results are expressed as means  $\pm$ SEM; the number of observations is given in parentheses.

low dose of triamcinolone (0.1 mg/130 g rat). In all experiments, it was observed that final values were significantly above those of the controls. The reason for this is unknown, but it may be related to retention of a very small proportion of triamcinolone in the kidney even after several half-lives (15).

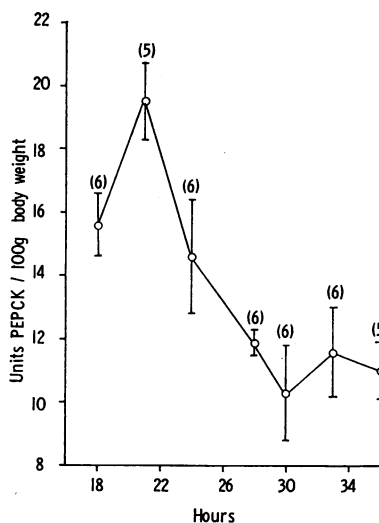


FIGURE 2 Time-course of the decay of kidney PEPCK activity after triamcinolone. Adrenalectomized rats (120–150 g) were given a single intraperitoneal injection of triamcinolone acetate at zero time (5 days postoperative). Tissue extractions and assays were performed as described in the Methods section. Results are expressed as means  $\pm$ SEM; the number of observations is given in parentheses.

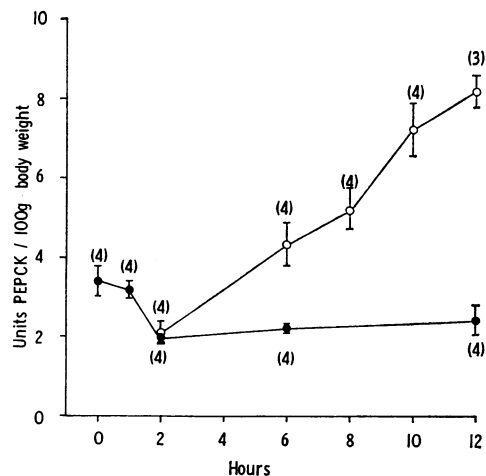


FIGURE 3 Time-course of the increase in kidney PEPCK activity after  $\text{NH}_4\text{Cl}$  feeding. Rats (approximately 200 g) were given either 5 ml 0.4 M  $\text{NH}_4\text{Cl}$  experimental (○), or 5 ml 0.4 M NaCl (controls, ●) per 100 g body weight by stomach tube. Tissue extractions and assays were performed as described in the Methods section. Results are expressed as means  $\pm$ SEM; the number of observations is given in parentheses.

*Time-course of PEPCK induction by  $\text{NH}_4\text{Cl}$ .* Oral admission of  $\text{NH}_4\text{Cl}$  leads to a significant degree of acidosis after 2 hr (16). PEPCK activity is only slightly increased over control values at 4 hr. The lag period of approximately 2 hr is similar to that seen with induction by steroids. After 4 hr activity increases rapidly (Fig. 3). Control values remain unchanged.

*Time-course of the decay of PEPCK activity after  $\text{NH}_4\text{Cl}$ .* The reduction in kidney PEPCK activity was followed in animals rendered alkalotic at zero time (Fig. 4). In a similar experiment, not shown, blood pH values measured 3 hr after  $\text{NaHCO}_3$  administration were  $7.54 \pm 0.07$  and  $7.62 \pm 0.02$  for the "acidotic" and control groups, respectively ( $n=4$  in both cases). PEPCK activity in "acidotic" kidneys fell rapidly and was only slightly greater than control values at 12 hr. Extracts of kidneys from control animals treated with  $\text{NaHCO}_3$  initially exhibited a reduction of PEPCK activity, presumably due to the onset of alkalosis (16); values later increased, and by 25 hr were the same as those in kidneys from experimental animals and those observed in "normal" control kidneys.

*Time-course of liver PEPCK induction by triamcinolone.* The activity of liver PEPCK after intramuscular injection of triamcinolone was monitored over a 12 hr period to determine whether PEPCK induction exhibited similar kinetics in both liver and kidney.

