to result from the loss of water. That increments in erythrocyte Na occurred is clear, and while it is very difficult to assess the importance of alterations in canine red blood cell electrolytes, because their chemical anatomy is quite different from other body cells, such increases could be mediated by the influence of excessive amounts of 17-hydroxycorticosteroids on membrane permeability.

Summary. Twenty-four dogs were subjected to an ambient temperature of sufficient magnitude to elevate rectal temperatures to 42.5°C for one hour. These animals were studied with regard to plasma 17-hydroxycorticosteroid concentration and plasma and erythrocyte electrolyte levels. Significant changes were observed in all measured variables with the exception of plasma Na concentration, which was not altered from the control value. The physiological implications of these data are discussed.

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Increased Activity of Renal Glutaminases in Guinea Pig Following Prolonged Administration of Acid or Alkali.* (22733)

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The relationship between ammonia excretion and renal glutaminase activity has attracted much attention in recent years. Davies(1) discovered a marked increase in the ability of kidney slices from chronically acidotic rats to deaminate glutamine and other amino acids. On the other hand, Handler(2) found no increased hydrolysis of glutamine in slices from rats chronically treated with acid or alkali. White(3) observed no increase in renal glutaminase activity in homogenates from acidotic rats, while Rector(4) demonstrated a sharp day to day rise in the glutaminase activity of kidney homogenates of rats treated with ammonium chloride. Contrary to observations in man, dog and rat, we observed that, in the guinea pig, not only does ammonia excretion increase in acute acidosis but also in acute alkalosis(5). It was thought that investigation of the activity of renal glutaminases in the two conditions might aid in elucidating the biochemical mechanisms responsible for the increased urinary ammonia excretion. As a basis for work on the enzymatic mechanism of ammonia production, it was mandatory to characterize the enzymes involved. This had not been done previously and might explain the controversial observations. We have been able to distinguish three enzymes involved in glutamine metabolism in

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the renal tubular cells‖: *Glutaminase I* is a hydrolyzing enzyme with an optimal pH of 7.4, is activated by phosphate and is inhibited by Sulfobromophthalein or Quinacrine (6). *Glutaminase II* is a hydrolyzing enzyme system with an optimal pH of 8.8, is dependent on the presence of α-keto acids, and is similar to the glutamine transaminase-deamidase of the liver studied extensively by Meister(7). *Glutamine synthesizing enzyme system* forms glutamine from glutamic acid and ammonia, has an optimal pH of 7.4 in homogenates, is dependent on adenosinetriphosphate and magnesium ions, and is inhibited by p-chloromercuribenzoate(7). Since it is not known which of these enzymes are involved in ammonia formation, all were investigated in this study.

