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Regulatory cell biology of growth hormone and prolactin in the cow mammary gland

David Andrew Walkup

REGULATORY CELL BIOLOGY OF PROLACTIN AND GROWTH HORMONE IN THE COW MAMMARY GLAND

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Janet M. Nolin

Prolactin (PRL) acts directly on milk secretory cell nuclei to stimulate lactation in rats. In the cow, PRL has been detected in Golgi apparatus, endoplasmic reticulum, and secretory vesicle subcellular fractions of mammary gland, but not nuclear fractions. However, growth hormone (GH) is the galactopoeitic hormone in cows, not PRL. The possibility of GH acting directly on the cow milk secretory cells, and PRL acting on their nuclei was investigated using an immunocytochemical method. PRL and GH were detected in endoplasmic reticular and Golgi apparatus regions of the milk secretory cells, as well as, on nuclear and fat globule membranes. PRL and GH were both detected in the alveolar milk. Therefore, it appears that GH and PRL act directly on the milk secretory cells of the cow.

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Prolactin and Growth Hormone
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by

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Approved

Chairperson of Thesis Committee

Member of Thesis Committee

Member of Thesis Committee

Member of Thesis Committee

Examining Committee

REGULATORY CELL BIOLOGY OF GROWTH HORMONE AND PROLACTIN IN THE COW MAMMARY GLAND

By

David Andrew Walkup

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Dedication

I would like to dedicate this thesis to Janet M. Nolin my thesis director and friend. She has been an excellent role model and has exposed me to more science than I ever dreamed of. My respect and trust in Janet is like that of my parents. The only thing I regret is that more science students will not have the opportunities I had because of her retirement.

Table of Contents

List of Tables and Figures

Introduction

In most mammals, the maintenance of galactopoiesis requires a hormonal complex consisting of oxytocin, prolactin, growth hormone, adrenal corticoids, thyroxine, parathyroid hormone, and insulin. Of these hormones, prolactin (PRL) is the rate limiting regulatory hormone of in most laboratory species (Kumaresan et al., 1966; Cowie, 1969; Guyette et al., 1979; and Flint et al., 1984). In the Holstein cow, this is not the case (Koprowski and Tucker, 1973; Smith et al., 1974). Instead, growth hormone (GH) is the rate limiting regulatory hormone in the cow. Physiological and pharmacological studies have demonstrated that plasma GH levels are highly correlated with milk yield from the galactopoietic cow (Bauman et al., 1985; Bines et al., 1980; Cotes et al., 1949; Davis et al., 1987; Hart et al., 1978; Machlin, 1973; Peel et al., 1981).

Since the discovery of the galactopoeitic role of GH in the Holstein cow, the physiology of GH's stimulatory effect on lactation has become the topic of several studies. GH increases plasma metabolites used by the milk secretory cells in the synthesis of milk: GH levels are highly correlated with circulating levels of non-esterfied fatty acids (Hart et al., 1978), and glucose (Russell, 1957). In addition, injection of GH leads to an eventual increase in plasma levels of PRL, thyroxine, insulin (Bines et al., 1980), and

IGF-I (Davis et al., 1987), which all appear to be synergistic hormones in galactopoiesis. Although the actions of GH described above suggest that milk synthesis and secretion are increased by the action of GH on non-mammary gland sites, the idea of GH acting directly on the mammary secretory tissue has been neglected. The primary reason for this neglect, appears to be related to an inability to detect GH receptors on the cow milk secretory cells (P. V. Malven, personal communication).

PRL acts directly on the milk secretory cells of the rat (Topper and Chomczynski, 1974; Nolin and Witorsch, 1976; Nolin and Bogdanove, 1980) where it is the major regulatory hormone of galactopoiesis. Because GH is the major regulatory hormone of galactopoiesis in the cow it seems possible that GH also could act directly on the milk secretory cells of the cow. This possibility was investigated in the present study using an immunocytochemical procedure. Immunocytochemical staining of PRL also was carried out for comparison to GH staining, as well as, to previous studies done in the rat which demonstrated the presence of PRL inside the nuclei of the milk secretory cells (Nolin and Bogdanove,1980).

Materials and Methods

During lactation, the milk secretory cells of the mammary gland undergo a secretory cycle. Immediately

following milk removal from the mammary alveoli, the milk secretory cells become columnar and initiate synthesis and secretion of the constituents of milk into the alveolar lumen (Nolin, 1981) (fig. 1a). As milk fills the lumen, the milk secretory cells progressively become cuboidal (fig. 1b), and finally squamous (fig. 1c). Since PRL is present in columnar milk secretory cell nuclei, at a time which coincides with the initiation of a secretory cycle in the rat, this experiment was designed to obtain the maximum number of milk secretory cells in this stage.

