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Morphological changes in the small intestine of the fetal pig after prenatal stimulation of the sow with ACTH

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Summary. Twelve pregnant primiparous sows were catheterized on day 102 of gestation and randomly allocated to receive ACTH (days 112-113 of gestation [2d], days 105-parturition [10d]) or saline. At parturition the 3rd, 4th, 6th and 7th piglet born alive were sacrificed either at birth or at 6 h. The later group was fed bovine colostrum through a stomach tube at 30 min, 2 and 4 h of life. Following sacrifice, the small intestine of each piglet was excised, extended and measured. Piglets from sows in the 2d group tended to be heavier with longer small intestines than either the control or 10d groups. When the small intestine length was expressed as a function of body weight, the 10d group had the greatest ratio, suggesting that the pre-parturition maintenance of elevated cortisol levels either enhanced small intestine growth or attenuated overall weight gain. Two types of enterocyte granules were described at the level of the electron microscope: granular and opaque. Opaque granules from piglets born to sows in the 2d treatment group had both a lower volume fraction and were fewer per unit area when compared to those of either the saline or 10d treatments. The process of macromolecule uptake from the intestinal lumen appeared to have been interfered with as a result of an acute prenatal stimulation with ACTH. The above results suggest that in piglets, the level of circulating cortisol differentially controls the processes of IgG absorption, enterocyte replacement and small intestine growth through separate mechanisms. Further, these processes can be manipulated in the prenatal piglet.

Key words: Piglet, Immunoglobulin G, Intestinal absorption, Development, ACTH, Glucocorticoids

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Introduction

In the early stages of independent life, the protective capacity of a piglet's immune system is dependent upon the ability to absorb immunoglobulin, specifically immunoglobulin G (IgG) from maternal colostrum (Butler et al., 1981). The absorption of IgGs from colostrum is in part, determined by the capacity of the small intestine to take up macromolecules from the intestinal lumen and transfer them to the circulatory system (Moog, 1979). Soon after birth, fetal enterocytes cease transferring IgGs into circulation and a process begins whereby fetal enterocytes are replaced by mature enterocytes that are incapable of taking up IgGs (Patt and Eberhart, 1974; Patt, 1977). The absorption of immunoglobulin from colostrum to piglet circulation must therefore occur during the first few hours of life (Speer et al., 1959; Payne and Marsh, 1962).

Little is known concerning the effects of hormones, specifically glucocorticoids, on the prenatal development of the small intestine in the pig. Glucocorticoids are catabolic on most organs of the body (Nelson, 1980; Wilcke and Davis, 1982) but have been shown to be anabolic on the rat digestive and reproductive tracts (Silber and Porter, 1953; Clark, 1971). There is now evidence suggesting that glucocorticoids may have similar actions on piglet small intestine (Patt and Eberhart, 1976; Bate et al., 1991). In sows, circulating glucocorticoid levels rise immediately prior to labour and the increased level is maintained until the end of parturition when levels quickly return to prepartum values (Molokwu and Wagner, 1973). Since glucocorticoids are capable of diffusing across the placental membrane (Martin, 1985) piglets are born with high levels of circulating glucocorticoids (Silver and Fowden, 1989). Patt (1977) has suggested that the efficient process of IgG absorption in piglets is dependant upon glucocorticoids reaching an optimal circulating level. The enhanced level of glucocorticoid production in the

sow immediately prior to parturition ensures that fetal enterocytes are exposed to a sufficiently high level of the hormone and are thus primed to absorb immunoglobulins immediately following birth. Following birth, glucocorticoid levels in the newborn piglet quickly decrease and it has been postulated (Bate, 1993) that this rapid decline may trigger a set of events leading to a) the cessation of immunoglobulin transfer from the enterocyte into circulation and b) the replacement of fetal enterocytes by mature enterocytes. However, following termination of the transfer process, enterocytes continue to absorb IgGs from colostrum (Clarke and Hardy, 1971) which accumulate within the enterocyte cytoplasm and are thought to be eventually catabolized within phagolysosomes (Brown and Moon, 1979). The molecular debris may then be discarded into the lymphatic circulation or remain within the enterocyte to be discarded when the cell is sloughed off during replacement.

Later studies where preparturition glucocorticoid levels have been manipulated lend support to the above ideas. Increasing prenatal levels of glucocorticoids in the sow by either cold stress (Bate and Hacker, 1985a) or administration of ACTH for 10 days prior to parturition (days 105 - parturition) (Bate and Hacker, 1985b) led to an enhanced level of serum IgG in the piglet. The increased level of serum IgG may have resulted from a glucocorticoid induced increase in the total absorptive area of the intestine or via an up-regulation of enterocyte absorptive mechanisms. Termination of ACTH administration 3 days prior to parturition (day 112) (Bate et al., 1991) resulted in a prenatal decrease in the artificially enhanced serum glucocorticoid level in the sow. This was followed by the naturally occurring increase and decrease in serum glucocorticoid levels at the time of parturition. This manipulation did not lead to a significant increase in piglet serum IgG levels as compared to when plasma IgG levels were measured in piglets born to sows that had received ACTH until parturition (Bate and Hacker, 1985b). Further, Bate et al. (1991) found that the total absorptive area of enterocytes of new born piglets was not affected, rather, the organization of the intestinal epithelium was influenced, presumably by speeding up the enterocyte replacement process. The effect of enhanced prenatal glucocorticoid levels in the piglet following continuous ACTH administration in the sow may be to stimulate or up-regulate the mechanisms involved in IgG absorption. An *in utero* decrease in artificially elevated glucocorticoid levels in the piglet as a result of the cessation of ACTH administration to the pregnant sow may be similar to the decrease in piglet glucocorticoid levels following parturition and may act as a signal for enterocytes to shut down the process of macromolecular transfer and begin the process of enterocyte replacement.

In the above study (Bate et al., 1991) piglets fed colostrum for 6 h following birth showed a significant accumulation of electrodense granules within the enterocyte cytoplasm. These granules have been described by others (Smith and Jarvis, 1978) and putatively identified as accumulations of IgGs (Széki et al., 1979). These morphological observations are consistent with the idea that the decrease in circulating glucocorticoid levels following the natural prenatal elevation of these levels attenuates the processes involved in the transfer of macromolecules out of the cell while leaving uptake mechanisms intact. Further, prenatal cessation of ACTH treatment resulted in piglets with a heavier small intestine when expressed as a proportion of body weight (Bate et al., 1991), perhaps a result of an increased accumulation of IgGs within the enterocytes. These data support the idea proposed by Silber and Porter (1953) that glucocorticoids are anabolic on the digestive tract.