As distinct from that in kidney, the situation in the liver is complicated by the diurnal rhythmicity of

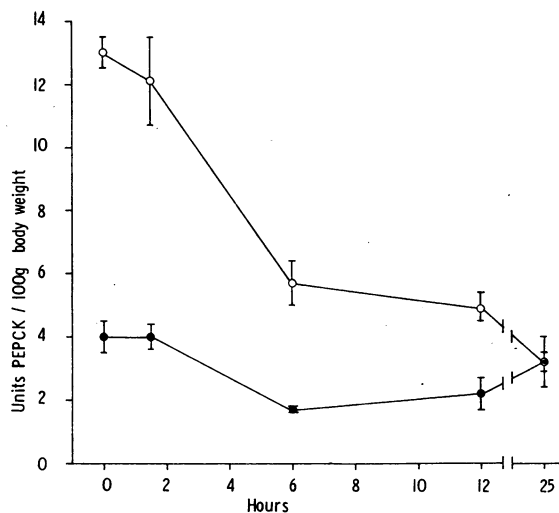


FIGURE 4 Time-course of the decay of kidney PEPCK activity after administration of  $\text{NaHCO}_3$  to acidotic rats. Rats (approximately 200 g) were rendered acidotic (O) by four administrations of 5 ml 0.4 M  $\text{NH}_4\text{Cl}$ /200 g body weight at 12-hr intervals. Controls (●) received simultaneously 5 ml 0.4 M  $\text{NaCl}$ /200 g body weight. At 48 hr (zero time, 0700 hr Greenwich Mean Time) all animals were given 10 ml 0.3 M  $\text{NaHCO}_3$  in 10% glucose, and were subsequently allowed access to 20 mM  $\text{NaHCO}_3$  in 0.18%  $\text{NaCl}$  ad lib. Tissue extractions and assays were performed as described in the Methods section. Results are expressed as the means  $\pm$ SEM of four observations in each case.

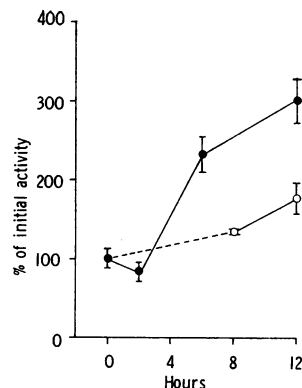


FIGURE 5 Time-course of the changes in rat liver PEPCK after triamcinolone. Rats (approximately 150 g) were injected intramuscularly with 2.5 mg/100 g body weight of triamcinolone acetamide (experimentals, O) or a similar volume of 0.9%  $\text{NaCl}$  (controls, ●) at zero time (0700 hr). Tissue extractions and assays were performed as described in the Methods section. Results are expressed as the means  $\pm$ SEM of four observations in each case.

PEPCK activity (17) (Fig. 5). The activity in control livers is increased 200% at 12 hr (1900 hr GMT). In contrast, triamcinolone, at the high dosage used abolishes this rhythm and activity at 12 hr is only slightly increased over that at zero time. Longer time-courses, not shown, reveal that PEPCK activity under these conditions rises more slowly in the first 24 hr

TABLE III  
Kinetic Parameters of the Decay of Kidney PEPCK Activity after Triamcinolone and  $\text{NH}_4\text{Cl}$

Experiment	Parameter	Predicted values for PEPCK activities (means $\pm$ SEM)	Rate constant for PEPCK decay ( $\text{hr}^{-1} \pm \text{SD}$ )	P	$t_{1/2}$ (hr $\pm$ SD)
Decay after triamcinolone	U/g kidney	Start 22.1 $\pm$ 1.3	0.51 $\pm$ 0.16	<0.02	1.37 $\pm$ 0.52
Decay after $\text{NH}_4\text{Cl}$		Final 11.6 $\pm$ 0.6			
Decay after triamcinolone	mU/mg soluble protein	Start 260 $\pm$ 17	0.57 $\pm$ 0.23	<0.02	1.21 $\pm$ 0.59
Decay after $\text{NH}_4\text{Cl}$		Final 133 $\pm$ 7			
Decay after triamcinolone	U/100 g body weight	Start 19.5 $\pm$ 1.4	0.41 $\pm$ 0.16	<0.05	1.69 $\pm$ 0.81
Decay after $\text{NH}_4\text{Cl}$		Final 10.7 $\pm$ 0.7			
Decay after triamcinolone	U/100 g body weight	Start 13.1 $\pm$ 1.1	0.21 $\pm$ 0.05	<0.05	3.32 $\pm$ 0.99
Decay after $\text{NH}_4\text{Cl}$		Final 3.5 $\pm$ 0.6			

The derivation of constants is given in the Methods section and is discussed in the text. Predicted values for PEPCK activities are those given in the computer output.

