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Development of the small intestine of piglets in response to prenatal elevation of glucocorticoids

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Summary. The effects of prenatal adrenal stimulation and synthetic glucocorticoid supplementation on development of the gastro-intestinal tract of the piglet were investigated. Twelve pregnant sows were treated with either ACTH infusion, Isoflupredone injection or Saline between days 105 and 112 of gestation. Neonatal pigs were weighed, bled and sacrificed at 0 or at 6 h. Piglets sacrificed at 6 h were fed bovine colostrum. Transverse sections were prepared from the duodenum, jejunum and ileum for measurement of the villus amplification factor (VAF) and basal membrane circumference. Sows in the ACTH group showed an elevation in cortisol in response to infusion; this decreased after infusion and then rose again at parturition. Piglets from both the ACTH and Saline groups had more villus surface area per unit of body weight (BW) than those born to Isoflupredone-treated animals. The BW of the ACTH piglets was lower ($P < 0.05$) than those of piglets in the other groups. When the weight of the stomach and the Small Intestine (SI) was expressed as a function of the body weight, the stomach and SI:BW ratio was larger ($p < 0.05$) in pigs born to ACTH-treated sows. The circumference of the ileum was larger at 6 h than at 0 h. Control pigs had a higher concentration of bovine IgG at 4 and 6 h ($P < 0.05$). Observations of the light microscopic preparations indicated a less organized epithelium in both ACTH and isoflupredone pigs sacrificed at 0 h. Light and EM preparations of ileum from ACTH pigs sacrificed at 6 h, showed an abundance of dark-stained vacuoles, characteristic of IgG-containing structures. These became less evident in piglets from the Isoflupredone group and even less so in the control groups. The consequences of these phenomena in terms of absorptive capacity are discussed.

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Introduction

Endocrine mechanisms controlling events leading to the cessation of immunoglobulin transfer to the circulation in the piglet have been extensively studied but are still not completely understood (Patt, 1977; Széky et al., 1979; Smith and Peacock, 1980). Glucocorticoids seem to play an important controlling role in this process (Patt and Eberhart, 1976). Prenatal stimulation of the sow with ACTH up to parturition, or elevation of cortisol by means of continuous exposure to cold from day 105 of gestation until parturition, have resulted in elevated serum immunoglobulins G (IgG) in piglets (Bate and Hacker, 1985a,b). The observed elevation in serum IgG could be attributed either to an increase in the absorptive area of the GI tract or to an increase in the absorptive capacity of it, or possibly both. Glucocorticoids are catabolic in most organs of the animal (Nelson, 1980). It has been found, however, that glucocorticoids are anabolic in the digestive and urogenital tract of rats (Silber and Porter, 1953). Therefore, if piglets respond to glucocorticoids in a similar way, it is conceivable that they could have a more developed small intestine with a larger absorptive area. Furthermore, Patt (1977) suggested that the process of IgG absorption in the pig may depend on an optimal concentration of glucocorticoids. The elevation of glucocorticoid in the sow prior to parturition, has resulted in several other physiological and behavioural changes which are considered beneficial to the piglet (Bate, 1991; Bate and Grimmelt, 1991). Therefore, the purpose of this study was to investigate the influence of prepartal elevation of cortisol, and supplementation with Isoflupredone (a synthetic glucocorticoids) on the development and absorptive characteristics of the GI tract in the neonatal pig.

Materials and methods

Animals and tissue processing

Pregnant, cross bred primiparous sows (n = 12) were randomly assigned to a control, or ACTH or Isoflupredone treatments. These animals were placed in gestation stalls on day 101 of gestation. They received a 14% protein commercial diet, at the same time being allowed free access to water through drinking nipples. The room in which the animals were housed was maintained at 18 to 20° C and subjected to a 14 h d^{-1} of fluorescent light at an intensity of 185-240 lux at eye level. On day 103 of gestation, the sows were catheterized through the ear vein, following the technique of Bate and Hacker (1985c). The catheters were kept patent with a saline IV drip (0.9% NaCl Travenol Company Ltd., Toronto) at a rate of 1 L d^{-1}. Treatment started on day 105 of gestation at 08:00 h. Control animals were continuously infused with saline at an average rate of 1 L d^{-1}. A second group received porcine ACTH (Sigma Chemical Ltd., St. Louis, Mo) at a rate of 1 IU kg^{-1} d^{-1} in a similar saline infusion. The third group was injected every day with 35.7 μg kg^{-1} of isoflupredone acetate IM (Predef R 2X, Upjohn Inter-American Corporation, Orangeville, Ontario). This group also received a continuous infusion of saline. All infusions were dispensed by a Gilson Miniplus 2 peristaltic pump. Blood samples were collected through the catheters every day between 08:30 and 9:00 h. Plasma was separated from the samples and stored at –20° C for later measurement of cortisol concentrations. In the following discussion, piglets born to sows treated with ACTH, saline or Isoflupredone will be referred to as ACTH, Control and Isoflupredone piglets respectively. At birth the piglets were divided into two groups; those to be sacrificed immediately and those to be sacrificed at 6 h of life (Piglets born 1st, 3rd and 5th). Prior to sacrifice by CO_2 inhalation in a sealed chamber, both groups had blood samples taken. In addition the piglets sacrificed at 6 h were kept in an incubator maintained at 33 to 35° C (Mount, 1968), fed 12.5 mL bovine colostrum kg^{-1} at 30 min, 2h, and 4h, and had blood samples taken at these times.