Nine Holstein cows were milked regularly, twice daily, prior to the sacrificial day (in collaboration with P.V. Malven, Purdue University). Milk yields ranged from 7 to 20 Kg per day, with a mean of 14.4 Kg/day. Three groups (n=3/group) of the cows were hand milked at 5, 30, or 180 min before scheduled slaughter, because the length of the bovine milk secretory cycle was not known. The cows ranged in age from two to seven years, and stages of lactation varied widely, but all cows were at least six weeks postpartum (mid-lactational). The cows were rendered unconscious by stunning and sacrificed by exsanguination. A pituitary gland and biopsies of mammary gland were immediatly excised, sliced into 1.0 cm cubes, and fixed in Bouin's fluid overnight.

After overnight fixation of the bovine tissues in

Bouin's fluid, all tissues were dehydrated by immersing in: 70% ethanol, three times, at least 30 min each time; 95% ethanol, three times, at least 30 min each time; 100% ethanol, three times, at least 30 min each time; xylenes, three times, at least 1 hr each time; and stored in cedar oil until processing for cytological examination. Mammary gland and pituitary gland tissues were removed from the cedar oil, sliced into 0.5 cm cubes, and immersed in: xylenes, three times, at least 1 hr each; molten paraffin, three times, at least 1 hr each; and embedded in paraffin blocks.

The pituitary gland tissue blocks were sectioned at a thickness of 5 um and mounted on gelatin coated slides. Four pituitary gland adjacent serial tissue sections were placed on each slide, with two of the serial sections on each slide oriented with their shared side up (mirror-images). Each tissue section was spaced at least 0.5 cm away from other tissue sections. Preparing mirror-image sections made it possible to characterize the antisera for PRL and GH staining, as well as, to study the occurrence of somatomammotrophs, which are pituitary gland cells thought to secrete both PRL and GH (Fumagalli and Zanini, 1985).

The mammary gland tissue blocks also were sectioned at a thickness of 5 um and mounted on gelatin coated slides, with 4 to 5 adjacent serial tissue sections per slide. As with the pituitary gland tissue sections, the mammary gland tissue sections were spaced at least 0.5 cm away from each other.

Because the major possible cross reactant with the antiserum to growth hormone would be the molecularly similar PRL, and vice-versa, specificity studies of PRL and GH staining were done in pituitary gland tissue sections, prior to the staining of either hormone in mammary gland tissue sections. Classical pituitary gland PRL secreting cells are large and polyhedral in shape, whereas classical GH secreting cells are small and ovoid. This morphological difference as well as staining of mirror image, adjacent serial tissue sections, were used as criteria for GH and PRL specific staining in pituitary tissue sections.

To stain pituitary tissue sections for GH, each tissue section was preincubated nine X 1 hr with 10 ul of polyclonal, affinity purified, goat anti-bovine IgG (ABIgG) (Southern Biotechnology Associates, Inc., Birmingham, Alabama) at a concentration of 1:10 (antiserum in PBS Buffer). After each incubation, and after all of the following incubations, the tissue sections were rinsed in tap water (pH 5.4) and PBS buffer (pH 7.0). Although preincubation of pituitary gland tissue sections with ABIgG was not required for the staining of GH and PRL in the pituitary gland tissue sections, it was used because it was required for the staining of GH and PRL in mammary gland tissue sections. After the nine incubations with ABIgG, each tissue section was incubated for three days with 10 ul of the

primary antiserum [polyclonal, affinity purified, rabbit anti-ovine growth hormone (AOGH), Accurate Chemical and Scientific Corp., Westbury, New York] at concentrations ranging from 1:500 to 1:5000 (antiserum in PBS-EDTA buffer). A three day incubation period was not required for the staining of GH or PRL in pituitary gland tissue sections, but it was used because it was required in the staining of GH and PRL in the mammary gland tissue sections. After the three day incubation, each tissue section was incubated six to nine times for 15 min with 10 ul of the secondary antiserum [polyclonal, affinity purified, goat anti-rabbit IgG conjugated to peroxidase (ARIgG-per)] at a concentration of 1:1000 (antiserum in PBS-0.1% Gel Buffer). After each of the 15 min ARIgG-per incubations, each section was incubated with the chromogenic substrate 2,3-diaminobenzidine-peroxide (DAB) for 1 to 2 min.

To stain PRL in pituitary gland tissue sections, each tissue section was treated exactly the same as in the GH staining protocol, except, 10 ul of the primary antiserum, rabbit anti-ovine prolactin (AOPRL) (NIH, Bethesda, Maryland) was used at concentrations ranging from 1:30 to 1:4800 (antiserum in PBS-EDTA buffer), instead of AOGH.

After characterization of the antisera used in GH and PRL staining in pituitary gland tissue sections, several tissue sections from several different areas of each bovine

mammary gland were H and E stained (Gill-1 Hematoxylin Protocol, Lerner Laboratories, Stamford, Ct.), to locate milk secretory cells in the columnar or initiation stage of their secretory cycle. Once areas containing a large number of columnar milk secretory cells were found, PRL and GH staining were carried out.

