EFFECT OF AGE AND GLUCOCORTICOID ADMINISTRATION ON THE PROTEOLYTIC ACTIVITY OF GASTRIC MUCOSA: A COMPARATIVE STUDY IN THE YOUNG RAT, CALF AND PIGLET

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Summary

The proteolytic activity of gastric mucosa markedly increased from 2 to 4 wk of age in rats and from birth to 8 wk in pigs. In calves it was at adult level at birth, then decreased until d 14 and remained at a relatively low level up to d 56 of age. Early postnatal glucocorticoid injections increased (P<.05) the proteolytic activity of gastric mucosa in rats, decreased (P<.05) the abomasal proteolytic activity in calves and had no significant effect in pigs. The high levels of proteolytic activity observed in the rat at earlier ages after glucocorticoid treatment suggested a precocious maturation of the gastric mucosa. Injections of different doses of hydrocortisone acetate and dexamethasone to calves and rats indicated that proteolytic activity of gastric mucosa is only sensitive to high doses (i.e., nonphysiological) of glucocorticoids. The response of gastric mucosa to glucocorticoid injections depended on the animal's age; maximum response was obtained in the early postnatal period while minimal response was observed after weaning.

(Key Words: Calf, Piglet, Rat, Proteolytic Activity, Gastric Mucosa, Glucocorticoids.)

Introduction

The development of digestive enzymes from birth to weaning differs among mammalian species. A marked increase in gastric proteolytic activity takes place between birth and 4 wk of age in rats (Furihata et al., 1972) and from birth to 8 wk of age in pigs (Hartman et al., 1961; Aumaître, 1971; Cranwell, 1977), whereas it does not seem to follow a definite pattern in calves. Huber et al. (1961) found that the level of calf abomasal protease activity was quite high at 1 d of age, increased to a maximum by d 8, decreased at d 15 and remained at this level up to d 44. Garnot et al. (1977) observed a threefold increase in pepsin activity of calf abomasal juice from 20 to 80 d of age while Henschel et al. (1961) and Henschel (1973) reported no marked change in the activity of pepsin secreted from 1 to 8 wk of age. In all these experiments on abomasal proteolytic activity, calves were separated from their dams at 2 or 3 d of age and were fed only whole milk or milk substitute without grain or hay.

Glucocorticoids are known to promote maturation of a variety of tissues (Ballard, 1979). These hormones are involved in the later stages of differentiation of the embryonic chick pancreas (Cohen et al., 1972) and fetal rat pancreas (McEvoy et al., 1976) and stomach (Yeomans et al., 1976). In the neonatal rat, glucocorticoids induced increases of digestive enzymes in the pancreas (Takeuchi et al., 1977; Hébert, 1978; Morisset and Jolicoeur, 1980), intestine (Doell and Kretchmer, 1964) and stomach (Furihata et al., 1972; Takeuchi et al., 1975; Pelletier et al., 1979). This response of the digestive enzymes to glucocorticoid injections was absent after weaning in rats (Sasaki et al., 1976) and mice (Kumegawa et al., 1978). A prolonged administration (19 d) of prednisone to pigs weighing 12 to 15 kg...
TABLE 1. PERCENTAGE COMPOSITION OF CONCENTRATE FED TO CALVES AND PIGLETS IN MEAL FORM

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Calf concentrate</th>
<th>Piglet concentrate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Creep feed</td>
</tr>
<tr>
<td>Corn (IFN 4-02-935)</td>
<td>10.00</td>
<td>55.00</td>
</tr>
<tr>
<td>Wheat (IFN 4-05-268)</td>
<td>11.75</td>
<td></td>
</tr>
<tr>
<td>Barley (IFN 4-00-549)</td>
<td>48.00</td>
<td></td>
</tr>
<tr>
<td>Oat groats (IFN 4-03-331)</td>
<td></td>
<td>20.00</td>
</tr>
<tr>
<td>Soybean meal (IFN 5-04-604)</td>
<td>16.00</td>
<td>21.00</td>
</tr>
<tr>
<td>Meat meal (IFN 5-09-323)</td>
<td>2.00</td>
<td></td>
</tr>
<tr>
<td>Limestone (IFN 6-02-632)</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>Sodium bentonite</td>
<td>.50</td>
<td></td>
</tr>
<tr>
<td>Salt</td>
<td>.50</td>
<td></td>
</tr>
<tr>
<td>Molasses (IFN 4-04-696)</td>
<td>10.00</td>
<td></td>
</tr>
<tr>
<td>Mineral and vitamin premix</td>
<td>.23^a</td>
<td>4.00^b</td>
</tr>
</tbody>
</table>