Materials and methods. Adult guinea pigs, averaging 450 g (400-485 g), in groups of 3 males and 3 females each were treated for a period of 3 weeks as follows: Each group was intubated daily with either 3 ml of 0.9% sodium chloride, 3 ml 1 M ammonium chloride, or 3 ml 1 M sodium bicarbonate. The latter 2 groups received either 0.5 to 1% ammonium chloride or 1 to 2% sodium bicarbonate respectively in their drinking water. All animals were fed Purina Rabbit Chow Checkers supplemented daily with cabbage. The animals were killed by a blow on the head. The kidneys were removed, decapsulated, chilled, and made into a 5% homogenate according to the procedure of Potter(8). The enzyme assays were carried out under optimal conditions as established by previous experiments. The following incubation mixtures were used: *Glutaminase I*: .1 ml 5% tissue homogenate, .1 ml .1 M l-glutamine. .05 ml 1 M sodium phosphate (pH 7.4), .2 ml .05 M 2-amino-2 - hydroxymethyl-1,3 - propandiol (tris buffer) (pH 7.4), .9% sodium chloride to make 1 ml. *Glutaminase II*: .3 ml 5% tissue homogenate, .1 ml .1 M l-glutamine, .1 ml .5 M sodium pyruvate, .2 ml .1 M Veronal-HCl buffer (pH 8.8), .9% sodium chloride to make 1 ml. *Glutamine synthesizing enzyme*: .3 ml 5% tissue homogenate, .2 ml .5 M sodium l-glutamate, .2 ml 2.94 mM ammonium chloride, .1 ml .8 M magnesium sulfate, .2 ml .05 M adenosinetriphosphate-sodium (ATP), .2 ml .05 M tris buffer (pH 7.4), 0.9% sodium chloride to make 1.5 ml. All incubations were carried out in 25 ml serum bottles at 37.5°C for 30 minutes. At the end of the incubation period, the bottles were stoppered, injected through the stopper with 1 ml 20% sodium carbonate and analyzed for ammonia by the micro-diffusion method of Seligson(9). Glutaminase I activity was derived by taking the difference between ammonia liberated in the presence and in the absence of phosphate and corrected for the phosphate-activated, non-enzymatic decomposition of glutamine. For Glutaminase II activity, the assay results in the absence of pyruvate were subtracted from those obtained in the presence of pyruvate. Glutamine synthesis was calculated from ammonia disappearance during incubation and corrected for preformed ammonia and ammonia liberated from endogenous substrates. All results are expressed as μM ammonia liberated per 100 mg desoxyribosenucleic acid, as suggested by Knox(10) since it reflects the number of cells present. The results are similar when wet or dry weight is used as a base but show somewhat greater variability. Desoxyribosenucleic (DNA) and ribosenucleic (RNA) acids were determined on a sample of the original homogenate. The method employed for DNA was a combination of the technic developed by Schneider(11) and of Dische's color reaction for desoxyribose(12). The method of Mejbaum(13) was used to determine RNA.

Results. The percent increase in body weight of the acidotic animals was significantly less than the control or alkali treated groups. The renal DNA concentration was lower in the alkalotic animals than in the controls (Table I). Since the kidney weights were essentially equal, cytoplasmic mass must have been greater in the kidney cells of the alkalotic animals than in the controls. Renal RNA concentration was similar in all 3 groups.

To justify the statement that an increase in enzyme activity is due to induction, the following criteria must be fulfilled(14,15).

‖ Unpublished data.

TABLE I. Effect of 3 Weeks Administration of Acid or Alkali on Body Weight, Kidney Weight and Concentration of Desoxyribosenucleic and Ribosenucleic Acids in Kidney of Guinea Pig.

Group (No. of animals)	Body wt		Kidney wt		Nucleic acids	
	Increase, %	Final, g	% body wt	g	DNA mg/1000 mg d.w.‡	RNA mg/1000 mg d.w.‡
Control (6)	20 ± 3†	533 ± 17	.79 ± .03	4.1 ± .3	45.0 ± 3.2	4.8 ± .3
Acidotic (6)	*10 ± 3	*476 ± 10	.86 ± .04	4.0 ± .2	43.2 ± 3.3	4.6 ± .2
Alkalotic (5)	23 ± 2	528 ± 26	.83 ± .04	4.3 ± .3	*36.9 ± 1.6	4.2 ± .2

* Differs from controls, P <.05. † Mean ± S.E. ‡ Dry wt.

First, there must be an increase in enzyme concentration. To rule out the effects of altered permeability, cofactors, etc., homogenates should be employed for assays. Second, to eliminate an increase in enzyme activity due to an increased number of cells, enzyme activity should be expressed on a per cell basis. Since the DNA content of each cell of a species is relatively constant, enzyme activity is best expressed in terms of DNA. Third, since enzyme induction is a form of protein synthesis, it is dependent on amino acid and energy supplies and can be blocked by amino acid antimetabolites or agents blocking oxidative phosphorylation. In the present study the first two criteria have been fulfilled, while the third will be studied in a succeeding investigation.