Initial staining of GH and PRL in mammary gland tissue sections was impossible because of the direct binding of ARIgG-per to immunoglobulin-like molecules (IGLM) present in the mammary gland. The mammary gland is a well known secretor of immunoglobulins during lactogenesis, and in the cow, apparently during galactopoiesis as well. In an attempt to mask IGLM present in mammary gland tissue sections and prevent the direct binding of ARIgG-per, the sections were preincubated with unlabeled goat anti-monkey IgG, goat anti-donkey IgG, or goat anti-bovine IgG. The goat anti-bovine IgG (ABIgG) was the only one that could prevent the direct binding of ARIgG-per to IGLM present in the mammary gland tissue, and was used in the GH and PRL staining protocols in this study.

The protocols used in the staining of GH and PRL in mammary gland tissue sections were the same as for each in the pituitary gland tissue sections (discussed above). The only difference is that concentrations of AOGH ranging from 1:500 to 1:1000, and AOPRL ranging from 1:300 to 1:900 were used.

Several controls were used to determine if GH or PRL specific staining was occurring in the mammary gland tissue sections. First, one pituitary gland mirror-image tissue section was stained for GH, the other mirror-image tissue section was stained for PRL, both were photographed, and one was made into a transparent overlay for the other (Table 1). If the staining was not specific for PRL or GH, then one would expect to see the same cells stained identically for GH and PRL. Since classical GH and PRL secreting cells can be distinguished on the basis of morphology, it is easy to determine GH and PRL specific staining. GH occurs in a population of cells distinct and different from the cells that contain PRL.

The second control used was the staining of three mammary gland sections on a slide for GH or PRL, whereas the fourth tissue section was treated with PBS-EDTA instead of the primary antibody in the incubation sequence (Table 2). If the staining reaction requires the affinity purified antisera to GH and PRL, then this is further evidence that GH and PRL are being stained specifically. Also, if similar staining is seen in triplicate then this would show that the results are at least consistent. On the other hand, if the affinity purified antisera are not required for staining the mammary gland tissue, then the staining is probably of something other than GH or PRL.

The third control used was the absorption of AOGH with purified bovine pituitary gland GH (PGH) (USDA-bGH-B-1, AFP-5200, Courtesy of D. Bolt, USDA-Reproduction Lab., Beltsville, Maryland), or recombinant methionyl bovine GH (RGH) (Courtesy of G. F. Hartnell and R. Collier, Monsanto Agricultural Company, St. Louis, Missouri), to prevent the staining of GH in the mammary gland tissue sections. In this control, one tissue section is stained for GH, one is treated with PBS-EDTA instead of the primary antiserum, and two tissue section are incubated with 10 ul of AOGH (1:1000) premixed with 6.25, 12.5, 25, or 50 ug/ml of PGH or RGH (Table 3). If staining does not occur when the antiserum is mixed with purified antigen, then this is further evidence that the antibodies specific for GH are staining tissue antigens that have GH epitopes.

Results

Both AOPRL and AOGH were specific for pituitary gland PRL and GH, respectively. The AOPRL stained large (10 to 30 um) polyhedral cells at concentrations ranging from 1:30 to 1:4800 (fig. 2). There was a discernible reduction in the intensity of the staining of PRL at concentrations of AOPRL less than 1:1200. The AOGH stained small (10 um) ovoid cells at concentrations ranging from 1:500 to 1:5000 (fig. 3), with a discernible reduction in the staining intensity at AOGH concentrations of less than 1:2500. Comparison of the

stained mirror-image pituitary gland tissue sections, demonstrated that AOGH and AOPRL stained different cell populations (fig. 4)(See Table 1 for staining protocol).

H and E staining of several areas of the mammary gland, demonstrated that the cows had cells in all stages of their secretory cycle (fig. 1a-c). After determining the areas of each mammary gland that contained milk secretory cells in the early part of their secretory cycle (columnar cells), these areas were used in the immunocytochemical localization of PRL and GH.

As stated earlier, initial immuno-staining for PRL and GH was impossible because the secondary antiserum, ARIgG-per bound directly to immunoglobulin-like molecules (IGLM) present in milk secretory cells. This is demonstrated by the intense staining of tissue sections incubated with buffer and ARIgG-per/DAB only (fig. 5a). However, by incubating the tissue sections nine X 1 hr with ABIgG (1:10), the direct binding of ARIgG-per was prevented, as seen by the absence of staining of the mammary gland tissue sections (fig. 5b)(See Table 2 for staining protocol).

The staining of immunoreactive PRL (IPRL) in the columnar milk secretory cells was positive at concentrations ranging from 1:300 to 1:900 (fig. 6a, c, and d), whereas the PBS-EDTA control was negative (Figure 6b). IPRL staining was noted on nuclear membranes, fat globule membranes, Golgi

apparatus, endoplasmic reticular areas of the milk secretory cells, and in the luminal milk (Figure 7).

The staining of the milk secretory cells early in their secretory cycle for GH was positive at AOGH concentrations of 1:500 to 1:1000 (fig. 8a, c, and d), whereas the PBS-EDTA controls were negative (fig. 8b). Immunoreactive GH (IGH) staining was similar but not identical to that of IPRL with the staining of nuclear membranes, fat globule membranes, Golgi apparatus, and endoplasmic reticulum areas of the milk secretory cells, as well as, in the luminal milk (fig. 9). The notable difference between IGH and IPRL staining in the milk secretory cells was a distinctly darker stain on nuclear membranes stained for IPRL. This staining of the nuclear membrane made it possible to identify IPRL stained mammary gland tissue sections by this criterion alone.