After sacrifice, all piglets were weighed prior to exsanguination, and their GI tract was removed. The stomach was then opened, emptied and weighed, while the small intestine (SI) was separated from its mesenteric attachments and weighed. The weight of the large intestine (LI) was also recorded. A sample of the ileum was removed and fixed in 1% glutaraldehyde and 4% formaldehyde in phosphate buffer, for electron microscopy following the McDowell and Trump (1976) technique. The rest of the SI was then fixed in formalin buffer for light microscopy. After fixation the SI length was recorded and the internal fluid was then removed and the tissue weighed again. Thereafter, samples from the duodenum, jejunum and ileum were subsequently excised.

These were then processed for LM, using a Fisher tissue processor model 166-MP, followed by wax embedding and sectioning at 6 μm with a Spencer microtome model 820. Subsequently the slides were stained with Hematoxylin and Eosin (Luna, 1960). Samples for EM were postfixed in 1% osmium tetroxide in 0.1M 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, using glass knives. The semi-thin sections were stained with 1% toluidine blue in 1% sodium borate, and analyzed to determine the best specimen area for EM viewing. Excess tissue was then trimmed off. Thin sections were placed in 200 mesh copper grids and stained with saturated uranyl acetate in 50% ethanol for 30 min, followed by lead citrate (Sato stain) for 2 min. The thin sections were viewed with a Hitachi H7000 electron microscope. The semi-thin sections were photographed on a Zeiss D-7082 Transmitted-Light Photomicroscope III.

Measurements

The Villus Amplification Factor (VAF) and the boundary of LM samples were determined following the method described by Mayhew (1982) with the use of a Leitz Wetzlar Neo Promar microscope model 512736. One Isoflupredone sow, was removed from the experiment because its farrowing was unobserved. Serum samples from all sows were analyzed for cortisol using a solid phase Radioimmunoassay (Diagnostic Product Corporation, Los Angeles CA.). Inter- and intra-assay coefficient of variations were 7.9 and 3.3% respectively. Bovine IgG was determined by radial immunodiffusion following the procedure developed by Mancini et al. (1965), with the modifications described by Bate and Hacker (1985a). All animals were handled in accordance with regulations of the Canadian Council on Animal Care.

The absorptive area of the villi in each section was calculated by multiplying the VAF times the boundary value for each section. All parameters were analyzed using general linear models from SAS, and the difference among treatments determined by Duncan's test within the same program (Spector et al., 1985).

Results

The concentration of cortisol in the ACTH sows increased in response to ACTH stimulation; it declined once the stimulation was removed on day 112 of gestation, and thereafter increased at the time of parturition (Table 1). Isoflupredone did not modify the levels of cortisol in sows. ACTH piglets weighed less ($P < 0.05$) than those on the other treatments (Table 2). The weight of the fresh stomach, SI and LI was similar in the two experimental groups; nor did the weight and the length of the SI after fixation differ among experimental groups ($P > 0.05$). When the stomach and SI were expressed in terms of units of body weight, however, the ACTH piglets had larger SI ($P < 0.05$) than those from the other groups. Circumference, VAF of the SI were not influenced by treatment ($P > 0.05$). The surface area of jejunum,

Table 1. Serum concentration of cortisol (ng/mL) in sows treated with ACTH, Saline or Isoflupredone during late gestation.

Day of Gestation*	Treatment		
	ACTH	Control	Isoflupredone
105	35.0 ± 8.0	29.6 ± 10.8	29.4 ± 5.1
106	121.4 ± 25.8	28.4 ± 10.2	20.4 ± 12.0
107	87.7 ± 27.8	23.1 ± 9.9	8.4 ± 3.2
108	79.2 ± 32.4	18.7 ± 2.4	20.7 ± 16.9
109	105.9 ± 33.5	29.5 ± 4.3	21.7 ± 7.8
110	93.6 ± 39.6	45.4 ± 5.5	9.8 ± 2.3
111	78.5 ± 37.2	26.6 ± 7.2	17.2 ± 5.2
112	75.1 ± 30.4	30.6 ± 3.1	35.5 ± 15.5
113	18.5 ± 6.5	32.4 ± 10.9	20.7 ± 3.5
–3	20.6 ± 5.4	27.7 ± 6.8	33.1 ± 15.9
–2	51.8 ± 23.9	25.2 ± 4.8	21.9 ± 6.9
–1	53.2 ± 39.1	14.1 ± 1.1	45.0 ± 18.0
0	95.0 ± 72.1	46.0 ± 2.8	37.3 ± 11.3

* Days 105-113 represent the period covering before, during and immediately after treatment was completed. Days -3 to -1 represent the period before parturition. Day 0 represents farrowing day.