It is likely, therefore, that the result of a decrease in glucocorticoid levels that occurs naturally following birth acts as a signal to a) stop the transfer of macromolecules from the enterocyte into circulation and b) stimulate the process of enterocyte replacement. It was the objective of this study to measure the effects of different prepartum regimes of ACTH administration to the sow on these processes. Various physical characteristics and morphological parameters of the piglet intestinal epithelium were used to define the effects of prepartum ACTH treatment on the process of IgG absorption.

Materials and methods

Twelve commercial crossbred Landrace and Yorkshire primiparous sows bred to Duroc boars were used. All sows were housed in gestation stalls with water available *ad libitum* through drinking nipples. Prior to parturition sows were fed 2 kg of commercial 14% protein diet per day and following parturition the same food was available *ad libitum*. On day 102 of gestation, the sows were catheterized through the ear vein according to an established procedure (Bate and Hacker, 1985c). The catheters were kept patent with a continuous IV drip (0.9% NaCl, Travenol Company Ltd., Toronto) at a rate of 1 L d^{-1}. Following parturition, piglets (except those to be sacrificed immediately) were kept in a box under infra red lighting with temperature maintained between 33° and 35° C. Lighting in the farrowing room was maintained on a 12 h light:12 h dark cycle. All procedures were carried out in accordance with Canadian Council on Animal Care guidelines.

Each sow was randomly allocated to be treated with porcine ACTH (Sigma Chemicals Ltd., St. Louis, Mo.) for 2 days on days 112 and 113 of gestation (2d), ACTH for 10 days, from day 105 of gestation until parturition (10d) or continuous infusion of saline. Treated sows received a dosage of 1 IU ACTH kg^{-1} d^{-1} on a continuous IV infusion. At birth all piglets were dried and weighed. A blood sample from the sub orbital sinus was collected with a 2.5 cm x 20 gauge needle and processed as follows: the plasma from each sample was separated by centrifugation, harvested and sotred at

-20 °C for later measurement of IgG concentration.

Piglet numbers 3, 4, 6 and 7 born alive were randomly divided into two groups: those to be sacrificed immediately or at 6 h. Piglets in the 6 h group were fed 12.5 mL kg^{-1} bovine colostrum through a stomach tube at 30 min, 2 h and 4 h. First milk colostrum from 5 multiparous cows was freshly pooled and frozen in 200 mL aliquots in plastic bags. Prior to parturition the colostrum was thawed and warmed in a water bath at 37 °C. At the time of sacrifice, 0 h or 6 h, blood samples were taken from the sub orbital sinus, processed as described above and assayed for bovine IgG concentration. Bovine IgG concentrations were determined by radial immunodiffusion following the procedures developed by Mancini et al. (1965) with modifications described by Bate and Hacker (1985c). Sacrifice was by CO_2 inhalation followed by immediate exsanguination. The small intestine (SI) was excised, extended, measured and weighed. Samples of ileum were collected from the same area of the SI in each piglet, at 20% of the total SI length from the distal end of the intestine.

The samples of ileum were fixed in a solution of 1.0% glutaraldehyde/4.0% formaldehyde (v/v) in 0.1M phosphate buffer. Samples were postfixed with 1.0% osmium tetroxide in 0.1 M phosphate buffer, dehydrated and embedded in an epon-araldite mixture. Semi-thin (0.5-0.6 µm) and thin (70-80 nm) sections were cut with a Reichert-Jung Ultracut microtome fitted with glass knives. Semi-thin sections were stained with 1.0% toluidine-blue in 1.0% sodium borate and analyzed to determine the best specimen area for electron microscope (EM) viewing. Thin sections were placed on 200 mesh copper grids and stained with saturated uranyl-acetate in 50% ethanol for 30 minutes followed by immersion in lead acetate (Sato stain) for 2 minutes. Semi-thin sections were photographed with a Zeiss D-7082 Transmitted Light Photomicroscope 111.

A preliminary investigation of the semi-thin sections was carried out at the light microscope level followed by morphological characterizations at the electron microscope level. A representative sample of 3 electron micrographs was chosen from each piglet and analyzed using Bioquant System IV software (R&M Biometrics Inc., Nashville, TE). Each micrograph chosen had to have at least 3 enterocyte cells that were visible from the brush border to the basal lamina. All micrographs were analyzed by an experimenter blind to the treatment group. Where applicable, granules visible within the cell cytoplasm were divided into two groups: 1) opaque granules - granules that were opaque or darker than the surrounding cellular cytoplasm, and 2) granular granules - granules that were not opaque and of similar background consistency as the surrounding cellular cytoplasm. The following measurements were made from each enterocyte: total cell area, total nucleus area, total number of opaque or granular granules and the area of each opaque and granular granule.

The volume fraction of both opaque and granular granules was calculated as the ratio between the total granule area and the difference between the total cell area minus the total area of the nucleus.

Analysis of variance (one way) was conducted to determine the effect of sow prenatal treatment on piglet birth weight, SI length, SI to body weight ratio and piglet plasma IgG concentrations. To determine the effect of sow prenatal treatment on piglet birth weight, the litter size was used as a covariate.

Analysis of variance (two way) was conducted to determine the effect of sow prenatal treatment and time of sacrifice on the following histological measurements: volume fraction of opaque and granular granules, the number of each type of granule per unit area of cell cytoplasm and the average area of each type granule.

The data were analyzed as a split plot design with prepartum treatment as the main plot and the time of sacrifice as the sub plots. The analysis was conducted using Statistical Analysis system software (Spector et al., 1985). Where appropriate, post hoc analysis was conducted using the Student Neuman Keuls test.

Results

Physical characteristics

Piglet body weight at birth was significantly negatively correlated with litter size ($F[1,72]=14,38$, $p<0.01$). The smaller the litter the more a piglet tended to weight at birth. An increase in litter size by one piglet tended to result in a 201.4 g decrease in the average piglet birth weight. When the effect of litter size on body weight was removed by using litter size as a covariate, the drug treatment effect on piglet body weight was not significant.

The prenatal treatment was not significant on piglet SI length ($F[2,9]=0.41$, $p>0.05$). However, the SI length of piglets born to sows in the 2d treatment group tended to be logner than those of piglets born to sows receiving saline while the SI lengths of piglets born to sows in the 10d treatment group tended to be shorter (Table 1). When intestine length was expressed as a function of body weight (length:body weight), the highest ratio was observed in the 10d treatment group while the average length to weight ratios of piglets born to sows in the 2d and saline treatment groups were similar. The effect of sow prenatal treatment, however, did not reach statistical

Table 1. Physical characteristics of piglets born to sows that have undergone preparturition treatment with saline, ACTH for 2 days or ACTH for 10 days. The number in parenthesis represents the standard error of the mean.