^a Mineral and vitamin premix contained per kg: vitamin A, 5,600,000 IU; vitamin D, 660,000 IU; vitamin E, 1,000 IU and contained (%): Mg, 4.4; I, .004; Zn, 4.4; Mn, 1.76; S, 10.5; Co, .20.

^b Mineral and vitamin premix contained per kg: vitamin A, 165,000 IU; vitamin D, 16,000 IU; vitamin E, 528 IU; vitamin K, 44 mg; riboflavin, 77 mg; thiamin, 33 mg; niacin, 550 mg; choline chloride, 10,560 mg; pyridoxine HCl, 37.4 mg; vitamin B₁₂, 275 mg and contained (%): salt, 10.0; Ca, 20.0; P, 8.5; Fe, .35; I, .0008; Zn, .2; Cu, .025; Mn, .125.

resulted in the development of stomach lesions without any significant change in pepsin secretion (Zamora et al., 1975a). The same authors also reported that plasma pepsinogen and plasma corticosteroid concentrations were not related to the presence or severity of gastric lesions in swine (Zamora et al., 1975b).

The objectives of this study were to determine 1) the pattern of change in the proteolytic activity of the developing calf abomasum as compared with that of rats and piglets and 2) the effect of glucocorticoid injections on the development of proteolytic activity in the gastric mucosa of neonatal rats, calves and piglets.

**Experimental Procedure**

Male Sprague-Dawley rats were used. The litters were adjusted to 10 males on the day of parturition by adoption of pups from another mother. The animals were housed in a room at 21°C with 40% relative humidity and alternative 12 h light and dark periods. The rats were weaned at 21 d postpartum unless killed at an earlier age. They were fed pelleted Purina Rat Chow ad libitum after weaning. The rats had free access to water. The animals were fasted overnight before being killed by decapitation. One-hundred-sixty Holstein-Friesian male calves weighing about 40 kg at birth were used. The calves remained with their dams for the first 2 d postpartum and then were moved to individual pens bedded with wood shavings in a building in which the temperature was maintained between 18 and 20°C with ventilation and supplemental heat when necessary. The calves were fed a calf concentrate (table 1) ad libitum and cow milk at the level of 10% body weight. The calves were killed by an injection of Euthanyl Forte⁵ in the jugular vein at a dose of .3 ml/kg body weight. Before killing, 1-, 3-, 7- and 14-d-old calves were fasted for 24 h while the 56-d-old calves were fasted for 48 h. Eighty-eight crossbred piglets (Landrace x Yorkshire x Duroc) from litters of eight piglets each were used. The piglets were raised on a commercial farm⁶ and housed in a barn maintained at 21 to 22°C with ventilation and supplemental heat when necessary. The piglets were weaned at 21 d of age and moved from the farrowing crates to metal rod cages of 1.2 x 1.8 m. They had access to creep feed (table 1) from 10 to 28 d and were fed ad libitum weaner diet (table 1) from 29 to 56 d of age. The animals had free access to water. The adult bovine and swine stomachs were obtained at a slaughter house from animals that were fasted overnight before

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⁵ M.T.C. Pharmaceuticals, Hamilton, Ontario.
⁶ Mr. Rolland Simard, North Hatley, Québec.
being killed. The proteolytic activity of gastric mucosa was determined on eight animals (pooled stomachs for rats) at each age of killing for the three species.

A single injection of hydrocortisone acetate\(^7\), dexamethasone\(^8\) or saline was given sc into the back of the neck of rats and im into a thigh of calves and piglets. Hydrocortisone acetate doses of 10 to 250 mg/kg of body weight and dexamethasone doses of .04 to 10 mg/kg of body weight were given between 7 and 17 d of age. Each litter of rats and piglets contained equal numbers of control and treated animals. Only four male piglets per litter were used to study the effect of glucocorticoid administration on gastric proteolytic activity.