Table 2. Paramenters of newborn piglets born to sows exposed to either ACTH, Isoflupredone or Saline prior to parturition (meon ± SD)

Parameter	Treatment		
	ACTH	Control	Isoflupredone
Body weight (g)	1170[a] ± 36.4	1330[b] ± 52.0	1319[b] ± 48.9
Stomach (g)	6.78[a] ± 0.18	7.26[a] ± 0.28	6.82[a] ± 0.23
Small Int. (g)	55.1[a] ± 1.9	56.1[a] ± 2.3	55.1[a] ± 2.6
Large Int. (g)	13.9[a] ± 0.72	14.5[a] ± 0.78	15.1[a] ± 1.02
SI length (cm)	283[a] ± 7.65	301[a] ± 6.83	303[a] ± 7.21
Stomach/BW (mg/g)	6.02[a] ± 0.16	5.48[b] ± 0.09	5.22[b] ± 0.12
SI/BW (mg/g)	48.1[a] ± 1.4	42.2[a] ± 1.2	41.8[a] ± 1.2
LI/BW (mg/g)	12.1[a] ± 0.55	10.9[a] ± 0.46	11.4[a] ± 0.55
Circ. Duod. (mm)	16.9[a] ± 0.59	16.9[a] ± 0.54	15.1[a] ± 0.64
Circ. Jej. (mm)	16.5[a] ± 0.48	16.9[a] ± 0.54	15.3[a] ± 0.54
Circ. Ile. (mm)	14.4[a] ± 0.42	14.9[a] ± 0.62	14.6[a] ± 0.52
VAF. Duod.	8.95[a] ± 0.87	9.49[a] ± 0.66	9.20[a] ± 0.81
VAF. Jej.	9.71[a] ± 0.77	9.61[a] ± 0.61	8.51[a] ± 0.53
VAF. Ile.	8.45[a] ± 0.65	8.89[a] ± 0.66	8.14[a] ± 0.50
Area Duod./BW	0.14[a] ± 0.01	0.12[a] ± 0.01	0.11[a] ± 0.01
Area Jej./BW	0.14[a] ± 0.01	0.12[ab] ± 0.01	0.10[b] ± 0.01
Area Ile./BW	0.11[a] ± 0.01	0.10[a] ± 0.01	0.09[a] ± 0.01

[a] Values with different superscript in a row are different (P < 0.05).

