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GASTRIC FUNCTION IN *CAIMAN CROCODILUS* (CROCODYLIA: REPTILIA)—II. EFFECTS OF TEMPERATURE ON pH AND PROTEOLYSIS

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Abstract—1. In *Caiman crocodilus*, the pH of the gastric secretions during digestion may reach 1·2 in specimens kept between 20 and 30°C. *C. crocodilus* feeds and digests at 15°C, but the pH of gastric secretions did not drop below 1·8.

2. The gastric secretion increased in rate and hydrochloric acid concentration after injections of histamine (400 μg/kg body weight, given in four hourly doses). The rate increased from 0·05 ml/kg . hr (resting rate, at 20°C) up to an average of 0·66 ml/kg . hr, at 30°C.

3. There is a latent period of 4–8 hr before the effects of histamine can be detected in tests carried out at 20, 25 and 30°C.

4. Peptic proteolysis of gastric secretions has pH optima in the range 2·0–2·6, when carried out at 25°C, using the hemoglobin method of Anson & Mirsky.

5. There were instances of more than one pH optimum for proteolysis, suggesting the occurence of isozymes.

6. Inside the range in which *C. crocodilus* feeds and digests, peptic proteolysis has an average Q_{10} of 1·62 (between 15 and 25°C) and of 1·50 (between 25 and 35°C).

7. In animals maintained at 25°C peptic proteolysis was highest at 24–48 hr after feeding.

INTRODUCTION

CROCODILIANS feed on a variety of relatively large food objects. The food is digested in a process that seems clearly to be temperature dependent inside the range 15–35°C (Diefenbach, 1975b).

Previous studies showed that *Caiman crocodilus* has a size (and age) dependent thermal preference, which does not relate to the digestive state (Diefenbach, 1975a). The availability of thermal energy permitting the attainment of different body temperatures does influence the willingness to feed (Diefenbach, 1975a).

The present study considers some simple chemical events, specifically hydrochloric acid activity and peptic proteolysis. It was again carried out on semi-restrained animals that were feeding freely (Diefenbach, 1975a). The gastric secretions were again sampled via in-dwelling catheters, providing a longitudinal record of individual animals when fasting and through a series of feeding cycles.

MATERIALS AND METHODS

Sixteen specimens of *Caiman crocodilus* coded as small (eight specimens ranging between 30·5 and 38·5 cm total length), medium (six specimens ranging between 56·0 and 65·0 cm total length) and large (two specimens, ranging between 84·0 and 96·0 cm total length) were used for this study. These animals were fed neonate mice to an amount equal to 5 per cent of the body weight at each feeding. Animals on this schedule of feeding appeared in good health, but were always ready to eat again less than 1 week after a feeding. In one series of experiments the medium animals were fed *ad lib.* The adult mice, skinned in order to avoid accumulation of hair in the stomach, were fed to the large animals only.

The gastric contents were sampled by the methods described in a previous publication (Diefenbach, 1975a) according to the following schedule: prior to feeding (fasting), 6, 12, 24, 36, 48 hr after feeding and at 24-hr intervals thereof.

In a series of pilot experiments all animals were fed once with beef muscle, in order to determine if the results obtained with a diet of mice could be influenced by (1) hydrochloric acid present in the stomach of the prey, or (2) the byproducts of digestion and proteolytic enzymes present in the stomach of the prey. Since the caimans may mechanically disrupt the prey while handling it, the contents of the prey's stomach could "spill" into the stomach of the caimans. As the pilot experiments showed no physiological events which could be attributed to these factors, all subsequent experiments used neonate and adult mice as food. In order to minimize any possible influence the mice were left without food for at least 4 hr prior to use.

The pH of the fluids was measured at a temperature of 25°C (±0·5°C) within less than 2 min after collection,

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with a Beckman Zeromatic pH meter, equipped with a Model 39030 Beckman Combination Electrode which, when used with a narrow bottom tube, allows measurements of samples as small as 0·2 m^1 to an accuracy of 0·1 units of pH. The accuracy of the pH meter was frequently checked with known concentrations of hydrochloric acid (0·1, 0·05, 0·01 and 0·005 N). Immediately after measuring the pH the samples were cooled to −1°C and centrifuged at 4000 rev/min, or frozen and stored for later centrifugation and analysis. The residue precipitated by centrifugation was discarded, as were any lipids, since it is the standard procedure to analyze only the aqueous fraction for peptic proteolysis.