TREATMENT	BODY WEIGHT (g)	SI LENGTH (cm)	SI LENGTH/ BODY WEIGHT (cm/g)
ACTH (2 Day)	1371.6±15.0	359.1±6.6	0.27±0.2
ACTH (10 Day)	1090.9±16.8	332.2±8.1	0.34±0.3
Saline	1275.0±18.0	339.8±7.7	0.29±0.2

significant for this measure (F[2,9]=2.20, p>0.05).

Sow prenatal treatment proved not to significantly influence plasma bovine IgG concentrations at both 0 and 6 h (F[2,8]=2.62, p>0.05). At 6 h, however, the piglets born to sows in the 2d group tended to have a lower plasma bovine IgG concentration. As expected, the effect of time was significant with plasma bovine IgG concentrations in piglets sacrificed at 6 h being higher than bovine IgG concentrations in plasma from piglets sacrificed at 0 h (F[1,2]=501.88, p<0.01).

Morphological Characteristics - Light Microscope

Light microscopy of sections of ileum of those animals sacrificed at 0 h reflected the state of the gut epithelium before it was exposed to colostrum. The enterocytes of all groups exhibited a columnar epithelium. The nuclei in these enterocytes tended to be closer to the cell apex regardless of the position of the enterocyte within the villus. Small clear vacuoles, approximately 3 μm or less in diameter, were found near the apices of many enterocytes, especially within piglets from the 10d treatment group. Goblet cells were observed randomly throughout all villi.

The ileal epithelium of piglets sacrificed at 6 h was characterized by enterocytes filled with multiple clear, lightly stained and heavily stained vacuoles of all sizes. These granules, especially the very large ones, were more prevalent near the base of the enterocyte while the nuclei in all groups were consistently located in the apical half of the enterocyte. There was no apparent difference in the distance between the brush border and the basal lamina between the three treatment groups.

Morphological Characteristics - Electron Microscope

The most recognizable characteristic of the enterocytes at the electron microscope level was the large number of darkly stained opaque granules in the enterocytes of piglets sacrificed at 6 h (Fig. 2) and the lack of these granules in those enterocytes of piglets sacrificed at 0 h (Fig. 1). Piglets sacrificed at both 0 and 6 h, however, exhibited a number of clear membrane bound granular granules. The granular granules, although few in number, tended to be positioned at the apical pole of the cell, whether they were from enterocytes from piglets sacrificed at either 0 h or 6 h. Further, they seemed to be relatively few in number and observed as often within all treatment groups. The opaque granules were of all sizes and observed only at 6 h in the enterocytes of all treatment groups.

The observation of numerous inwardly budding vesicles from the apical membrane in enterocytes of all treatment groups at both 0 h and 6 h suggested that a process of pinocytosis was actively occurring.

Morphological Quantification

The mean area of the cell was significantly greater at 6 h compared to 0 h (F[1,24]=8.60,