TABLE IV  
Effect of Triamcinolone and Acidosis on the Rates of  
Synthesis of Kidney PEPCK

Inducer	$t_0$	$t_f$	$P_0$	$P_f$	$S'$	$S'/S$
	hr	hr	U/g	U/g		
Triamcinolone	2	6	2.61	5.02	2.74	1.79
	6	8	5.02	6.81	3.99	2.61
	8	10	6.81	9.37	5.51	3.60
	10	12	9.37	10.89	5.99	3.92
NH <sub>4</sub> Cl	4	8	3.82	7.21	2.05	1.06
	8	10	7.21	8.66	2.40	1.24
	10	12	8.66	10.35	2.85	1.47

The method of calculation was as discussed in the text.  $t_0$  is the lower time point,  $t_f$  the higher time point;  $P_0$  is the enzymic activity at  $t_0$ , and  $P_f$  is the activity at  $t_f$ .  $S$  is the normal rate of enzyme synthesis (as given in the text) and  $S'$  is the "induced" rate of synthesis.

than in the 24–48 hr period. Maximum activity is reached at 48 hr.

*Derivation of kinetic constants for PEPCK turnover.* Computer analysis of the PEPCK activity decay curves (9) after triamcinolone and NH<sub>4</sub>Cl yields the kinetic constants shown in Table III. The first order rate constant,  $k'$ , for the decay after steroid is approximately 0.5 hr<sup>-1</sup>, corresponding to a half-life of only 1.4 hr.

The normal rate of synthesis ( $S$ ) is given by

$$S = kP \quad (2)$$

where  $k$  is the zero order rate constant for synthesis and  $P$  is the steady-state level of enzyme activity. Since triamcinolone-induced enzyme is similar to normal "noninduced" PEPCK on the basis of antibody crossreactivity and molecular weight studies (1, 18), it is reasonable to assume that  $k'$  equals  $k$ . By substitution of the value for the normal noninduced PEPCK activity (3.0 U/g) in equation 2, the normal rate of synthesis is found to be 1.53 U/hr. In the experiment involving measurement of the decay of PEPCK activity after NH<sub>4</sub>Cl, the normal noninduced level of PEPCK activity was 3.8 U/g kidney. Using the value of  $k$  already determined, the normal rate of synthesis in this case was 1.94 U/hr.

The new rates of synthesis after induction with both steroid and NH<sub>4</sub>Cl were calculated as suggested by Haining (19), using equation 3,

$$S' = \frac{k'(P_0 e^{-k't} - P_f)}{e^{-k't} - 1} \quad (3)$$

where  $S'$  is the new rate of enzyme synthesis,  $P_0$  is the value for enzyme activity at the lower time point

chosen,  $P_f$  is the value for enzyme activity at the higher time point chosen,  $t$  is the time interval separating  $P_0$  and  $P_f$ , and  $k'$  is the rate constant for degradation as determined. The results of these calculations are given in Table IV. Triamcinolone induces an increase in the rate of PEPCK synthesis which is nonlinear; this resembles the situation observed with other enzyme activities in rat liver (19). The new rate of synthesis approaches a value of approximately 4 times the normal rate at 12 hr. With NH<sub>4</sub>Cl induction, however, the "induced" rate of synthesis is only slightly greater than the normal rate, indicating that NH<sub>4</sub>Cl has a minimal effect on *de novo* PEPCK synthesis rates, and must therefore exert most of its "inductive" effect on other processes including enzyme degradation.