The measurements of pH were carried out at 25°C because: (1) the available tables for conversions from pH to concentration of hydrogen ion give coefficients of activity at 25°C, and (2) no differences could be detected between the pH of the gastric samples at 30, 25, 20 or 15°C. Since all of the samples were measured at the same temperature, the information obtained could serve for comparisons of the concentrations of hydrochloric acid secreted. The activity of the hydrogen ion increases with increasing temperature, but in the range of these temperatures no differences were apparent. Furthermore, conversion to the actual pH at a given temperature may be calculated, given the coefficient of activity of the hydrogen ion at this temperature.

Peptic activity was analyzed by the hemoglobin method of Anson & Mirsky (1932) as described in Hawk *et al.* (1954), with bovine hemoglobin (Worthington Corp.) dissolved in potassium chloride–hydrochloric acid buffer at the required pH used as substrate. The stock hemoglobin solution was in a 2% concentration, at pH 2·0. The desired pH was achieved by adding to the stock solution 0·1 N hydrochloric acid (for pH's below 2·0) or potassium hydroxide (for pH's above 2·0). The volume ratios were of respectively 1 : 5 : 10 for sample to be tested–hemoglobin solution–trichloroacetic acid solution. A parallel test was carried out for every incubation using mammalian (hog) pepsin, at a concentration of 25 mg/100 ml, in pH 2·0 hydrochloric acid solution. This concentration was chosen after pilot tests showed that it yielded absorbances in a range similar to the absorbances of the tested samples, and for the same dilutions. This procedure was taken to provide a basis for future comparisons of proteolytic activities, since porcine pepsins are the most studied among mammalian pepsins (Frutton, 1971). The concentration of amino acids of the incubations was determined by measuring the absorbance at 280 μm, in a Beckman Model DU spectrophotometer.

Most samples were diluted to half the original concentration (as obtained from the animals) with a 0·01 N solution of hydrochloric acid (because the samples are acid gastric juice) to extend the small amounts of each sample available. Dilution allowed more tests under different conditions.

The following tests were carried out on the samples: (1) proteolysis as a function of temperature, (2) proteolysis as a function of the pH of the medium and (3) proteolysis as a function of the time since feeding. In the first test the samples were incubated at 15, 25 and 35°C. When a sufficient sample was available tests were also carried out at 20 and 30°C. The expected pH was always 2·0, and the pH of every sample was checked during incubation. The samples were incubated in a water-bath at controlled temperature. Both hemoglobin substrates were brought to the desired temperature prior to mixing. Since 80 sec of stirring blank solutions (without enzymes) in the water-bath attained more than 99% thermal equilibration, this time was used. Before stirring, samples, as well as hemoglobin substrates, were kept below 2·0°C.

In the second test each sample was split into at least four fractions, each incubated at 25°C in the controlled temperature bath. The hemoglobin substrates were brought to the desired pH by careful addition of hydrochloric acid or potassium hydroxide. The buffer had its greatest power in the range of 1·2–2·0. Nevertheless, successful buffered incubations could be obtained up to a pH of 3·5. The pH of every sample was checked once during incubation.

In the third test several sequential samples from every animal were incubated at 25°C and with hemoglobin substrate at a pH of 2·0.

In order to obtain samples uncontaminated by food experiments were carried out on the effects of histamine. Four medium size animals that had the stomach empty of food were injected into the tail muscles with histamine (free base) to a total of 400 μg/kg body wt. The histamine was administered in a concentration of 100 μg/ml of a solvent containing 7·5 g of sodium chloride per 1000 ml of water, at a dosage of 100 μg/kg body wt per hr, over a 3-hr period. The saline concentration approximated the slightly lower osmolarity of the body fluids of crocodilians, compared with those of mammals (Dessauer, 1970). Gastric contents were sampled immediately prior to the first injection, and at intervals of 2 hr after the last injection. As controls each animal subjected to the histamine injections was checked without injection and with hourly injection of equivalent volumes of physiological saline. The first control established possible effects due to handling and sampling the animals. Those animals most easily handled and most docile were chosen for these experiments.