The staining of IGH in milk secretory cells with AOGH (1:1000) was prevented by preincubation of AOGH with 6.25 ug/ml of PGH or RGH (fig. 10b), and reduced using 12.5 ug/ml. Although AOGH (1:1000) also was mixed with 25 and 50 ug/ml of PGH or RGH, the staining was more intense using these concentrations than in control tissue sections. The control tissue sections were incubated with pure AOGH (1:1000) and stained GH positive. (fig. 10a)(See Table 3 for staining protocol).

Discussion

The results of my experiments demonstrate the presence of immunoreactive growth hormone (IGH) inside active milk secretory cells and in alveolar milk of mid-lactational Holstein cows. In the columnar milk secretory cells, IGH was found in the basal region where endoplasmic reticulum is known to be abundant, in the apical region where the Golgi apparatus is the major subcellular organelle, in apical fat globule membranes, and in association with nuclear membranes.

Although it is clear from this work that IGH is transcellularly transported by the milk secretory cell as a milk component, these findings also provide evidence for the first time, that IGH can and does get inside the active milk secretory cells, and that the mechanism of action of GH on the milk secretory cell is probably direct.

The specificity of the immunocytochemical staining assay for GH was determined in several ways. As there is a high degree of homology between pituitary gland GH and PRL, it seemed clear that PRL would be the major possible cross-reactor with the primary antibody, AOGH. Therefore, preliminary tests of specificity were carried out in bovine pituitary gland tissue sections, using AOGH and AOPRL as the primary antisera.

In pituitary gland tissue sections, AOGH bound specifically to small ovoid cells, which is the classical

morphological characteristic of the growth hormone secreting cells. At concentrations of AOGH ranging from 1:500 to 1:5000, the polyclonal antibodies bound specifically to the GH present in the GH secreting cells. There was no discernible difference in the staining of the GH secreting cells at concentrations ranging from 1:500 to 1:2500. However, at concentrations less than 1:2500, there was a significant decrease in the intensity of the staining. This indicates that the AOGH concentration of 1:2500 was sufficient to bind all of the available epitopes of pituitary gland GH, whereas concentrations less than 1:2500 were not able to saturate all of the available epitopes of pituitary gland GH. Conversely, concentrations greater than 1:2500 contained excess AOGH which could not bind to the GH epitopes, due to the saturation of all of the available epitopes of pituitary gland GH. Even at the highest concentrations of AOGH (1:500), the AOGH did not bind to PRL secreting cells.

The PRL secreting cells were stained using AOPRL as the primary antibody. AOPRL bound to larger polyhedral cells, which is the classical morphological characteristic of PRL secreting cells. Specific staining of pituitary gland PRL using AOPRL, occured at all concentrations ranging from 1:30 to 1:4800. There was no discernible difference in the intensity of staining of the PRL secreting cells at

concentrations of AOPRL ranging from 1:30 to 1:1200, however at concentrations less than 1:1200, there was a significant decrease in the intensity of staining. This indicates that the AOPRL concentration of 1:1200 was sufficient to bind all of the available epitopes of pituitary gland PRL, whereas, concentrations less than 1:1200 did not saturate all of the available PRL epitopes. Conversely, at concentrations greater than 1:1200, there was an excess of AOPRL, due to the saturation of all of the available epitopes of pituitary gland PRL by AOPRL. Even at the highest concentrations of AOPRL (1:30), there was never any immunological identification of GH secreting cells.

Fumagalli and Zanini (1985) reported the presence of both PRL and GH in the same pituitary gland cell in Holstein Friesian cows. Despite numerous experiments, the method used in this study was only able to demonstrate the occurrence of three possible pituitary gland cells, out of several hundred, containing both PRL and GH. Even when the pituitary gland tissue sections were incubated with excess AOGH (1:500) or AOPRL (1:30), for three days, AOGH and AOPRL identified different populations of pituitary gland cells.

The second piece of evidence of AOGH and AOPRL specificity was an important difference in staining of mammary gland tissue sections. Like IGH, IPRL was detected in the endoplasmic reticulum and Golgi apparatus regions, in

apical fat globule membranes, and in association with nuclear membranes of columnar milk secretory cells. All of these findings except nuclear PRL have also been shown by Malven and Keenan (1982) in the cow. In the rat, Nolin and Bogdanove (1980) found the same staining pattern as was demonstrated here. However, the main difference, was seen in the staining of IGH and IPRL on nuclear membranes. The nuclear membranes consistently stained more intensely for IPRL than IGH in the columnar milk secretory cells.