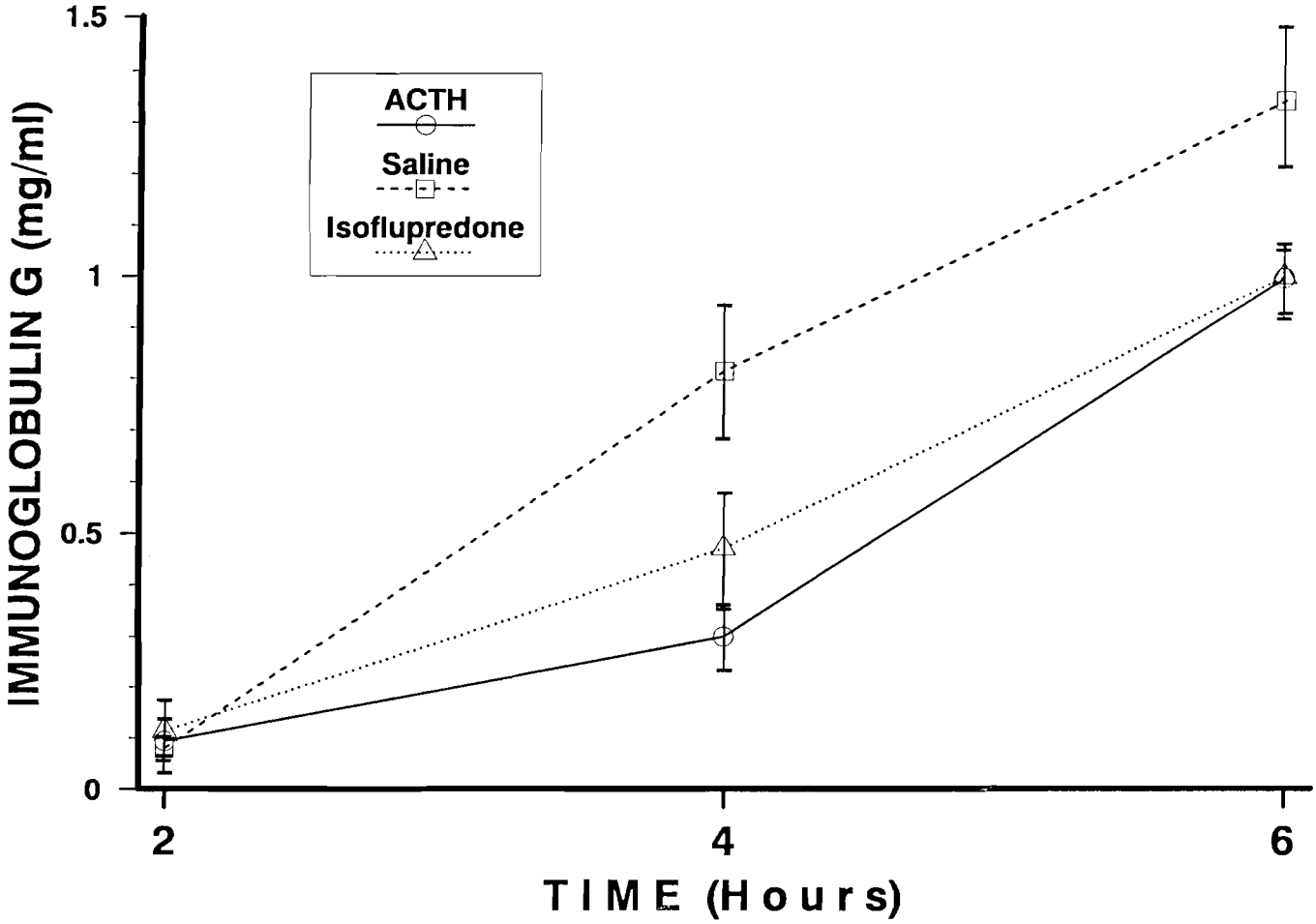

Fig. 1. Plasma concentration of Immunoglobulin G for the first 6 h of life in piglets born to sows treated with ACTH, isoflupredone or Saline prior to parturition.

when expressed in terms of units of body weight, was larger in ACTH piglets ($P < 0.05$). The serum concentration of Bovine IgG was similar in all groups at 2 h but at 4 and 6 hours the concentration of IgG was lower in ACTH and Isoflupredone piglets ($P < 0.05$) (Fig. 1).

Light microscopy of sections of ileum of those animals sacrificed at 0 h reflected the state of the gut epithelium before it is exposed to colostrum. Control animals exhibited a columnar epithelium. Nuclei in the cells at the bottom of the villi, were basal, whereas nuclei in cells near the tip of the villus tended to be closer to the cell apex. Small, clear vacuoles (3 μm) were found near the apices of many cells (Figs. 2, 3). In some sections, goblet cells were found in a ratio of one goblet cell per ten enterocytes. Enterocytes in one of the control animals contained large clear vacuoles that completely filled the cytoplasm except for a thin rim.

There was little difference between the epithelium observed in ACTH or Isoflupredone animals sacrificed at 0 h. The epithelium in these animals appeared to be more disorganized than in the controls. In some places the nuclei of these cells gave the appearance of that of a pseudo-stratified columnar epithelium. Some enterocytes had a triangular outline rather than being columnar. Small clear vacuoles were present near the apices in these cells.

The epithelium of the control animals sacrificed at 6 h was characterized by cells that had multiple, clear vacuoles of all sizes. These cells appeared more prevalent near the base of the villus. A few cells contained small (1 μm) dark vacuoles in the region of the nucleus. Nucleoli were predominant and sometimes double. In some animals of this group, the gut epithelium appeared similar to control animals killed at 0 h. Isoflupredone animals sacrificed at 6 hours, had ileal epithelium different from that of the controls. While there were many small, clear, apical vacuoles, these cells also contained numerous circular, dark-staining vacuoles. ACTH piglets sacrificed at 6 h had more evident dark-staining vacuoles, and less prevalent clear-staining vacuoles than Isoflupredone animals sacrificed at 6 h (Figs. 4, 5). These dark-stained vacuoles represent the presence of the colostrum immunoglobulins which are either in transit to the basal membrane for transfer to the circulation or are in the process of being internally digested by