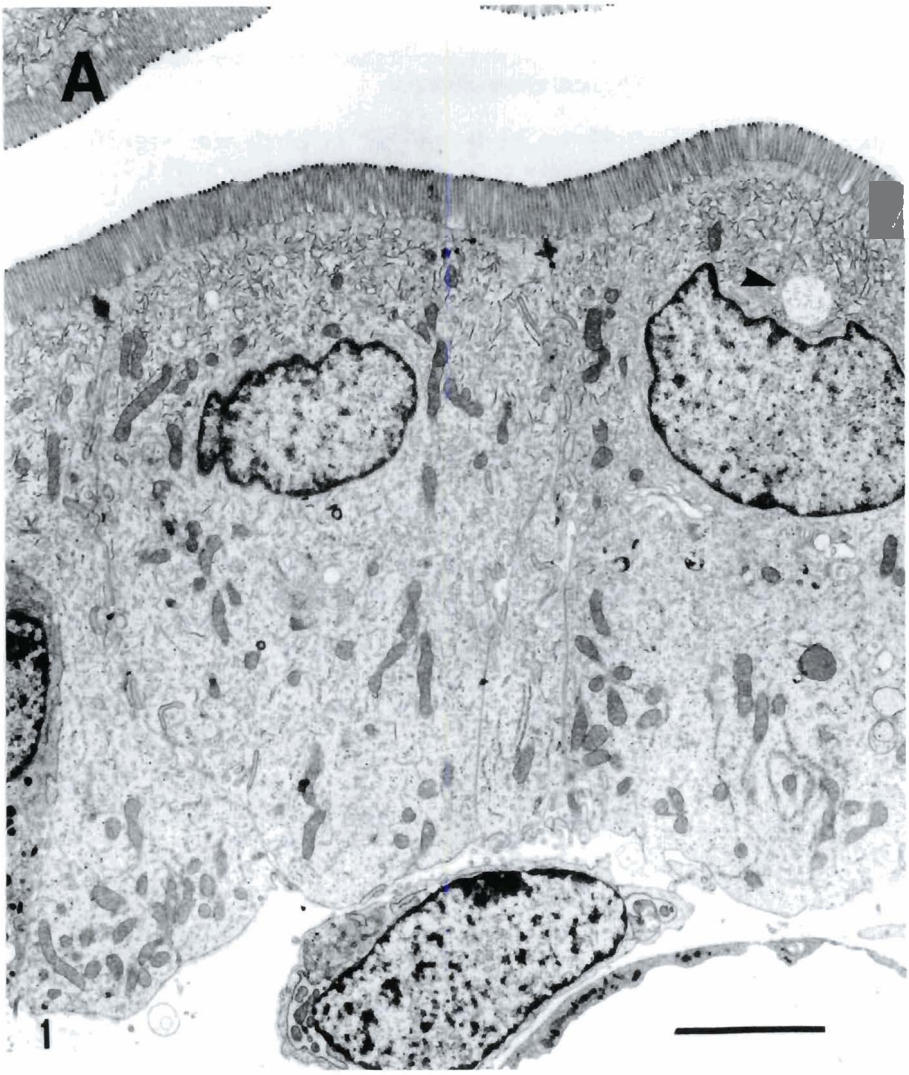


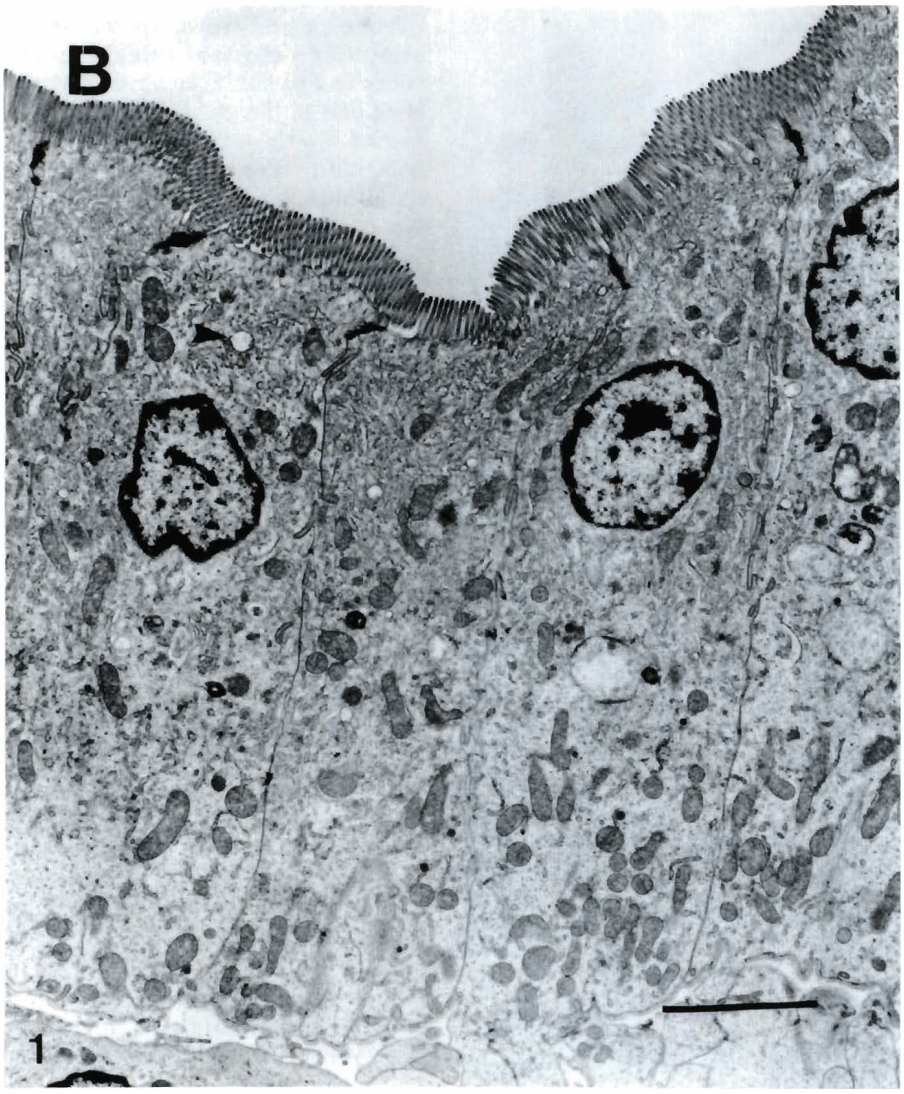
Fig. 1. Electron micrograph of ileum taken from piglets born to sows treated with Saline **(A)**, ACTH for 2 days **(B)**, ACTH for 10 days **(C)** and sacrificed at 0 hours. Arrowheads indicate granular granules characteristics of newborn gut. Bar=4 μm.

Table 2. The area (μm^2) of enterocyte cytoplasm, total granules and each type of granule (opaque and granular) in samples of ileum from piglets sacrificed at either 0 or 6 hours following birth. Piglets sacrificed at 6 hours were fed bovine colostrum prior to sacrifice.

PARAMETER	6 h	0 h	DIFFERENCE
Cytoplasm	540	410	130
Total granules	1222	8	114
Opaque	112	2	110
Granular	10	6	4

p<0.01), however, treatment and treatment by time interaction effects were not significant (F[2,9]=0.85, p>0.05 and F[2,24]=0.70, p>0.05, respectively). To determine if the increase in cell area was a result in an increase in either nuclear or cytoplasmic area, each cellular component was analyzed separately. There were no significant differences in nuclear area due to prenatal treatment or time of sacrifice (F[2,9]=0.16, p>0.05 and F[1,24]=4.70, p>0.05 respectively), nor was the interaction significant (F[2,24]=2.01, p>0.05). cytoplasmic area was greater at 6 h than at 0 h (F[1,24]=11.22, p<0.01) and neither prenatal treatment (F[1,9]=0.90, p>0.05) nor its interaction with time of sacrifice (F[2,24]=1.17, p>0.05) were significant.