The third and most definitive line of evidence of AOGH specificity for GH was demonstrated by preincubating AOGH with PGH or RGH, prior to its application to the bovine mammary gland tissue sections. Preincubation of AOGH with 6.25 ug/ml PGH or RGH resulted in the loss of IGH staining in the milk secretory cells. However, preincubation of AOGH with 25 or 50 ug/ml of RGH or PGH resulted in darker GH staining than in pure AOGH treated controls. This increase in staining could be the result of excess RGH or PGH binding to unoccupied GH receptors present in the milk secretory cells (personal communication, R. Collier, G. Mulheron, and J. Nolin). Since PGH and RGH (6.25 ug/ml) were able to bind all of the AOGH antibodies (1:1000), it appears that AOGH was specific in its staining of bovine IGH, and was not the result of a cross-reaction with IPRL, even though both hormones were clearly present inside the same cells in the mammary gland.

Since polyclonal antisera to GH and PRL were used in this study, IPRL and IGH could represent several modified forms of PRL and GH, respectively. IGH may only be a fragment of bovine GH, and IPRL may only be a fragment of bovine PRL. This study was not designed to determine if a fragment of bovine GH and PRL were present inside the columnar milk secretory cells, but to determine if GH could reach the interior of the milk secretory cells, and if PRL reached the nucleus of these cells. It is possible that a fragment of GH and PRL are stimulating the milk secretory cells. Previously, a fragment of GH was found to have a lipolytic effect on adipocytes (Yudaev et al., 1976), and a fragment of PRL has been shown to have an inhibitory effect on oocyte maturation and ovulation (Nolin, 1982).

With regard to the effect of GH on milk production by the Holstein cow, the work presented here strongly suggests that GH also acts directly on the mammary gland. Exactly how it acts on the mammary gland milk secretory cell is speculative at the present time. However, one might suggest that GH acts to stimulate one or more of the many points of protein synthesis, as an increase in protein synthesis has the potential to drive milk synthesis and secretion. For example, an increase in alpha-lactalbumin, a key protein in milk synthesis and secretion has already been reported to occur in response to injection of PGH and RGH (Eppard et al.,

1985). It seems likely that GH does not use a membrane-derived mediator, such as cAMP, but that it acts directly on subcellular organelles and/or cellular enzymes. In the last few years, several other anterior pituitary hormones including: prolactin (Nolin and Witorsch, 1976), adrenocorticotropin (Nolin, 1979; Nolin 1980), follicle stimulating hormone, and luteinizing hormone (Mulheron et al., 1987), have all been shown to enter their target cells. Bovine GH can now be added to that list.

Table 1. Incubation sequence for the staining of pituitary gland flip-flop tissue sections for prolactin (PRL) and growth hormone (GH). All pituitary gland tissue sections were preincubated with goat anti-bovine IgG (ABIgG). Sections 2 and 3 were sections with their shared side up (mirror-images). Section 2 was incubated with rabbit anti-ovine prolactin (AOPRL), and section 3 was incubated with rabbit anti-ovine growth hormone (AOGH), while sections 1 and 4 were incubated with PBS-EDTA buffer. All sections were then incubated with affinity purified anti-rabbit IgG conjugated to peroxidase (ARIgG-per) followed by the chromogenic substrate 2,3-diaminobenzidine-peroxide (DAB).

Section #	1	2	3	4
Preincubation	ABIgG	ABIgG	ABIgG	ABIgG
Primary Antibody	PBS-EDTA	AOPRL	AOGH	PBS-EDTA
Secondary Antibody Chromogen	ARIgG-per/ DAB	ARIgG-per/ DAB	ARIgG-per/ DAB	ARIgG-per/ DAB

Table 2. Incubation sequence for the staining of mammary gland tissue sections for immunoglobulin-like molecules, prolactin, and growth hormone. Tissue sections 1 thru 4 are preincubated with goat anti-bovine IgG (ABIgG), while tissue section 5 is incubated with PBS buffer. Tissue sections 1 thru 3 are incubated with PBS the primary antibody, rabbit anti-ovine growth hormone (AOGH), or rabbit anti-ovine prolactin (AOPRL), while sections 4 and 5 are incubated with PBS-EDTA buffer. Finally, all of the tissue sections are incubated with the secondary antibody conjugated to peroxidase, goat anti-rabbit IgG-Peroxidase (ARIgG-per), followed by the chromogenic substrate 2,3-diaminobenzidine-peroxide (DAB).