Since drinking during the tests on histamine could introduce uncontrollable effects (the water in which the animals were kept had at times a pH of 8·5–9·0), the animals were placed in a "moist" cage without drinking water 3 hr prior to the onset of the injections. To minimize integumentary and ventilatory losses of water, several layers of wet paper towelling were placed on the floor and walls of the cage, and its top was covered with a cotton cloth. After each set of injections or samplings the animals were sprinkled with a fine spray of water. A check with a psychrometer showed that a 90–95 per cent relative humidity can be maintained in this manner.

RESULTS

Changes in pH during digestion

In the samples collected 6 hr after feeding, the pH was invariably above 2·0, even in those animals in which the pH of the gastric fluids was below 2·0 before feeding (see Fig. 1). Twelve hr after feeding, when the next sample was taken, the pH was consistently lower. The results of these changes in pH as a function of time are graphed in Fig. 1. Since the pH of the gastric contents was similar for all sizes of animals under all conditions, and for both sampling methods, the results were combined

for the calculation of the means. After conversion of the pH to the product

(concentration of hydrochloric acid) × (coefficient of activity)

according to the formula given by Robinson (1972), the mean value was calculated. This product, calculated for every pH recording, was then averaged, and the result converted back to pH, for plotting in the graphs. This procedure is necessary since pH is logarithmic, and it is incorrect to calculate averages of pH values directly (Davenport, 1971).

The pH changes during gastric resident time followed a similar pattern at all temperatures at which the animals were kept. There was an initial rise, recorded at the sixth hour, and a drop between the sixth and the twelfth hour; this drop in pH continues, for a temperature-dependent period (Fig. 1). After some time, during which the animals still have food in the stomach, the pH starts to rise again. Paralleling this rise in pH, the samples start to show less residue after centrifugation. The amount of residue during gastric residence was very variable. It ranged from approximately 10 per cent up to 40 per cent of the sampled volume.

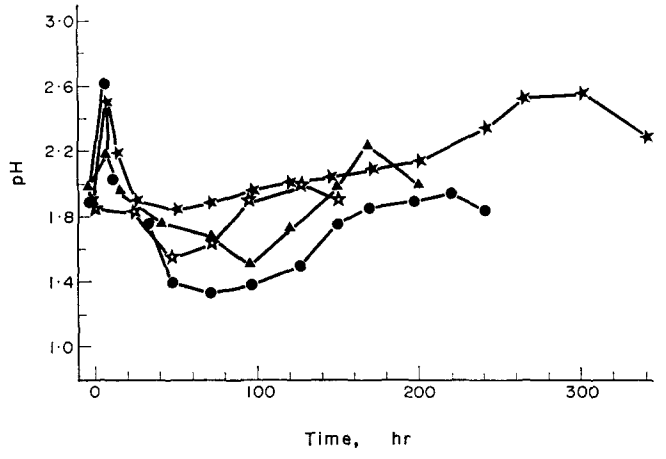

Fig. 1. Illustration of the mean pH of gastric contents during digestion, at 15, 20, 25 and 30°C. ★, 15°C; ●, 20°C; ▲, 25°C; ☆, 30°C.

Table I. Peptic activity as a function of time of digestion.

Sample No.	Time of sampling (hours before or after feeding)						
	−2	12	24	36	48	72	96
1	0·080	0·115	0·165	0·230	0·250	0·175	0·180
2	0·050	0·090	0·215	0·200	0·210	0·160	0·135
3	0·110	0·125	0·160	0·270	0·255	0·200	0·165
4	0·065	0·170	0·210	0·215	0·290	0·235	0·190
5	0·090	0·145	0·160	0·185	0·200	0·180	0·155
6	0·100	0·190	0·245	0·255	0·230	0·190	0·110
7	0·030	0·095	0·175	0·215	0·200	0·180	0·130
8	0·050	0·130	0·165	0·220	0·225	0·205	0·140
9	0·010	0·110	0·240	0·305	0·270	0·255	0·220
$\bar{X}$	0·065	0·130	0·190	0·230	0·235	0·195	0·160
S.D.	0·030	0·030	0·050	0·035	0·030	0·025	0·030
$\bar{X}$/tyr.	0·175	0·351	0·513	0·621	0·635	0·527	0·432