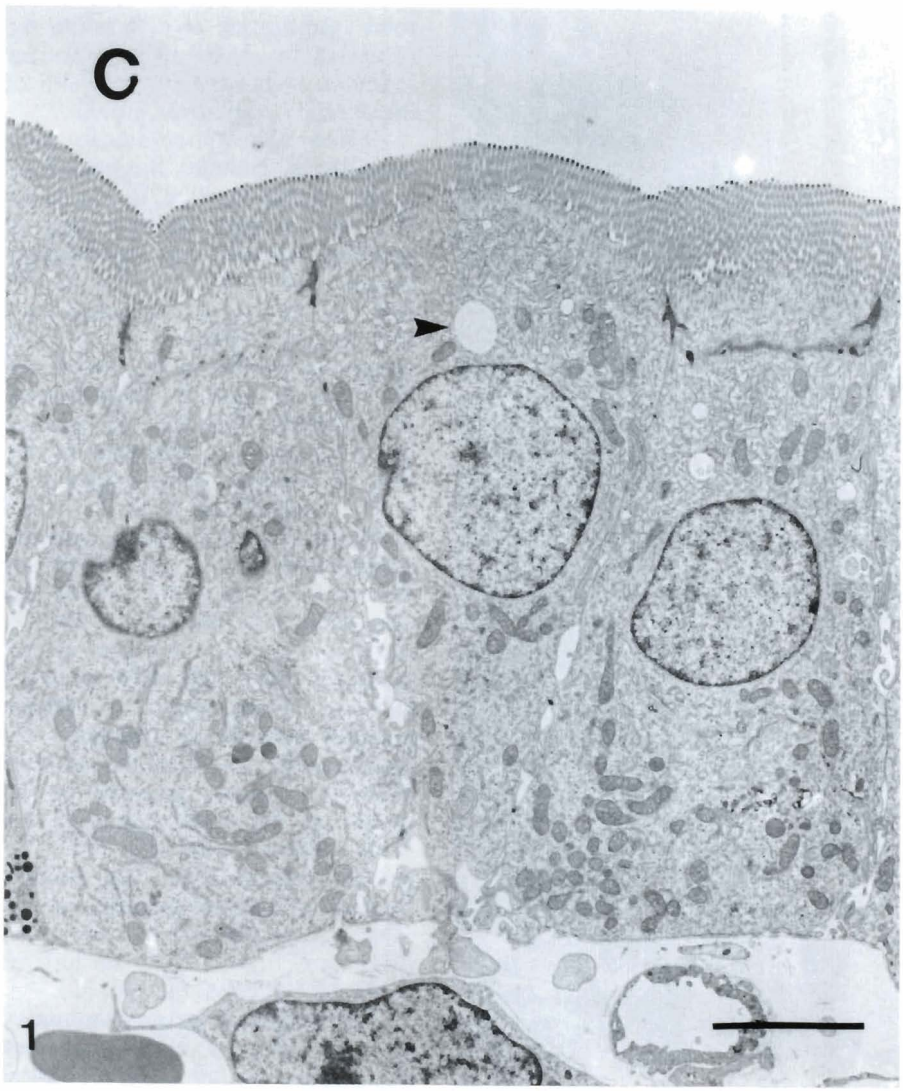
The total mean area of enterocyte cytoplasm occupied by either granular or opaque granules was greater at 6 h compared to 0 h (F[1,24]=11.67, p<0.01 and (F[1,24]=19.55, p<0.01, respectively) (Table 2). There were no significant treatment or interaction effects for either the granular (F[2,9]=0.64, p>0.05 and F[2,24]=0.99, respectively) or opaque granules (F[2,9]=0.16, p>0.05 and F[2,24]=0.20, p>0.05, respectively). The average area of the enterocyte cytoplasm at 6 h increased on average by 130 μm^2 (Table 2) compared to the average cytoplasm area of enterocytes at 0 h. The increased area of all granules at 6 h accounted for 88% of the cytoplasmic growth. The increase in area due to opaque granules accounted for 85% of the total cytoplasmic growth while the increase in area due to granular granules accounted for only 3% of the overall cytoplasmic growth.



When the cytoplasmic area occupied by granules was calculated as the volume fraction, it was found that the volume fraction due to granular granules was significantly greater at 6 h (F[1,24]=5.05, p<0.05), however, treatment and interaction effects were not significant (F[2,9]=0.04, p>0.05 and F[2,24]=0.32, p>0.05, respectively). The volume fraction of opaque granules was also affected by the time of sacrifice, being greater at 6 h (F[1,24]=57.85, p<0.01). The treatment effect on opaque granule volume fraction was also significant (F[2,9]=4.82, p<0.05) while the interaction was not (F[2,24]=2.77, p>0.05). Post hoc analysis of the treatment effect with SNK revealed that the volume fraction of opaque granules from the 2d treatment was significantly less than the volume fraction of opaque granules in the saline and 10d treatments (q[3,9]= 3.98, p<0.05) and q[2,9]=3.52, p<0.05, respectively). The volume fraction of opaque granules in the 10d treatment group was not different, however, from saline treatment (q[2,9]=0.62, p>0.05).

The number of granular granules per unit area of cytoplasm was significantly greater at 6 h (F[1,24]=

7.24, $p<0.05$) while treatment and treatment by time effects were not significant ($F[2,9]=1.64$, $p>0.05$ and $F[2,24]=0.77$, $p>0.05$, respectively). A similar pattern was observed with the number of opaque granules per unit area of cytoplasm, being greater at 6 h ($F[1,24]=46.42$, $p<0.01$). Treatment and treatment by time interaction effects, however, were also significant for the number of opaque granules per unit area ($F[2,9]=9.48$, $p<0.01$ and $F[2,24]=4.06$, $p<0.05$, respectively). The significant interaction can be explained at least in part by the observation that at 6 h the number of opaque granules per unit area was lower in the 2d group when compared to both the saline ($q[2,9]=4.62$, $p<0.01$) and 10d ($q[3,9]=4.53$, $p<0.05$) treatments. Further, the number of opaque granules per unit area in both the 10d and saline treatments was significantly greater at 6 h than at 0 h ($q[6,24]=7.73$, $p<0.01$ and $q[3,24]=5.38$, $p<0.01$, respectively). The difference in the number of opaque granules per unit area between 0 and 6 h was not significant for the 2d treatment ($q[3,24]=3.45$, $p>0.05$).



Discussion

Glucocorticoids are known to be catabolic on most tissues and organs in the body (Nelson, 1980; Wilcke and Davis, 1982), however, there is evidence that glucocorticoids have anabolic effects on the digestive tract of the rat (Silber and Porter, 1953) and piglet (Patt and Eberhart, 1976; Bate et al., 1991). In the present study, piglets born to sows treated with ACTH for the final 10 days of gestation tended to be lighter than piglets born to sows treated with saline (Table 1). It must be noted, however, that in the present study this difference did not reach significance. The trend of the present findings is consistent with previous results (Bate et al., 1991) which demonstrated that piglets born to sows treated with ACTH for 8 days (days 105 to 112 of gestation) were lighter than piglets born to sows treated with saline. The decreased weight of these piglets might be explained by an overall catabolic effect on piglet growth by the ACTH stimulated cortisol release in the sows. In this study, however, all or at least part of the observed decrease in piglet birth weight in the 10d treatment group might also be explained by the larger sow litter size.

Bate et al. (1991) have proposed that a decrease in *in utero* piglet cortisol levels following an experimentally induced increase, may mimic the natural increase and decrease which occurs in piglet cortisol levels surrounding the time of parturition. The decrease in cortisol levels, either experimentally induced *in utero* or naturally occurring postnatally, might act as an endocrine signal for the piglet gastrointestinal system to enter a new developmental growth stage. As long as the *in utero* cortisol levels are maintained at an artificially high level, the natural *in utero* growth of the piglet small intestine would be enhanced while the overall development of other tissues and organs would be retarded. This theory could partially explain both the decrease in birth weight and the increase of the SI length to body weight ratio for piglets in the 10d treatment group. Further evidence of the overall catabolic effect of elevated cortisol levels *in utero* is provided by Bate and Hacker (1985a) where pregnant sows

exposed to cold temperatures, which raise cortisol levels in *in utero* piglets, resulted in a decreased piglet body weight at birth. Although the 10d piglets were lighter at birth, the ratio of SI length to body weight tended to be larger than the ratios for both the 2d and saline groups. Increasing piglet cortisol levels prenatally may be catabolic on the overall piglet growth rate but might be either anabolic or at least less catabolic on SI growth. These results are in agreement with those of Silber and Porter (1953), Clarke and Hardy (1971) and Bate et al. (1991) who have all suggested a potential cortisol mediated anabolic effect on the mammalian digestive tract.