The stomachs (abomasums for calves) were removed after killing without delay, opened up and washed with cold tap water. Secretory antrum and fundus areas were cut into large pieces (two pieces in the case of rats) and placed in Erlenmeyer flasks containing Krebs-Ringer phosphate (KRP) buffer at pH 6.9 at 40 °C. The gastric mucosa was scraped from the muscle layer with a microscope cover glass. To get enough tissue from rats for biochemical assays, tissue scraped from stomachs of animals were pooled; 10, 5 and 2 stomachs for pups under 18 d, at 18 d and older than 30 d of age, respectively. For the measurement of total proteolytic activity of the abomasum, the mucosa was separated from the muscularis for the whole abomasum. The scraped material or pieces of mucosa were placed in the KRP buffer at pH 6.9 for homogenization. The concentration of tissue in the KRP buffer for homogenization was 15% w/v for rats and pigs and 30% w/v for calves. Samples of homogenate (5 ml) were centrifuged at 235,000 \(\times\) g at 4 °C for 150 min and the supernatants were used to measure proteolytic activity.

Proteolytic activity was determined according to the method of Lanoë and Dunnigan (1978), which was based on the classic assay by Anson (1939). The enzymatic preparation was incubated at 37 °C at pH 2.0, which is optimum for pepsin activity, with an excess of bovine hemoglobin substrate\(^9\). The extent of proteolysis was determined by measuring the formation of trichloroacetic acid-soluble material as an increase in absorbance at 280 nm. One unit of proteolytic activity was the amount of enzyme that produced an increase in absorbance of .001/min of incubation at pH 2. Protein was assayed by the method of Lowry et al. (1951) and DNA according to Volkin and Cohn (1954) using calf thymus DNA as the standard. Portions of abomasal mucosa were fixed in 10% neutral buffered formalin and embedded in paraffin according to conventional methods. Histological sections of 6 μm thickness were stained with hematoxylin, phloxin and safran, and Periodic Acid Schiff stains.

Data were analyzed statistically by the analysis of variance (Steel and Torrie, 1960). A completely randomized design was applied on the rat and calf data and a split-plot design on the pig data (litter as a main plot). Two models were used, one including only one factor at a time (either age or hormone concentration), the other model included two factors (doses or types of hormones and age of injection).

**Results and Discussions**

The proteolytic activity of rat and pig gastric mucosa increased with age (figure 1). In the rat,
proteolytic activity remained relatively constant between birth and 16 d of age; it increased sharply up to 30 d of age and leveled off thereafter to adult levels at 56 d of age. In the pig, proteolytic activity increased progressively from birth to 56 d of age reaching adult levels. In the calf, the proteolytic activity was high on d 1, decreased until d 14 (significant differences being observed between d 7 and 14) and remained at a low level up to 56 d of age. The adult level was just slightly higher than that observed in 1-d-old calves. By 56 d of age rats have reached about 60% of the physiological development they achieve before the onset of puberty, whereas by 56 d of age calves and pigs have only reached approximately 10 and 25% of their prepubertal development, respectively.

The changes in proteolytic activity of gastric mucosa with age are in agreement with other reports on rats (Furihata et al., 1972) and on pigs (Hartman et al., 1961). Our data on the development of proteolytic activity of calf abomasum are in agreement with those of Huber et al. (1961), except for a higher level of proteolytic activity observed at 1 d of age in our calves. From d 14 to 56 there was no marked change in the proteolytic activity, which is in agreement with findings of other authors (Henschel et al., 1961; Henschel, 1973; Garnot et al., 1977).