Samples collected from animals kept in water at 25°C. The values given are the absorbance attributed to peptic activity as described in the text. The values of absorbance are rounded to the nearest 5/1000 (limits of accuracy of the spectrophotometer). The values of means and S.D. are also so rounded. For comparison, tyrosine, 50 μg/ml, had an absorbance of 0·370. The line $\bar{X}$/tyr. gives the ratios between the mean absorbances of the samples of one column (time of sampling) and the absorbance of the amino acid tyrosine, in the concentration mentioned above. The animals were fed at time zero.

Fasting (resting) samples and pH

Attempts, successful in 60 per cent of the cases, were made to collect samples from all animals prior to every feeding, at each test temperature. The volumes collected ranged from a few drops (less than 0·3 ml, barely enough to measure the pH) to 2·0 ml. The pH of these fluids from fasting animals of all sizes ranged between 1·7 and 3·0 These fluids were frequently light yellow and sometimes (less than 10 per cent of the cases) green colored. The residue after centrifugation of the fasting samples was very small, less than 10 per cent, and as a rule less than 5 per cent of the collected volume.

Proteolytic activity with time after feeding

Samples collected from animals kept at 25°C were incubated at 25°C, with hemoglobin substrate buffered at pH 2·0 (Table 1). Only those cases where the pH remained at this value during incubations of samples from a single animal were used in the numerical analysis of the results. The peptic activity (i.e. concentration of proteolytic enzymes) was highest in samples collected between 24 and 48 hr after feeding. The mean highest activity occurred 36 hr after feeding.

Proteolytic activity and pH

The samples used in these tests were collected from animals maintained at 25°C and were collected within 24 or 36 hr after feeding. The pH dependence of peptic proteolysis is illustrated in Fig. 2. Some samples had more than a single pH optimum (see Fig. 2 for an example). Most samples successfully analysed had pH optima between 2·0 and 2·2. In 20 per cent of the cases the pH optimum was between 2·4 and 2·6. In a few cases (four out of twenty-six incubations) the peptic activity was very similar at pH's of 2·0–2·2 and 2·4–2·6.

Figure 2 shows that the slope of the plot is steeper below pH 1·8. The increment in peptic activity (measured as the ratio between absorbances) is between 0·8 and 1·3 between the pH's of 1·4 and 2·0, while the negative increment (absolute value of the decrement) is only on the order of 0·1–0·3 between the pH's of 2·0 and 3·0. The illustration shows only those incubations yielding at least an interval of 0·3 units of pH between the incubations of the same sample. It was sometimes impossible to obtain the desired pH for an incubation, especially for pH's above 2·0, since the hydrochloric acid–potassium chloride buffer loses most of its buffering power above 2·0.

Proteolytic activity and temperature

Between 15 and 35°C the peptic activity is a direct function of temperature. With a few exceptions the slope (Q_{10} effect) is apparently steeper in the interval 15–25°C than in the interval 25–35°C. In a few cases (see samples 2, 5 and 7 in Table 2) the increment is greater in the last 10°C interval. These differences in Q_{10} effect were apparently independent of any known variable during the experiments.

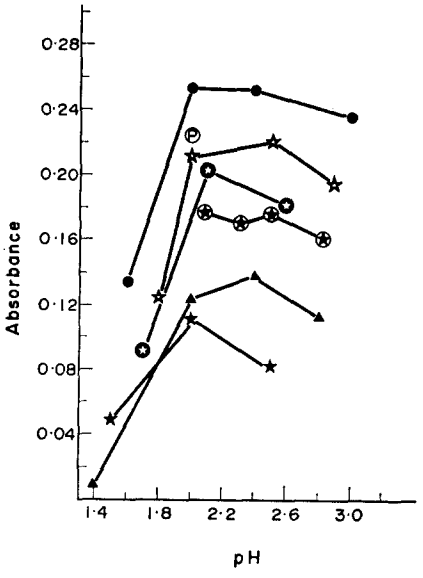

Fig. 2. Graph of the peptic activity as a function of pH of the incubating medium. Each symbol represents the incubations of one sample. The letter P, encircled, represents the absorption yielded by the mammalian pepsin, incubated in parallel. Some samples incubated at four different pH's and others at only three different pH's (see explanation in the text). For comparison, tyrosine, 50 μg/ml, had an absorbance of 0·370.