To further characterize the ACTH induced effects on piglet intestine development, morphological analysis of several enterocyte characteristics was carried out. The observed increase in the mean enterocyte area in piglets sacrificed at 6 h as compared to 0 h can be mostly explained by the accumulation of granules and not by a significant increase in non-granular cytoplasmic area as neither of the drug treatments resulted in an increase of non-granular cytoplasmic area (Table 2). In fact, the accumulation of opaque granules (Fig. 2) accounted for 85% of the increase in cytoplasmic area measured at 6 h, while the accumulation of granular granules (Fig. 1) accounted for only 3% of the cytoplasmic growth over 6 h. There was however a minimal increase in cytoplasmic area as 12% of the measured increase could not to be attributed to the accumulation of granular granules (Table 2). The ACTH induced anabolic effect on the piglet small intestine can not be explained by an increase in non-granular cytoplamsic area since most of the increase in cytoplasmic area measured at 6 h is due to the presence of opaque granules.

The opaque granules observed in the enterocytes of piglets sacrificed at 6 h were similar to the granules described by Széky et al. (1979) as being IgG filled vacuoles. Other research (Kraehenbuhl and Campiche, 1969) has raised the possibility that these are phagolysosomes. However, the opaque granules described in this study are not membrane bound and the dark staining is characteristics of lipid droplets (Fawcett, 1994). This does not preclude the possibility that IgG molecules are contained within the lipid droplets. The different shades of darkness or level of opaqueness observed between the opaque granules most probably represents the variability in the degree of unsaturation of the lipid's constituent fatty acids (Fawcett, 1994). Both the opaque and granular granules, especially those observed in enterocytes of piglets sacrificed at 6 h might also contain IgG. Specific immunoglobulin cytochemistry of the enterocyte must be used to answer the question of exactly where within the enterocyte the immunoglobulins reside.

The decrease in the volume fraction of the opaque granules measured in the 2d ACTH treatment group may be in part a cause of the corresponding decrease in plasma concentration of bovine IgG in piglets born to sows

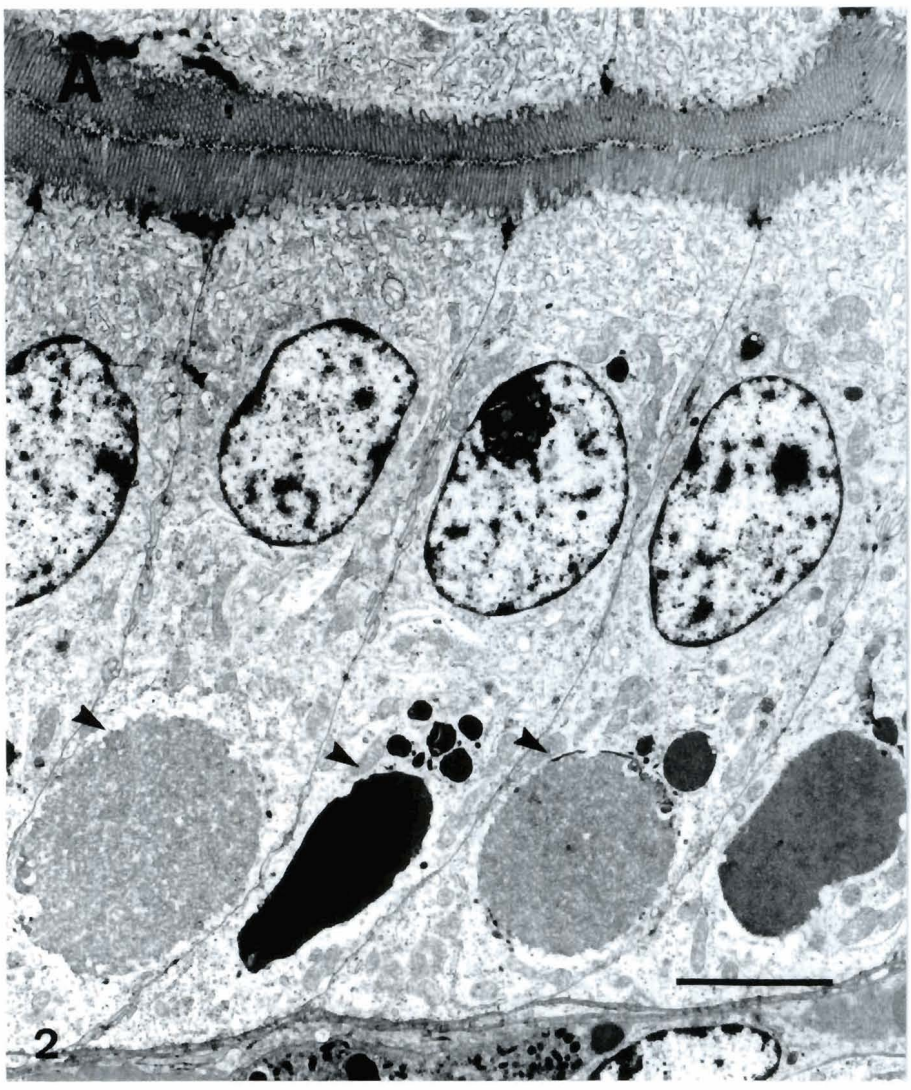
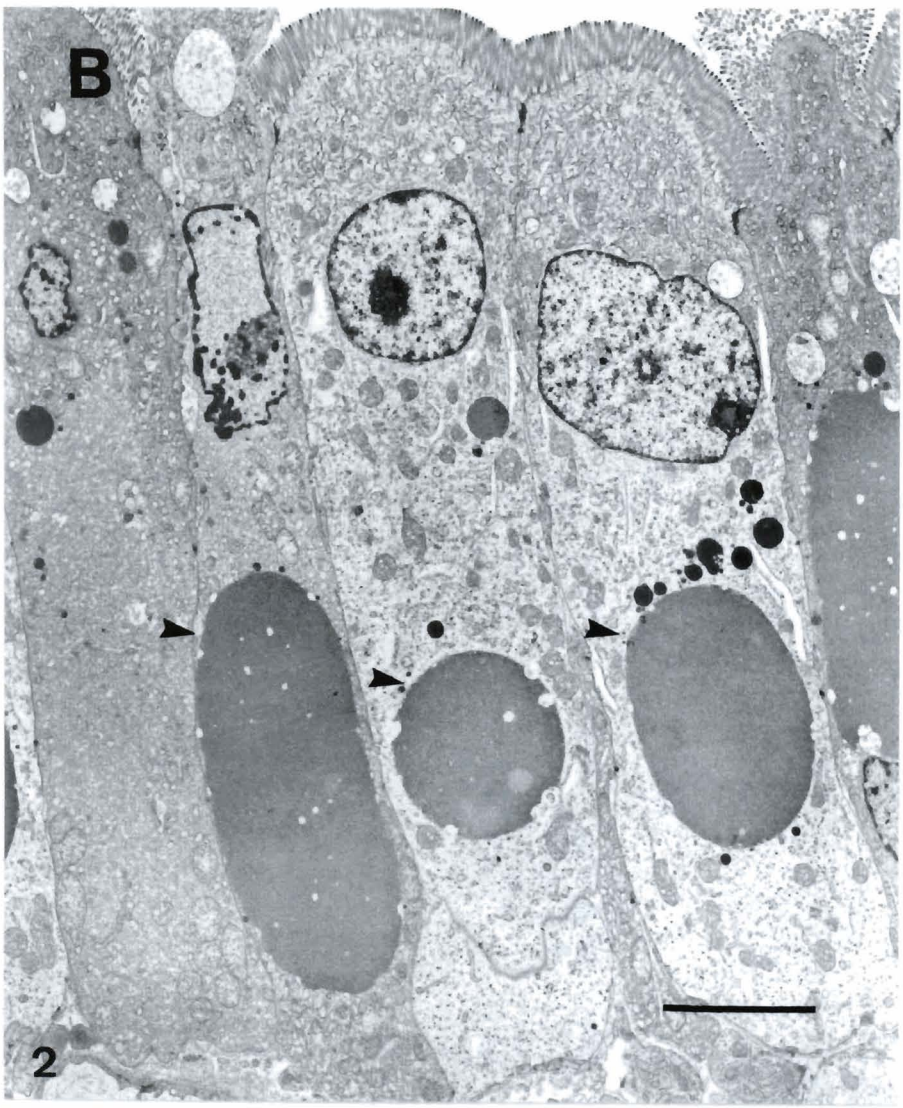