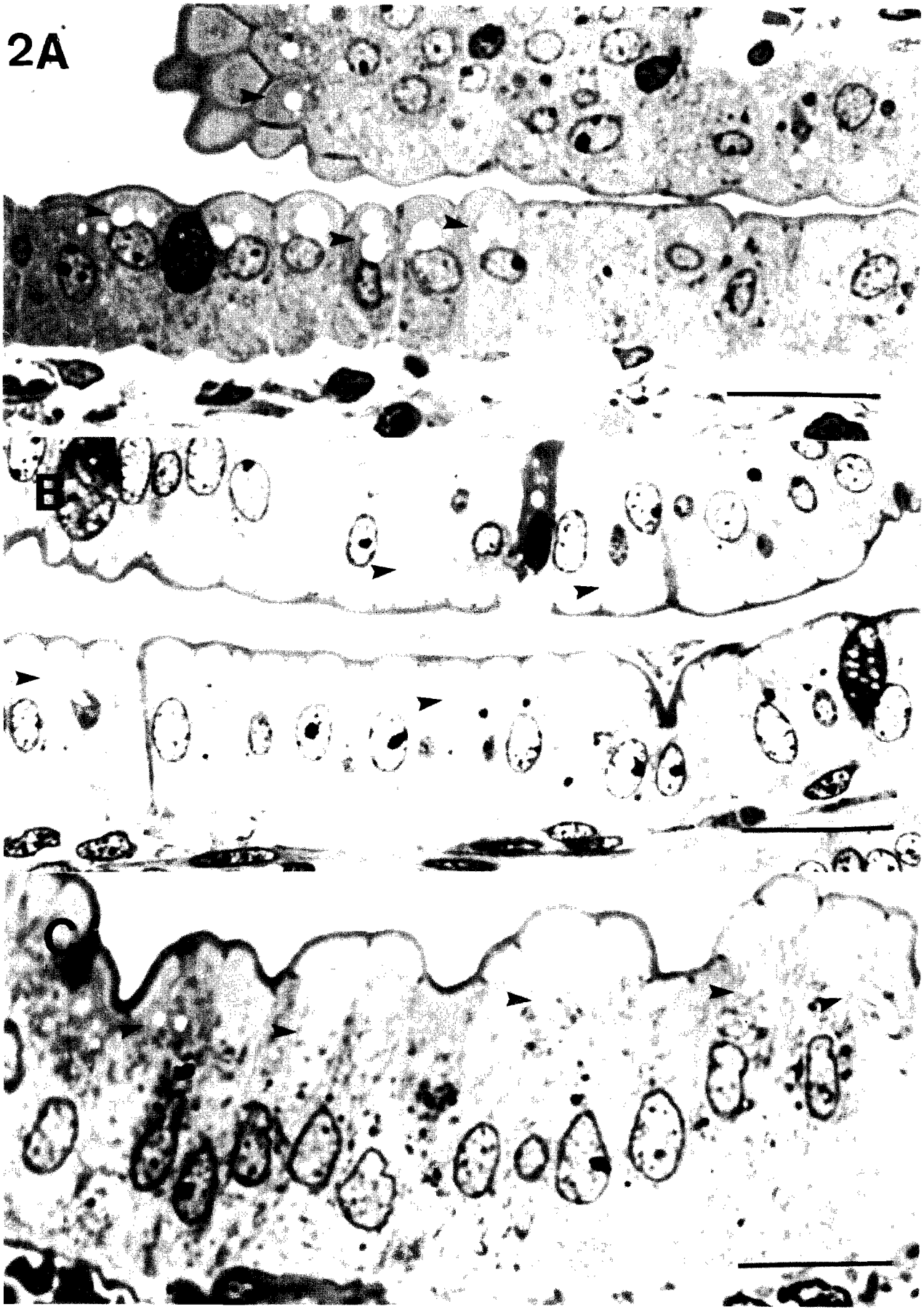

Fig. 2. Light micrograph of ileum villus of piglets born to sows treated with Saline (A), Isoflupredone (B), ACTH (C) and sacrificed at 0 hour. Arrowheads indicate clear vacuoles characteristic of newborn gut. Bar represents 100 μm.