Tests were carried out on samples from animals kept at 25°C, and collected at the thirty-sixth hour after feeding. The hemoglobin substrate was again buffered at a pH of 2·0, but the expected pH of 2·0 could not always be obtained. The results hence include only those incubations where all tests of one sample yielded the same pH.

Histamine administration

Initially, injections of histamine yielded unclear results. The gastric fluids of the medium-sized animals sometimes had a marked fall in the pH. Other experiments did not show any change, either in pH or in volume secreted. A careful check of the conditions of the experiments revealed that consistent results could only be obtained from animals in which the stomach had been empty for at least 5 days. Therefore, all subsequent experiments on the effects of histamine were carried at least 7 days past the gastric residence time. This procedure led to positive and replicable results from histamine injections.

Table 2. Absorbances as a function of temperature

Sample	Q_{10} and temperature of incubation (°C)				
	15	Q_{10}	25	Q_{10}	35
1	0·130	1·73	0·225	1·37	0·310
2	0·090	1·61	0·145	2·03	0·295
3	0·140	1·53	0·215	1·34	0·290
4	0·165	1·69	0·280	1·25	0·350
5	0·110	1·40	0·155	1·80	0·280
6	0·140	1·67	0·235	1·31	0·310
7	0·085	1·52	0·130	1·88	0·245
8	0·185	1·59	0·295	1·35	0·400
9	0·150	1·60	0·240	1·35	0·325
10	0·155	1·87	0·290	1·50	0·435
11	0·105	1·71	0·180	1·41	0·255
Average Q_{10}		1·62 (±0·11)		1·50 (±0·25)	

The absorbances (at 280 μm) are an indication of the concentration of products of peptic proteolysis. The columns of Q_{10} give the increment in absorbance between the immediately preceding and the immediately following temperatures of incubation for the same sample. For comparison, tyrosine, 50 μg/ml, had an absorbance of 0·370.

The results of these experiments are summarized on Table 3. Figure 3 illustrates one case with one animal, showing pH changes after the injections. As noted previously, in 60 per cent of the fasting animals gastric fluids could be obtained. The pH of these fluids was sometimes as low as 1·7. Nevertheless, a statistical analysis showed a significantly greater decrease in pH after histamine injections from the normal levels found in the fasting animals ($P<0·005$, F-test). A comparison of the results of histamine stimulation with the two controls also indicates that the stimulus is effective (Fig. 3 for an example).

Table 3. Secretory rates and resting gastric pH

	After histamine	Controls
20°C	r: 0·08–0·25 $\bar{X}$: 0·16	r: 0·00–0·12 $\bar{X}$: 0·05
25°C	r: 0·20–0·50 $\bar{X}$: 0·34	r: 0·00–0·23 $\bar{X}$: 0·06
30°C	r: 0·35–1·20 $\bar{X}$: 0·66	r: 0·07–0·24 $\bar{X}$: 0·13

Resting pH's at 25°C

Small	(N: 15) r: 1·7–2·9 $\bar{X}$: 2·0
Medium	(N: 14) r: 1·8–3·1 $\bar{X}$: 2·2
Large	(N: 14) r: 1·9–2·5 $\bar{X}$: 2·1

Values of secretory rates in ml/kg . hr.

It takes several hours for the response to histamine injections to become detectable. Generally, the marked fall in the pH appeared only 5–6 hr, or even 8 hr, after the first injection. The injections of histamine also increase the volumes secreted (collected) over control levels (0·05, $P>0·005$, F-test).