The differences observed between the three species in the developmental pattern of the proteolytic activity of gastric mucosa may be the result of differences in management; calves were weaned at 2 or 3 d of age and piglets and rats were weaned at 21 d of age. Higher pancreatic amylase and chymotrypsin activities, and lower lipase activity have been reported for early weaned rats fed concentrate (Dumont et al., 1978). Higher stomach proteolytic activities have been reported for early weaned piglets fed milk substitutes (Decuypere et al., 1978). However, frequency of feeding (hourly vs twice daily) had no marked effects on the proteolytic activity of gastric mucosa of early weaned piglets when fed milk substitutes (Braude et al., 1970). Suckling piglets showed increases in pepsin secretions when creep feed was offered (Cranwell, 1977). In summary, early weaning or creep feeding generally increase the level of digestive enzymes in the gastrointestinal tract. Therefore, these factors would not appear to be responsible for the decrease in the proteolytic activity of calf abomasum from birth to 14 d of age. In addition, differences in dietary protein sources may not affect the proteolytic activity of the gastric mucosa in calves because replacement of casein by fish or soya proteins in the milk substitute did not influence the content (Garnot et al., 1974) or the secretion (Garnot et al., 1977) of pepsin, but only that of chymosin. Furthermore, liquid or dry feeding of concentrate did not seem to influence the secretion of the pancreatic enzymes (Gen Asher et al., 1981). Consequently, the high level of proteolytic activity of the calf abomasum at birth followed by a decrease in activity during the first 2 wk of life could merely be a natural phenomenon that is similar to the normal development of pancreatic chymotrypsin observed in rats (Larose and Morisset, 1977).

Hydrocortisone acetate injections resulted in an increased protease activity of rat gastric mucosa (figure 2). The 25 mg/kg dose produced a maximum effect at d 4 postinjection (13 d of age), while the increase continued up to d 7.
with the 100 mg/kg dose. The greatest effect was observed with the 250 mg/kg dose on d 7 postinjection. Dexamethasone at doses of 1 and 10 mg/kg was effective at 3 and 4 d after injection, but only the dose of 10 mg/kg increased the proteolytic activity of rat gastric mucosa at 7 d postinjection.

Gastric protease activity seems to be much less sensitive to glucocorticoids than other tissues. For example, 11-d-old rats receiving hydrocortisone acetate at a dosage of 5 mg/kg injected on three consecutive days showed an increased amylase activity in the pancreas (Hébert, 1978). In this study, the lowest dose of hydrocortisone acetate (25 mg/kg), which had very little effect on rat gastric protease activity, was above the physiological range; Loeb (1976) reported that 3 mg of hydrocortisone·kg of body weight⁻¹·d⁻¹ is a dosage equivalent to about twice the basal secretory rate of corticosterone, the principal glucocorticoid in the rat.

The long term effect of hydrocortisone acetate on rat gastric mucosa is shown in figure 3. An injection of hydrocortisone acetate on d 9 of age increased the proteolytic activity for the following 9 d only. The gastric proteases appeared to be much less sensitive to hydrocortisone when the steroid was given at an older age (Pelletier et al., 1979). In fact, animals injected at 30 d and killed at 34 d of age showed only a 35% increase (P > .05) in gastric proteolytic activity over that of control rats.

The specific proteolytic activity per mg of protein can be compared with the specific proteolytic activity per µg of DNA. The DNA content is generally recognized as a good indicator of cell number. These two methods of reporting specific activity gave similar results when comparing the evolution with age in figures 1 and 3 and the response to hydrocortisone acetate in figures 2 and 3.

Calves injected with 1.0 mg/kg body weight of dexamethasone 3 d after birth and killed 4 d later showed a decrease (P < .01) in abomasal proteolytic activity as compared with untreated calves (figure 4). At the .04 mg/kg dose, there was a nonsignificant reduction in protease activity. There was also a decrease (P < .05) in abomasal proteases after injection of .2 mg/kg dexamethasone at 3 d of age (figure 5). The effect was not significant when injection was given at 17 d of age. An injection of hydrocortisone acetate at 3 d of age also resulted in a marked reduction (P < .01) in proteolytic activity, but little effect was observed when the hormone was injected at 10 d of age (figure 6).

The specific proteolytic activity of the fundic mucosa from calf abomasum decreased (P < .05) after glucocorticoid injection at 3 d of age.
RESPONSE OF GASTRIC MUCOSA TO GLUCOCORTICOID INJECTIONS

Figure 5. Proteolytic activity of abomasal fundic mucosa of calves sacrificed 4 d after a single injection of dexamethasone (DEX); effect of age of injection. Proteolytic activity differed (P<.05) or did not differ (NS) from control values (n = 6). BW = body weight.

age (table 2). The total proteolytic activity of the abomasal mucosa was also decreased (P<.05) after hormone injections at 3 d of age. No effect on the proteolytic activity was observed when the glucocorticoid was injected at 17 d of age resulting in a significant glucocorticoid treatment x age interaction. Histological observations did not show degenerative changes of surface epithelium or the chief or parietal cells of the abomasal mucosa after glucocorticoid injection.