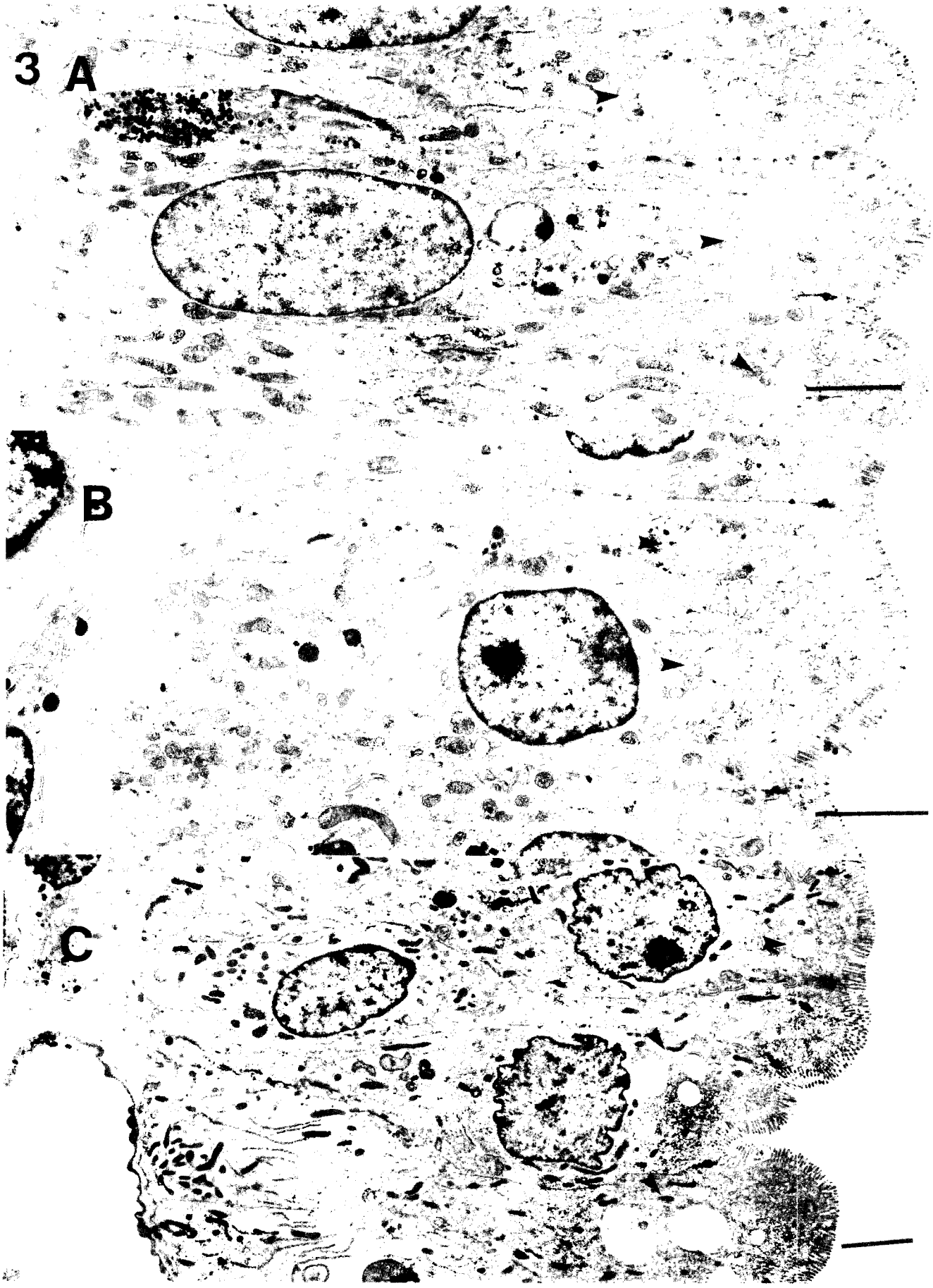

Fig. 3. Electron micrograph of ileum cell taken from piglets born to sows treated with Saline (A), Isoflupredone (B), ACTH (C) and sacrificed at 0 hour. Arrowheads indicate clear vacuoles characteristic of newborn gut. Bar represents 3 μm.