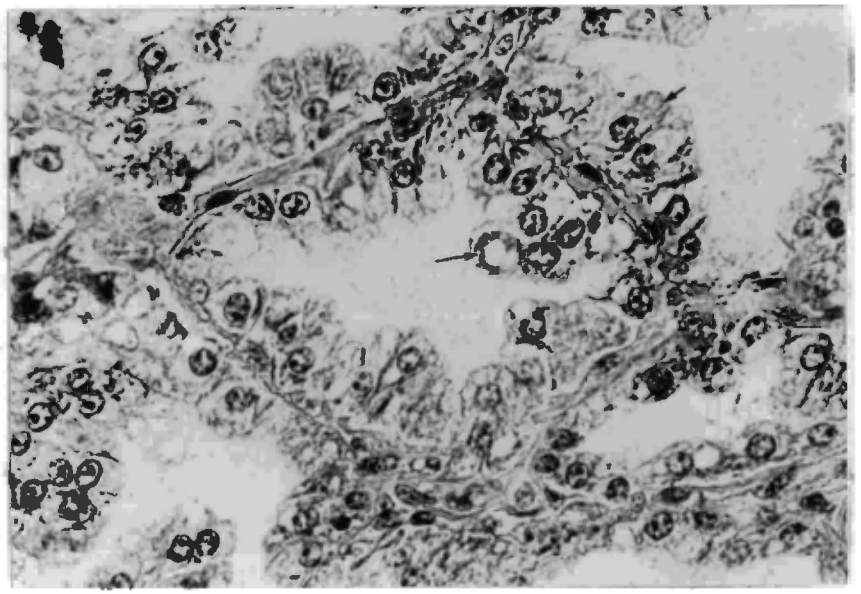
Section #	1	2	3	4	5
Pre-incubation	ABIgG	ABIgG	ABIgG	ABIgG	PBS
Primary Antibody	AOGH or AOPRL	AOGH or AOPRL	AOGH or AOPRL	PBS-EDTA	PBS-EDTA
Secondary Antibody/ Chromogen	ARIgG-per /DAB	ARIgG-per /DAB	ARIgG-per /DAB	ARIgG-per /DAB	ARIgG-per /DAB

Table 3. Incubation sequence for preventing the staining of growth hormone (GH) in mammary gland tissue sections, by absorbing the rabbit anti-ovine growth hormone (AOGH) with purified bovine pituitary gland GH (PitGH), or recombinant bovine GH (RGH). All 4 tissue sections are preincubated with goat anti-bovine IgG (ABIgG). Preincubation with ABIgG is followed by section 1 being incubated with PBS-EDTA buffer, section 2 and 4 with AOGH mixed with different quantities of PGH or RGH, and tissue section 3 with AOGH. All tissue sections are then incubated with goat anti-rabbit IgG conjugated to peroxidase (ARIgG-per), followed by the chromogenic substate, peroxide-2,3-diaminobenzidine (DAB).

Section #	1	2	3	4
Preincubation	ABIgG	ABIgG	ABIgG	IBIgG
Primary Antibody	PBS-EDTA	AOGH abs. PitGH or RGH	AOGH	AOGH abs. PitGH or RGH
Secondary Antibody Chromogen	ARIgG-per/ DAB	ARIgG-per/ DAB	ARIgG-per/ DAB	ARIgG-per/ DAB

Figure 1a. H and E stained mammary gland tissue section showing the columnar milk secretory cells (arrows), actively synthesizing and secreting the constituents of milk, early in a secretory cycle. (600 X)

Figure 1b. H and E stained mammary gland tissue section showing alveolar milk, and cuboidal milk secretory cells (arrow), mid-way through a secretory cycle. (600 X)



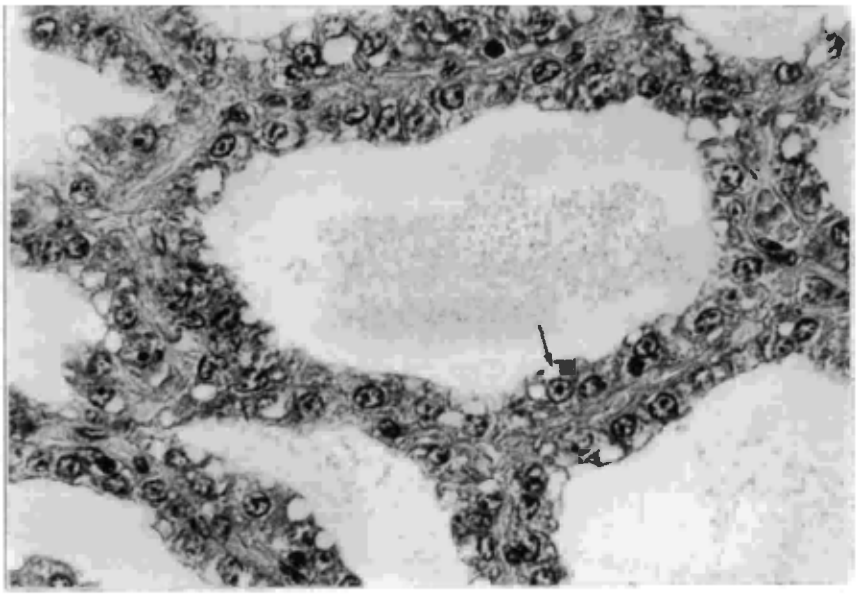


Figure 1c. H and E stained mammary gland tissue section showing the squamous milk secretory cells (arrow), at the end of a milk secretory cycle. (600 X)