Glucocorticoid injections at different doses and ages of injection had no significant effect on the proteolytic activity of the gastric mucosa in piglets (table 3).

Figure 6. Proteolytic activity of abomasal fundic mucosa of calves killed 4 d after a single injection of hydrocortisone acetate (HA); effect of age of injection. Proteolytic activity differed (P<.01) or did not differ (NS), from control values (n = 6). BW = body weight.
### TABLE 3. PROTEOLYTIC ACTIVITY OF GASTRIC MUCOSA OF PIGLETS: EFFECT OF THE TYPE OF GLUCOCORTICOID, DOSE AND THE AGE OF ANIMALS

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose, mg/kg BW</th>
<th>Age, d</th>
<th>Proteolytic units/ mg protein</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>100</td>
<td>Injected</td>
<td>3</td>
<td>1,081</td>
</tr>
<tr>
<td>Hydrocortisone acetate</td>
<td>100</td>
<td>Killed</td>
<td>7</td>
<td>1,038</td>
</tr>
<tr>
<td>Saline</td>
<td>100</td>
<td>Injected</td>
<td>10</td>
<td>1,480</td>
</tr>
<tr>
<td>Hydrocortisone acetate</td>
<td>100</td>
<td>Killed</td>
<td>14</td>
<td>2,052</td>
</tr>
<tr>
<td>Saline</td>
<td>100</td>
<td>Injected</td>
<td>17</td>
<td>3,570</td>
</tr>
<tr>
<td>Hydrocortisone acetate</td>
<td>100</td>
<td>Killed</td>
<td>21</td>
<td>4,410</td>
</tr>
<tr>
<td>Saline</td>
<td>50</td>
<td>Injected</td>
<td>3</td>
<td>1,310</td>
</tr>
<tr>
<td>Dexamethasone</td>
<td>50</td>
<td>Killed</td>
<td>7</td>
<td>1,530</td>
</tr>
<tr>
<td>Saline</td>
<td>100</td>
<td>Injected</td>
<td>3</td>
<td>809</td>
</tr>
<tr>
<td>Dexamethasone</td>
<td>100</td>
<td>Killed</td>
<td>7</td>
<td>1,021</td>
</tr>
</tbody>
</table>

*None of the results (n = 4) reached the 5% level of significance.

BW = body weight.

The response of gastric mucosa to glucocorticoid injection differed in the three species studied. Glucocorticoid injections increased proteolytic activity in the rat, decreased activity in the calf and had no effect in the piglets. The high levels of proteolytic activity observed in the rat at earlier ages after glucocorticoid treatment suggest a precocious maturation of the gastric mucosa. The decrease in abomasal proteolytic activity observed in treated calves could also be interpreted as a precocious maturation because the injected 7-d-old calves ended with levels of proteolytic activity similar to those of control calves at 14 d of age.

The differing response of gastric mucosa to glucocorticoid injection among species may be the result of different plasma glucocorticoid concentrations among these species. The levels of plasma corticosterone at birth were 70 ng/ml in calves (Fairclough et al., 1975), 250 ng/ml in rats (Cohen, 1976) and 500 ng/ml in piglets (Dvorak, 1972).

Responses of rat gastric mucosa (figure 3) and calf abomasal mucosa (figure 5 and 6) to glucocorticoid injections were dependent on the age of animals, which is in agreement with the observations on the pancreas (Hébert, 1978) and the parotid glands (Sasaki et al., 1976).

These results showed that proteolytic activity of gastric mucosa follows a different pattern of development from birth to weaning in different mammals. This development does not seem to be under the control of glucocorticoids alone. However, a combination of hormones may be responsible for the development of proteolytic activity of gastric mucosa. In mice for example, a combination of thyroxine and hydrocortisone increased proteolytic activity of gastric mucosa and their effect was additive (Kumegawa et al., 1978).

**Literature Cited**


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