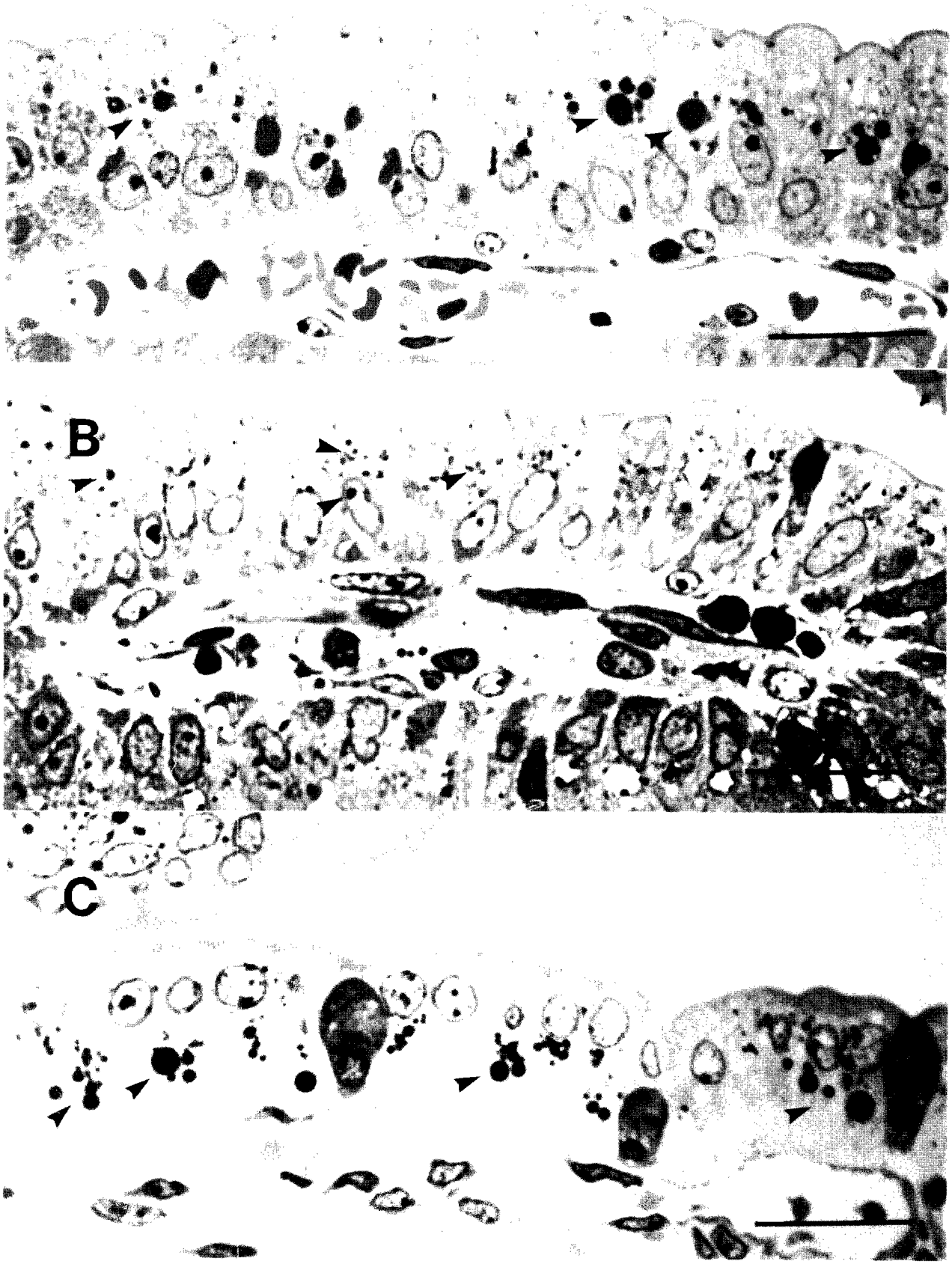

Fig. 4. Light micrograph of ileum villus of piglets born to sows treated with Saline (A), Isoflupredone (B), ACTH (C) and sacrificed at 6 hours. Arrowheads indicate dark stained vacuoles containing IgG. Bar represents 100 μm.