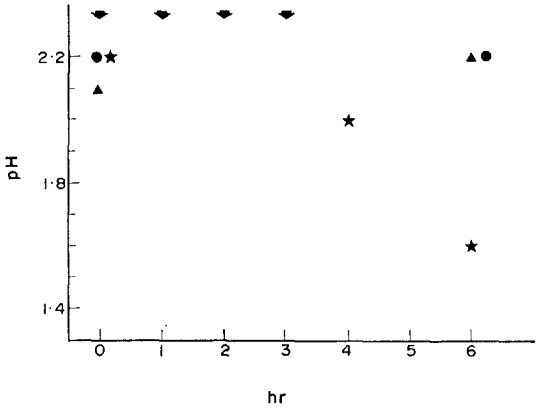

Fig. 3. Illustration of one experiment on the effects of histamine upon gastric secretion in an animal kept at 20°C. The solid circles and triangles indicate the controls carried out with the same animal. ●, Results of injections of saline alone; ▲, collection of gastric secretion without any injection. The stars indicate the results after histamine injection. The arrows at the top indicate the times of injection of histamine.

In 40–45 per cent of the cases the gastric fluids collected after histamine treatment were of marked green color, in contrast to the generally yellow and translucent colored samples obtained immediately before the histamine administration. Only 7 per cent of these samples were green. The green color of fasting (empty stomach) secretions is an indicator of bile pigments, suggesting a reflux of duodenal contents (Hawk *et al.*, 1955; Oser, 1965).

As can be seen from Table 3, the fall in the pH after histamine at all temperatures tested, never reached the low values recorded while digesting the standard diet. This difference is most clear at 20°C (one recalls that at this temperature the pH of gastric contents went as low as 1·2 during digestion).

DISCUSSION

Assuming that pure hydrochloric acid enters the gastric secretion (Table in Moore, 1968), the levels of pH reached during digestion (down to 1·2) indicate that *C. crocodilus* is able to elaborate a hydrochloric acid secretion that is at least seven times more concentrated than that reported for *Alligator mississippiensis* (Coulson & Hernandez, 1964). However, the concentrations of sodium, potassium and hydrochloric acid in the gastric secretions are dependent upon the secretory rate (Davenport, 1971). Levels of both sodium and potassium are higher at low secretory rates, the reverse being true for the concentration of hydrochloric acid. Sodium and potassium cause an underestimate of the level of hydrogen ion in the gastric juice (Moore, 1963, 1968; Moore & Scarlate, 1965). If one assumes that crocodilians also secrete other inorganic ions the concentration of acid may

actually be higher than suggested by the pH measurement. Even if *C. crocodilus* did not secrete sodium and potassium, these ions will certainly be released by the decomposition of the bolus. Thus, ionization of hydrochloric acid will be partially repressed as the coefficient of activity diminishes. The ions secreted into the stomach will only be recovered after the gastric contents pass to a site of intestinal resorption. Experimental evidence from *A. mississippiensis* suggests that crocodilians may attain plasma chloride levels as low as 7 m-equiv/l., and a parallel blood pH of 8·0 (Coulson & Hernandez, 1964). Further secretion of acid may therefore become limited by the availability of plasma chloride and/or tolerance to higher blood pH's.

The minimum pH of crocodilian gastric secretions (1.2) is lower than that reported for turtles. *Chrysemys picta* (killed) has a pH of 1·8 at one point during digestion (Fox & Musacchia, 1959). In theory at least, *C. picta* could reach still lower pH values, since its pH was not measured longitudinally along time, as done in the present study. The lowest pH recorded in *A. mississippiensis* was slightly below 2 (Coulson & Hernandez, 1964). *Tachysaurus rugosus* (killed immediately after feeding) showed a pH as low as 1·5 (Wright *et al.*, 1957); injections of histamine caused the pH to drop to 1·0. In *Natrix natrix* the pH was 3·9, 2 hr after feeding (Skokzylas, 1970); the rate of secretion, as well as the concentration of hydrogen ion was higher at 25°C than at either 15 or 35°C. Measurements made with dye indicators are doubtful (Moore, 1963, 1968). Only direct (by electrodes) measurement of pH can give a reliable indication of the activity of gastric acid.