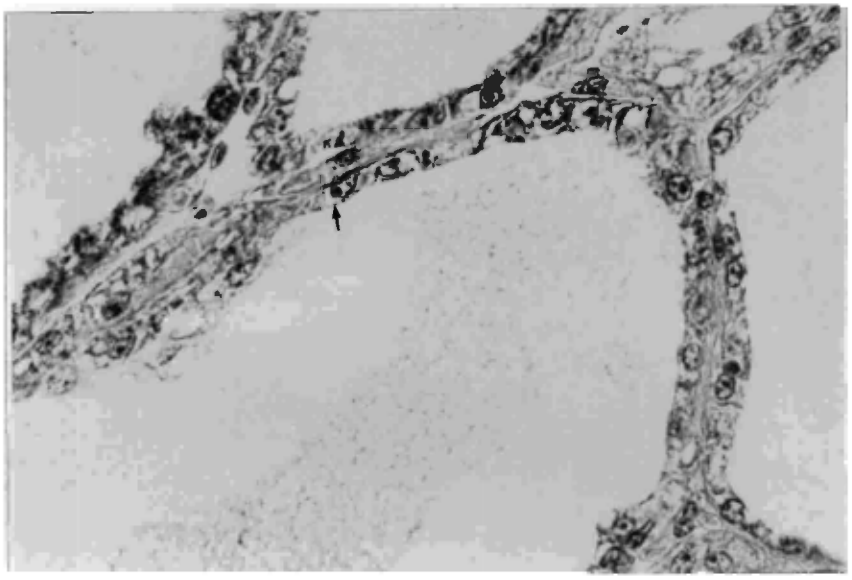


Figure 2. Pituitary gland tissue section stained for prolactin using rabbit anti-ovine prolactin (1:900). The darkly stained polyhedral cells are prolactin secreting cells which range in size from 10 to 30 um (arrows), depending on the plane of sectioning (See Table 1 for staining protocol). (350X)

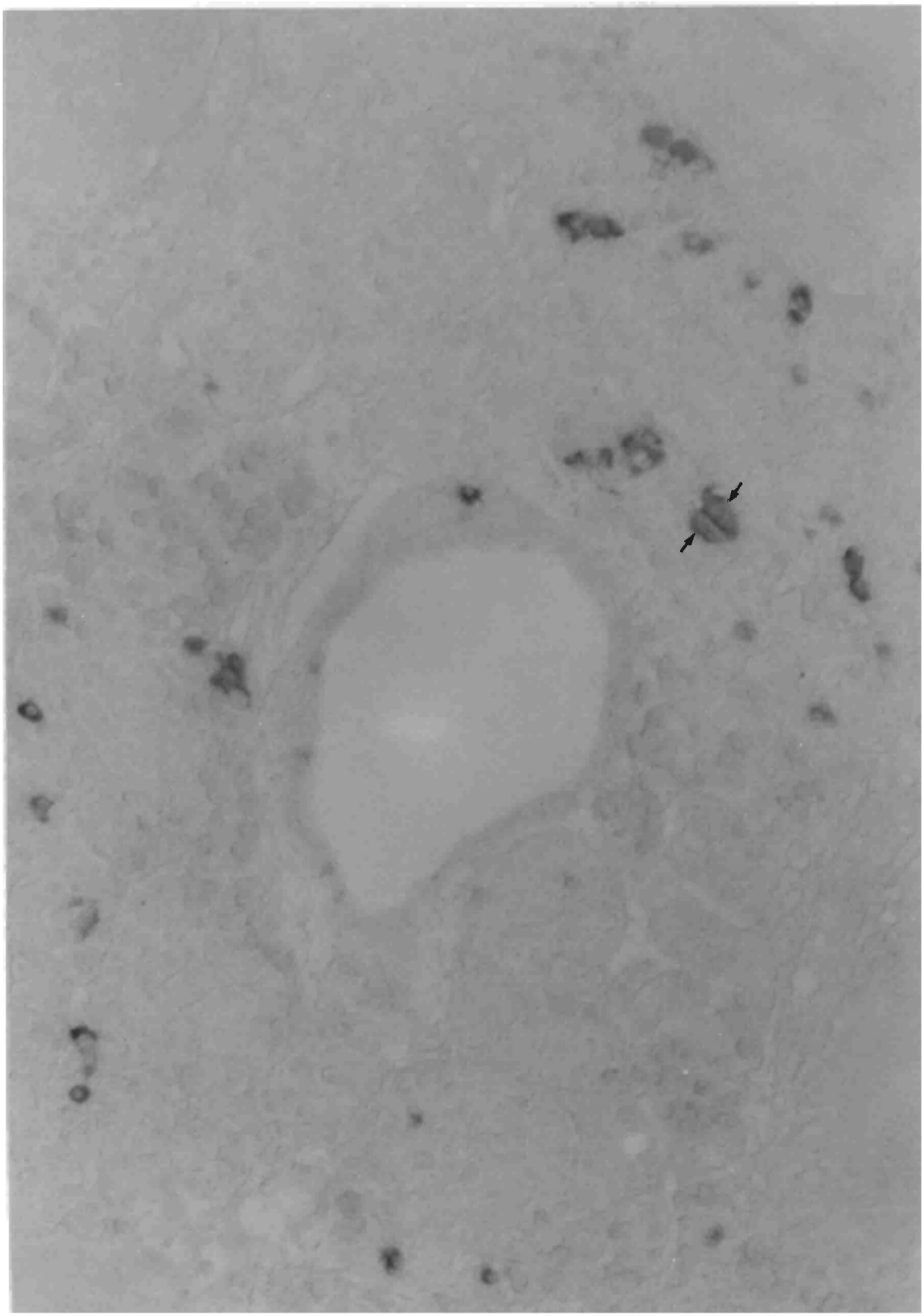


Figure 3. This is a pituitary gland tissue section stained for growth hormone using rabbit anti-ovine growth hormone (1:1000). The darkly stained ovoid cells are growth hormone secreting cells and are about 10 um in size (arrows), depending on the plane of sectioning (See Table 1 for staining protocol). (350X)