Fig. 2. Electron micrograph of ileum taken from piglets born to sows treated with Saline **(A)**, ACTH for 2 days **(B)**, ACTH for 10 days **(C)** and sacrificed at 6 h. Arrowheads indicate opaque granules characteristic of 6 h old gut. Bar=4 μm.

in the 2d group. If the presence of opaque granules are associated with the uptake of IgG from the intestinal lumen, as suggested by Széky et al. (1979), then it could be concluded that piglets born to sows having received two days of ACTH treatment would have taken up less IgG from their intestinal lumen. Support for this idea comes from the fact that a significant decrease in the number of opaque granules per unit area was measured in the 2d treatment group. If less IgG was taken up from the bovine colostrum that was fed to the piglets then less IgG would be available to transfer from the enterocyte into the vascular system. If, on the other hand, the uptake process was not effected and it was the transfer process that was affected, then one would expect a decrease in blood plasma IgG levels along with a corresponding increase in the volume fraction of opaque granules in the enterocytes of piglets in the 2d treatment group. Since 1) the volume fraction of the opaque granules was decreased, 2) the number of opaque granules per unit area of enterocyte cytoplasm was decreased and 3) the circulating levels of IgG in piglet blood plasma was decreased, the effect of the 2d ACTH treatment was to interfere with both the processes of macromolecule uptake from the intestinal lumen and IgG transfer from the enterocyte into the vascular system.

The interpretation of the above results are in accordance with a theory proposed by Bate (1993) whereby a decrease in circulating cortisol levels which naturally occurs postnatally would trigger the beginning of a stage of small intestine development. At least one aspect of this stage would be the process of immature enterocyte replacement by mature enterocytes which are incapable of taking up IgGs from colostrum. Our results lend further support to this theory. The prenatal and premature decrease in artificially enhanced circulating cortisol levels (2d group) led to a decrease in both the volume fraction and the number per unit area of opaque granules within the piglet enterocyte, presumably by stimulating the processes leading to the cessation of IgG uptake and enterocyte replacement.



In conclusion, the above results demonstrate that the prenatal administration of ACTH to pregnant sows can significantly effect both the volume fraction and the number of opaque granules per unit area that are visible within the enterocytes of newborn piglets. These results support the theory that there is a dissociation between the anabolic effects that ACTH, through glucocorticoids, exerts on the perinatal development of the small intestine. First, a prolonged prenatal enhancement of circulating cortisol levels leads to a longer small intestine relative to piglet body weight. Second, a sharp decline of artificially elevated circulating cortisol levels can trigger 1) the cessation of macromolecular transfer from the enterocyte into circulation and 2) the process of immature enterocyte replacement.