In approximately 60 per cent of the samples successfully obtained from fasting animals the pH of the stomach fluids was below 2·5, and sometimes as low as 1·7. A similar low fasting pH is reported in *C. picta* (Fox & Musacchia, 1959). These findings imply that there may be a minimal or "baseline" constant secretory rate, producing and maintaining a "pool" of acidic medium in the non-digesting (empty) stomach. Since the present study required handling of the animals for the sampling process, there is a possibility that this acid resting secretion may have been induced by the handling. On the other hand, the sampling process generally took less than 5 min, which makes it an improbable cause. After a few experiments the animals could be sampled without visible signs of stress or excitement, provided that care was taken to avoid sudden movements and loud sounds.

Hydrochloric acid secretion of *C. crocodilus* responds positively to dosages of four injections of histamine (100 μg/kg body wt per hr) provided that the stomach has been empty for at least 5 days. This report differs from the findings in *A. mississippiensis*, in which the rate of hydrochloric acid secretion supposedly does not respond to histamine, even when dosages as high as 10 mg/kg body wt. are used (Coulson *et al.*, 1950; Coulson & Hernandez, 1964). However, these authors do not specify the times of sampling after the injections, nor the feeding conditions prior to the experiments. The difference in the response to histamine could also be an interspecific one, but higher dosages were not tried in *C. crocodilus* as it was beyond the scope of the present study, and these are known to disturb the ventilatory control (Coulson & Hernandez, 1964).

A cephalic phase of gastric secretion is known to occur in Carnivora (dogs in particular), where anticipation of food stimuli causes the stomach to secrete. Thus, the arrival of food in the stomach is preceded by a "pool" of hydrochloric acid at low pH. *C. crocodilus* often feeds in water (though swallowing with the head above water) and the ingested water would raise the pH of any resting secretion. Furthermore, crocodilians may drink voluntarily during the ingestion. These several kinds of water intake may well explain the rise in pH during the first hours after feeding. Such aspects must be considered when evaluating the gastric changes of pH of animals moving freely in water. The ingestion of water will lower the concentration of ions and enzymes; it may simultaneously create pH conditions nearer the optimum for peptic proteolysis. Even though the animals secrete less acid at lower temperatures, the presence of an initial "pool" of secretions may initiate digestion until the presence of the bolus stimulates further secretions.

Because the fasting secretions were sometimes green, one may assume reflux into the stomach of duodenal contents, including bile. The process of collection may be responsible for the findings, or, the reflux may be normal, as in mammals (Hawk *et al.*, 1954; Davenport, 1971). The bile (Hofmann, 1968) and the alkaline pancreatic secretion cause partial neutralization of gastric contents.

There is a suggestion in *Caiman* ($0{\cdot}05 < P < 0{\cdot}5$) that the pH of the gastric contents reaches lower values at a temperature of 20°C. There are two ways of explaining this finding. The first is that the animals secrete more concentrated hydrochloric acid at this temperature. The second is that the neutralizing effects are slower at this temperature than at either 25 or 30°C, and better explain the low pH values recorded (H. W. Davenport, personal communication). At 15°C the secretory rate in those animals that do eat is very slow, and the concentration of hydrogen ion small; no pH values below 1·8 were recorded.

Peptic proteolysis

The present study shows that the pH of the gastric contents, at all temperatures, reaches and remains for long times at levels which are well below the pH optimum for peptic proteolysis. The rate of proteo-

lysis attributed to the pepsin(s) was found to be up to 2·5 times lower at pH levels actually measured during more than 60 per cent of the gastric residence time. The proteolytic activity of the samples was highest from 24 to 48 hr after feeding (from animals at 25°C body temperature) (see Table 1). At this time during the gastric residence period the pH was always below 2·0 (see Fig. 1), generally in the vicinity of 1·6–1·7. As the digestive process proceeded the pH rises again and the proteolytic activity of the sample decreases. These changes in proteolytic activity were interpreted as changes in concentration of enzymes, since the pH and the temperature of incubation were held constant during these tests. The higher concentration of proteolytic enzymes at the times of lower pH could, perhaps, compensate for the suboptimal pH conditions of the medium. Apparently it is advantageous for the animal to attain and maintain a high hydrogen ion concentration during gastric digestion, in spite of the fact that the environment will be suboptimal for the proteolytic action. One must consider also the possibility that at the initial stages of digestion the proteolysis occurs only at a relatively small surface area. During this initial phase, the actual microenvironment for the action of enzymes may be at a higher pH than what is recorded in a fluid sample (Barrington, 1957). The acid in the stomach cavity will be at higher concentrations at the sites near the mucosa. In the boundary between the large pieces of bolus and the bathing juice, neutralization is occurring. Thus there may be a gradient of acidity. In the sampling process used, such a gradient would not be detected.