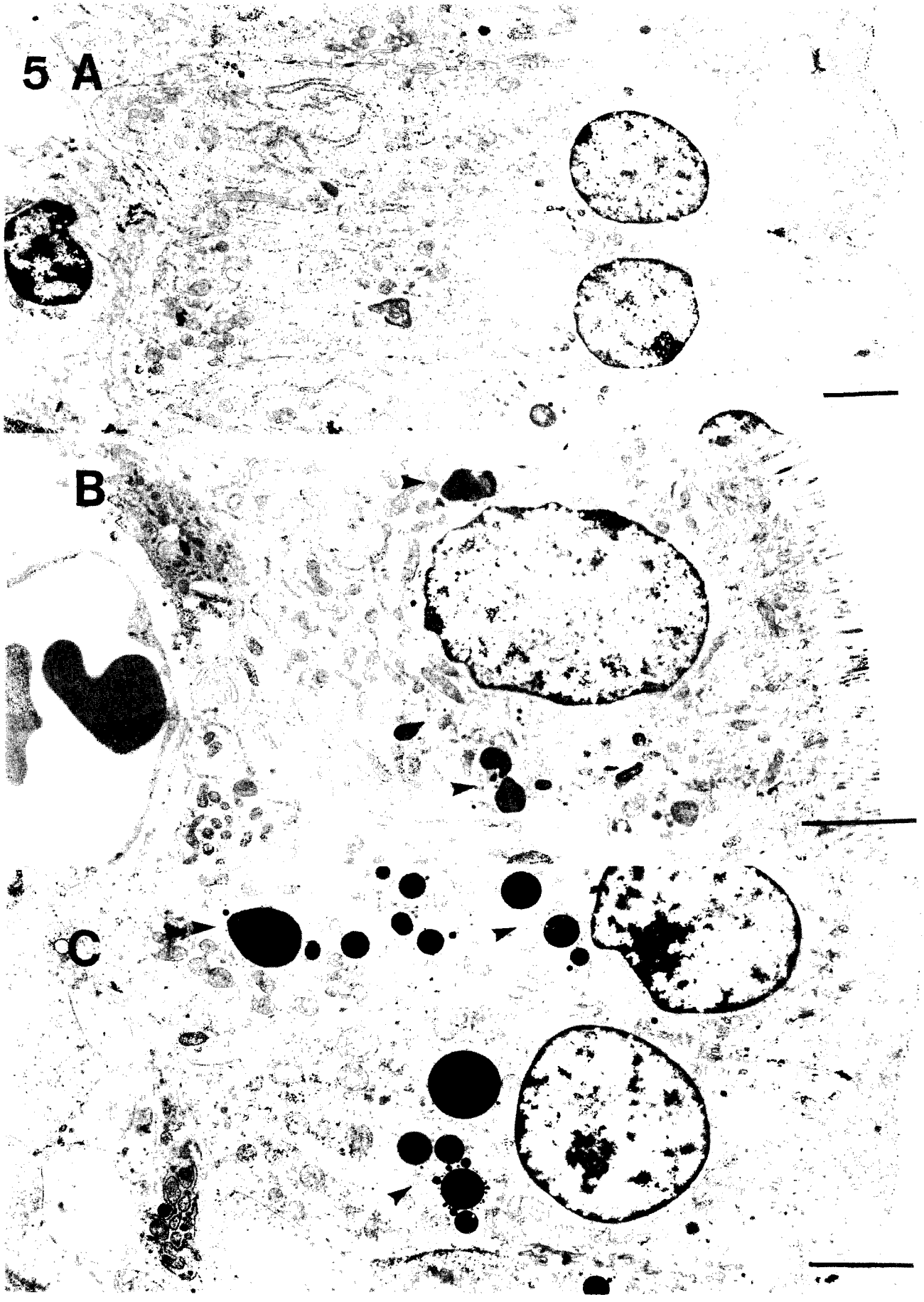

Fig. 5. Electron micrograph of ileum cell taken from piglets born to sows treated with Saline (A), Isoflupredone (B), ACTH (C) and sacrificed at 6 hours. Arrowheads indicate dark stained vacuoles containing IgG. Bar represents 3 μm.

phagolysosomes (Kraehenbuhl and Campiche, 1969).

Discussion

The observed decrease in body weight of ACTH piglets occurred as a result of exposure to glucocorticoids (Nelson, 1980). The absolute weight of the stomach and SI was similar in all groups, therefore, proportionally, ACTH animals have a higher stomach: BW and SI:BW ratio than controls. The stomach:BW and SI:BW ratios confirm this and suggest that the elevation in cortisol resulting from the ACTH treatment, may have triggered some anabolic process in the digestive tract, consistent with the findings of Silber and Porter (1953). However, this elevated ratio did not translate into an elevation of circulating IgG after 6 h of life as was expected, based upon previous observations by Bate and Hacker (1985a, b).

The time during which the new born epithelium is able to absorb macromolecules is limited (Lecce, 1973; Murata and Namioka, 1977; Frenyo, 1987). This period can be lengthened by not exposing the animal to food (Lecce and Morgan, 1962; Vellenga et al., 1988). It is well established that only fetal enterocytes have the capacity for absorbing macromolecules from colostrum and transferring them to the circulation (Smith and Jarvis, 1978; Moog, 1979). The termination of this process, which results in the incorporation of immunoglobulins into the circulation, can be attributed to one of two phenomena: first, the epithelium loses the ability to transfer macromolecules to the circulation, although absorption still takes place (Patt, 1977); secondly, the fetal enterocytes are replaced by mature ones (Moog, 1979). Before this process of cell replacement is completed however, the competence of the fetal enterocyte to transfer the absorbed macromolecules is impaired (Patt, 1977). It is well documented that the process of incorporation of IgG into circulation stops when the enterocytes are unable to discard the absorbed IgG through the basal or lateral membranes (Clarke and Hardy, 1971), rather than by cessation in the uptake of IgG from colostrum. In the meantime, the enterocytes accumulate IgG in their cytoplasm to be catabolized within phagolysosomes (Brown and Moon, 1979). The dark-staining vacuoles, so abundant in ACTH and partially abundant in Isoflupredone piglets, have been identified by Széky et al. (1979) to be IgG-filled vacuoles, which may be phagolysosomes (Kraehenbuhl and Campiche, 1969). Therefore, it appears that this phenomena was triggered to a large extent in ACTH and to a lesser extent in Isoflupredone piglets.

Cell replacement, leading to the termination of the absorptive period, has also been attributed to glucocorticoid influences (Clarke and Hardy, 1971). This theory fits well the sequence of events taking place around parturition (Molokwu and Wagner, 1973) where a normal elevation of cortisol seems to initiate the process of cell replacement which culminates in the total inability to absorb further IgG after about 10 days. The disorder observed in the epithelium of ACTH and Isoflupredone piglets, characteristic of enterocyte replacement (Smith and Peacock, 1980), suggests that this process started in these pigs while *in utero*, probably in response to the decline of glucocorticoids which followed stopping the treatment.

The main difference between this experiment and previous ones (Bate and Hacker, 1985a,b) was that in this study the treatment finished on day 112 of gestation instead of at parturition. This resulted in a decrease of maternal cortisol, prior to the peak occurring at parturition, as opposed to a sustained elevation in glucocorticoid levels lasting until parturition.

It would appear that once the levels of glucocorticoid decrease, at the end of stimulation, the process of transferring IgG to the circulation becomes impaired and results in the accumulation of IgG-filled vacuoles. Patt (1977) suggested that there may be an optimal glucocorticoid range below which immunoglobulin absorption is impaired in piglets. In view of the findings in this study, it is possible that such a threshold may be crossed as the concentration of glucocorticoid between in circulation.

Studies whereby cortisol concentration peaks at different times prior to parturition could help clarify the relationship between *in utero* levels of cortisol, and absorptive capacity. It can be tentatively concluded that the process leading to the termination of absorptive capacity in the newborn pig is controlled to a marked degree by a decrease in glucocorticoids.

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