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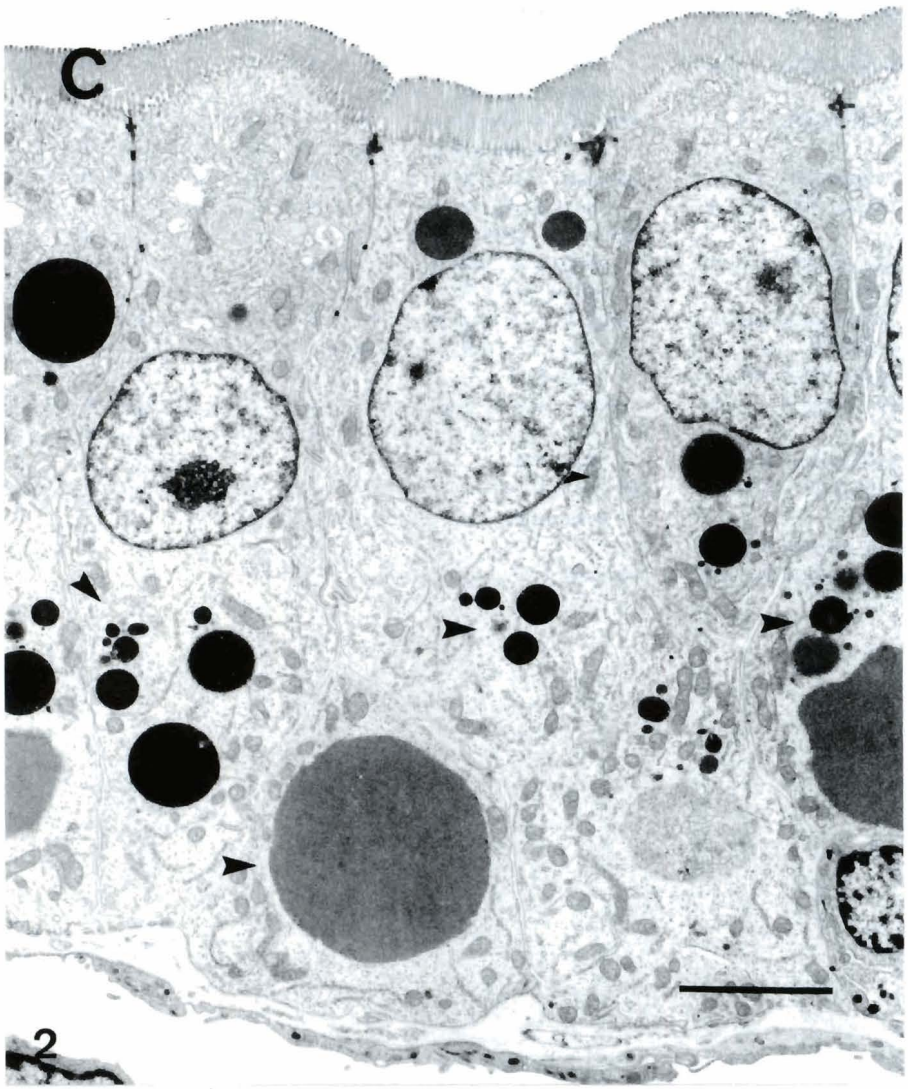

References

Bate L.A. (1993). Endocrine influences on several aspects of development and behaviour of the piglet which may influence its survival. Pig News Inform. 14, 45N-49N.

Bate L.A. and Hacker R.R. (1985a). Influence of environmental temperature during late gestation and soon after birth on IgG absorption by newborn piglets. Can. J. Anim. Sci. 65, 87-93.

Bate L.A. and Hacker R.R. (1985b). The influence of the sow's adrenal activity on the ability of the piglet to absorb IgG from colostrum. Can. J. Anim. Sci. 65, 77-85.

Bate L.A. and Hacker R.R. (1985c). Effect of cannulation and environmental temperature on the concentration of serum cortisol in pregnant sows. Can. J. Anim. Sci. 65, 399-404.

Bate L.A., Ireland W., Connell B.J. and Grimmelt B. (1991). Development of the small intestine of piglets in response to prenatal elevation of glucocorticoids. Histol. Histopathol. 6, 207-216.

Brown H.H. and Moon H.W. (1979). Localization and activities of lysosomal enzymes in jejunal and ileal epithelial cells of the young pig. Am. J. Vet. Res. 40, 1573-1577.

Butler J.E., Klobasa F. and Werhahn E. (1981). The differential location of IgA, IgM and IgG in the gut of suckled neonatal piglets. Vet. Immunol. Immunop. 2, 53-65.

Clark S.L. (1971). The effect of cortisol and BUDR on cellular differentiation in the small intestine in suckling rats. Am. J. Anat. 132, 319-338.

Clarke R.M. and Hardy R.N. (1971). Histological changes in the small intestine of the young pig and their relation to macromolecular uptake. J. Anat. 108, 63-77.

Fawcett D.W. (1994). In: Bloom and Fawcett. A Textbook of Histology. 12th ed. Fawcett D.W. and Raviola E. (eds). Chapell Hill. New York. pp 31-32.

Kraehenbuhl J.P. and Campiche M.A. (1969). Early stages of intestinal absorption of specific antibodies in the newborn. An ultrastructural, cytochemical and immunological study in the pig, rat, and rabbit. J. Cell Biol. 42, 345-365.

Mancini G., Carbonara A.O. and Heremans J.F. (1965). Immuno-

chemical quantization of antigens by single radial immunodiffusion. Immunochemistry 2, 235-256.

Martin C.R. (1985). Endocrine Physiology. Oxford University Press. New York.

Molokwu E.C.I. and Wagner W.C. (1973). Endocrine physiology of the puerperal sow. J. Anim. Sci. 36, 1158-1163.

Moog F. (1979). The differentiation and redifferentiation of the intestinal epithelium and its brush border membrane. Devel. Ciba Found. Series 70, 31-51.

Nelson D.H. (1980). The adrenal cortex. Physiological function and disease. Smith L.H. (ed). W.B. Saunders Co. Toronto.

Patt J.A. (1977). Factors affecting the duration of intestinal permeability to macromolecules in newborn animals. Biol. Rev. 52, 411-429.

Patt J.A. and Eberhart R.J. (1974). Effect of elevated corticosterone levels on serum gamma globulin concentration in newborn rats. Proc. Soc. Exp. Biol. Med. 145, 200-202.

Patt J.A. and Eberhart R.J. (1976). Effects of metyrapone and ACTH on intestinal absorption of immunoreactive bovine IgG in cesarean-derived pigs. Am. J. Vet. Res. 37, 1409-1413.

Payne L.C. and Marsh C.L. (1962). Gamma globulin absorption in the baby pig: the non-selective absorption of heterologous globulins and factors influencing absorption time. J. Nutr. 76, 151-158.

Silber R.H. and Porter C.C. (1953). Nitrogen balance, liver protein repletion and body composition of cortisone treated rats. Endocrinology 52, 518-525.

Silver M. and Fowden A.L. (1989). Pituitary-adrenocortical activity in the fetal pig in the last third of gestation. J. Exp. Physiol. 74, 197-206.

Smith M.W. and Jarvis L.G. (1978). Growth and cell replacement in the new-born pig intestine. Proc. R. Soc. B. 203, 69-89.

Spector P., Goodnight J.H., Sall J.P. and Sarle W.S. (1985). SAS Institute Inc. SAS/STAT Guide for Personal Computers. Version 6 Edition. Carry, NC.

Speer V.C., Brown H., Quinn L.Y. and Catron D.V. (1959). The cessation of antibody absorption in the young pig. J. Immunol. 83, 632-634.

Széky A., Rátz F., Tuboly S. and Nagy G. (1979). Absorption of colostral immunoglobulin in suckling piglets. Acta Microbiol. Acad. Sci. Hung. 26, 99-110.

Wilcke J.R. and Davis L.E. (1982). Review of glucocorticoid pharmacology. Vet. Clin. N. Am. 12, 3-17.

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