Proteolytic activity as a function of temperature shows the expected positive slope between 15 and 35°C. Nevertheless, the Q_{10} is generally greater between 15 and 25°C than between 25 and 35°C. A statistical analysis of significance between the increments at the two 10°C intervals did not give significance ($P > 0{\cdot}05$) because there were three out of eleven cases in which the Q_{10} was greater between 25 and 35°C than between 15 and 25°C.

One criticism about the incubations in this study is that the samples were used in a rather crude state, with unknown concentrations of inorganic and organic substances. In order to characterize the chemical properties of an enzyme or mixture of similar enzymes (isozymes), one should work with the purest possible samples. However, the secretions of the crocodilian stomach are mixed with the products of the decomposition of the bolus and perhaps with reflux of duodenal contents. These admixtures can introduce uncontrollable variables into the results. Nevertheless, the main objective of the present work was an analysis of the actual process of proteolysis rather than a characterization of the proteolytic enzyme(s). The proteolytic enzymes in the stomach must act in a changing environment, in which factors such as electrolytes and digestion end-products appear and disappear, introducing depressing or facilitating effects on proteolysis (Smit, 1968).

The enzymes in the living animal will rarely be in an optimal environment. The present study suggests, for instance, that proteolysis may most of the time proceed under suboptimal conditions at least with respect to pH. The gastric contents are neutralized as digestion proceeds. This neutralization is due to the release of digestion end-products and, probably, associated with a slowing down of gastric secretion. The release of calcium ions from the ingested bones probably contributes largely to this neutralization, particularly in cases where a crocodilian eats prey with high calcium contents, like turtles (Coulson & Hernandez, 1964). Nevertheless, the present work shows that gastric contents of *C. crocodilus* only approach the pH optimum for peptic proteolysis (here established as 2·0–2·4) when the gastric residence period is terminating.

Since the present study is based on animals which ate spontaneously, no conclusions can be reached as to how digestion in larger animals would proceed at 15°C. The results obtained with small animals, even though limited in number, suggest that the secretory ability for hydrogen ion diminishes drastically as the temperature drops below 20°C.

If one assumes that larger animals tend to feed on larger prey, one has to ask about the advantages of not eating at low temperatures. The enzymatic and hydrolytic action of gastric secretions can only be exerted at the exposed surfaces of the bolus. Larger prey, with a relatively smaller surface-to-volume ratio, would take longer to be digested, than would the proportionally equal objects ingested by a smaller animal. Consequently, the core of the bolus may not actually be attacked until putrefaction has already set in.

The tests for peptic activity as a function of pH show some instances of two pH optima (see Fig. 2), leading to the suspicion that peptic isozymes may have been involved. Peptic isozymes have been described in other vertebrates, generally having different pH optima, as well as different resistance to denaturation with alkali (Buchs, 1971). Peptic isozymes have been described in fishes (Merrett *et al.*, 1969), birds (Donta & van Vunakis, 1970), man (Etherington & Taylor, 1969; Samloff & Townes, 1970; Samloff, 1971) and other mammals (Chiang *et al.*, 1967; Chow & Kassel, 1968). Nevertheless, caution must be exercised before asserting the existence of isozymes. Some products of peptic autolysis, for instance, still have proteolytic activity, with a different pH optimum (Bovey & Yanari, 1960). Of course, the possibility of several different pepsins, with different pH optima cannot be ruled out. Proof of the existence of isozymes, on the other hand, will require more refined techniques, involving electrophoresis of purified samples.

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