## DISCUSSION

Some previous reports have suggested that the activities of liver gluconeogenic enzymes are increased in a concerted manner in adrenalectomized animals treated with triamcinolone (20, 21). This phenomenon has been attributed to the direct stimulation of a "functional genome" by the inducer. In normal animals, in contrast, the response of these same enzymes is more complex. Of the liver enzymes, PEPCK activity alone is sensitive towards induction by triamcinolone, while in the kidney both glucose-6-phosphatase and PEPCK are responsive. The differences between liver and kidney glucose-6-phosphatases may possibly be attributed to the properties of two distinct protein entities controlled by separate genes. Liver and kidney PEPCK, on the other hand, are immunologically indistinguishable (1), and presumably represent the activity of a single protein species. Despite this, the time-courses of PEPCK induction by triamcinolone in the two tissues are clearly different (see Figs. 1 and 5). In view of these findings, it is apparent that the processes of induction *in vivo* may be considerably more complex than would be expected from a simple, single, action of the hormone upon either transcription or translation.

The rates of degradation of enzyme protein are preferably determined from labeling and immunoprecipitation data (22), although recent caveats have been made (23, 24) regarding this approach. In the absence of sufficient specific antisera, however, calculations based on detailed measurements of activity alone may serve as a useful first approximation. The apparent half-life of kidney PEPCK after single triamcinolone injections is 1.4 hr. It may be objected that this figure represents merely the decline in enzyme activity parallel with and related to the decline in the tissue steroid content. Florini, Peets, and Buyske (15), however, have shown that the half-life of triamcinolone in rat blood is 52 min, and that a very small percentage only is retained

in the kidney after 24 hr. Nevertheless, in view of the initial pharmacological dosage given to each animal, this residual steroid may be sufficient to explain the observation that PEPCK activity decays only to a point significantly above the control value. The figure of 1.4 hr should thus more properly be considered a mean *maximal* value for the half-life of kidney PEPCK. This value is much shorter than the 9–16 hr reported by Reshef, Ballard, and Hanson, (25) for the apparent half-life of the immunologically similar adipose tissue PEPCK (18), but is in the range reported for several enzymes of regulatory significance in rat liver (26–28).

The half-life of PEPCK after acidosis is considerably longer (3.4 hr). Since acidosis may be terminated rapidly with NaHCO<sub>3</sub>, it is concluded that the observed rate of decay of PEPCK activity truly reflects the turnover of active enzyme and is not limited by the rate of removal of the “inducer.”

It is apparent that the two processes whereby kidney PEPCK total activity is increased are clearly distinguished kinetically. The increased rate of synthesis of PEPCK with triamcinolone, together with the known sensitivity of this process to actinomycin D (1, 2) and ethionine (2) indicate that steroid effects are most likely mediated through *de novo* production of enzyme protein. In acidosis, however, changes in the rate of “synthesis” appear to be small in comparison with changes in the rate of degradation. The insensitivity of PEPCK “induction” in acidosis to RNA synthesis inhibitors (1, 2) indicates that *de novo* synthesis is not obligatory for increased ability. Since the “induced” PEPCK activity exhibits a similar antibody titer to that of normal PEPCK, the presence of an inactive precursor molecule must be considered (1). Inhibition of the rates of protein degradation alone is insufficient to explain an *increase* in immunotitrable enzyme activity; it is simultaneously required that synthetic activity is maintained. If *de novo* synthesis is ruled out, the present results are consistent with the postulate of an inactive polypeptide precursor. Under these circumstances the premises upon which equation 1 are based are no longer valid, and the rate of change of enzyme activity is dependent on the concentration of precursor molecules (E\*) (equation 4),

$$\frac{dE}{dt} = k_1 E^* - k' E \quad (4)$$

where  $k_1$  is the first order rate constant for the activation reaction. (Equation 4 is, of course, further modified if the activation process involves either association or dissociation of precursor subunits.) If E\* is relatively large in comparison with E, as suggested by the insensitivity of the process to actinomycin D for a period of as long as 48 hr, then equation 4 may be

treated in a manner similar to equation 1 and the values obtained experimentally for  $k'$  may be taken to approximate those of the true decay rate constant.

The pathways by which PEPCK activity decays in alkalosis are not distinguished in the present study. At least three possibilities present themselves, viz., (a) conversion to “normal” PEPCK (1), (b) proteolysis by a pathway distinct from that of the “normal” enzyme, and (c) reconversion into the inactive species. Further kinetic analysis must therefore await the characterization of the enzymic species involved, together with an understanding of their interconversion.

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