Increased renal glutaminase activity occurred in the alkalotic as well as in the acidotic group (Table II). Activities of Glutaminase I, Glutaminase II, and the glutamine synthesizing enzyme were elevated in alkalosis and Glutaminase I in acidosis. The scattering of data with reference to Glutaminase I in alkalosis and Glutaminase II in acidosis was conspicuous and may, in some way, be connected with the metabolic alterations produced by the two conditions. The increase of Glutaminase I in acidosis is in agreement with the observations of Davies (1), who employed slices, and Rector(4) who used homogenates of kidneys from rats treated with acid. The contradictory results of Handler(2) and White(3) are probably due to the small number of animals used (only one or two acidotic animals were investigated) and the equivocal assay methods employed (non-optimal conditions).

TABLE II. Effect of Prolonged Administration of Acid and Alkali on Renal Glutaminases.

Group (No. of animals)	Glutaminase I*	Glutaminase II	Glutamine synthesizing enzyme
Control (6)	47 ± 10†	104 ± 9	98 ± 22
Acidotic (6)	§ 96 ± 12	140 ± 30	159 ± 24
Alkalotic (5)	‡141 ± 41	§172 ± 12	‡189 ± 29

* All enzyme activities expressed as μM NH_3 liberated or disappearing (glutamine synthesizing enzyme) from the incubation medium per 100 mg DNA per 30 min.
† Mean ± S.E.
‡ Statistically different from controls (P <.05).
§ *Idem* (P <.01).

The observation that glutaminase activity in homogenates from alkalotic animals is higher than the controls differs from the observation of Davies(1) that the activity of this enzyme in slice preparations from alkali treated animals was lower than the controls. He, however, employed slices whereas homogenates were used in the present study. Slices not only hydrolyze, but even in the absence of added ATP, synthesize glutamine. Since our studies show for the first time an increased glutamine synthesizing enzyme activity following prolonged administration of alkali, the decreased formation of ammonia from glutamine which Davies found may have been a consequence of assaying for glutaminase in the presence of increased glutamine synthesizing enzyme. Glutaminase II activity, which was observed in the present study to be increased by treatment with alkali, was not studied by Davies.

Whether glutamine from the blood is the only substrate for ammonia production or whether glutamine synthesized intracellularly is an additional source of ammonia is not known. Since both acidosis and alkalosis stimulate the ammonia production and in-

crease the glutaminase activity of the kidney, it is possible that prolonged treatment with either acid or alkali could diminish both blood and renal glutamine stores and increase the glutamate available to the synthesizing enzyme. This in turn would stimulate the synthesis of glutamine by the kidney and lead to increased activity of the glutamine synthesizing enzyme.

Summary. Increased activity of the enzymes involved in ammonia metabolism in the kidneys of guinea pigs was produced by prolonged treatment with acid or alkali. When the results were expressed on a per cell basis, Glutaminase I was increased in acidosis while in alkalosis Glutaminases I and II and the Glutamine synthesizing enzyme were increased. These increases in activity are suggestive of enzyme induction though all the criteria for this process have not yet been fulfilled.

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Lipide Analysis of Cellular Fractions in Liver Necrosis.* (22734)

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Within the past few years considerable information has been obtained concerning the pathogenesis of necrotic liver degeneration. It may be produced by dietary(1), chemical (2), or virus(3) means. The biochemical changes occurring in the liver cell during the production of necrosis are probably different for each agent. The necrotic livers of rats fed a *Torula* yeast diet are characterized by a progressive failure of oxygen consumption, ketogenesis, lipogenesis and oxidation of labeled acetate to CO_2(4). Popper *et al.*(5,6) have observed that necrosis caused by bromobenzene administration produces a decrease in the concentration of liver esterase, succinic dehydrogenase and total lipides. However, no analyses were made on the phospholipide content of the liver. Biochemical detoxification of bromobenzene causes depletion of cysteine and its precursors such as methionine from the liver(5). Koch-Weser *et al.*(7) have suggested that the condition produced in the bromobenzene treated rats is an example of a chemical conditional amino acid deficiency. In view of the above findings, experiments were undertaken to determine the lipide content of the various cellular fractions of the liver following bromobenzene administration to ascertain whether the lecithins and cephalins are involved in this process.

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Experimental. Male albino rats of the