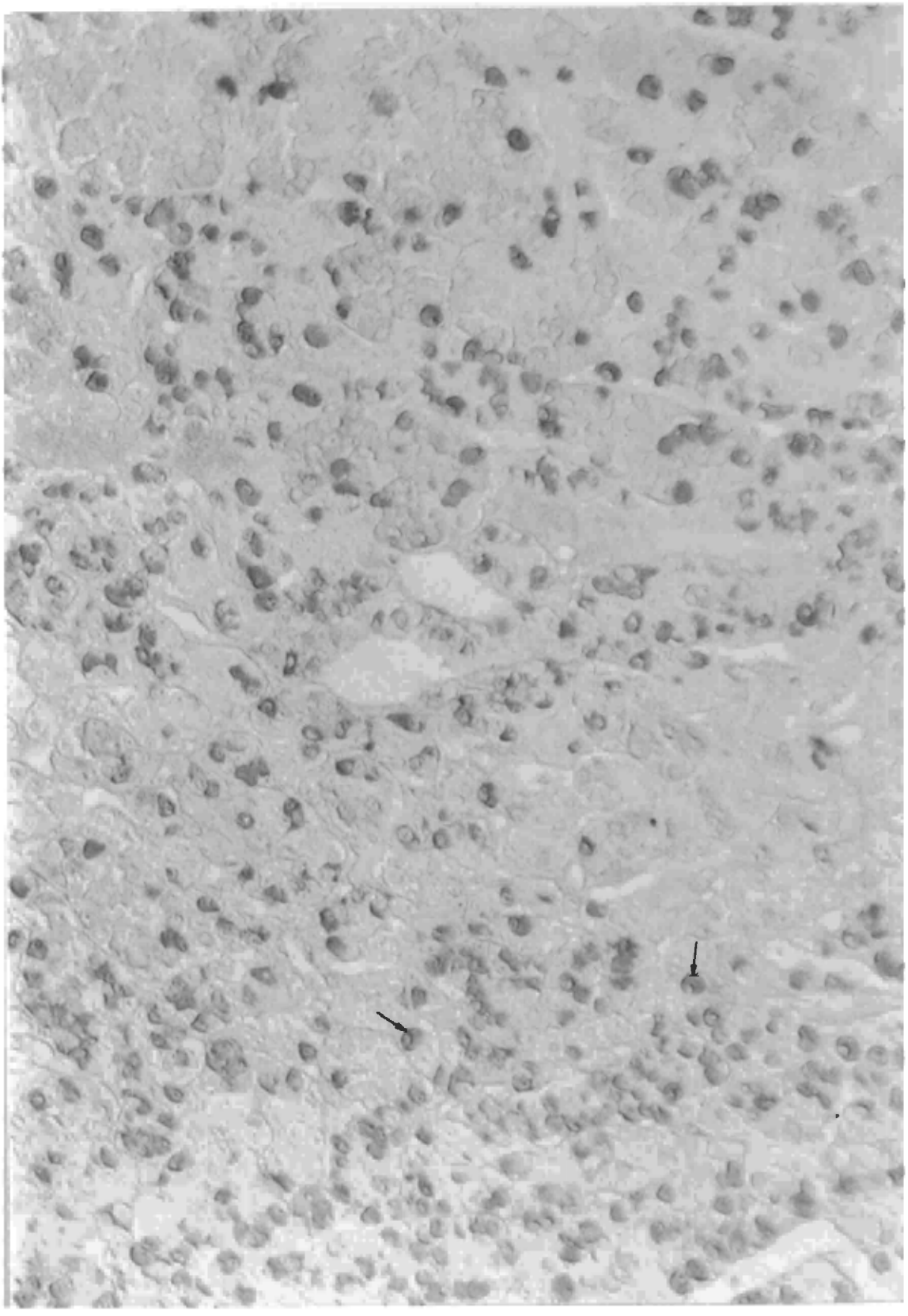


Figure 4. Pituitary gland mirror-image tissue sections stained for prolactin (overlay), and growth hormone (underlay). Using concentrations of rabbit anti-ovine prolactin ranging from 1:30 to 1:4800, and concentrations of rabbit anti-ovine growth hormone ranging from 1:500 to 1:5000, the staining of each antigen demonstrated that prolactin and growth hormone are in different and distinct cell populations. Although a different variety of cows have been reported to contain pituitary gland cells which secrete both prolactin and growth hormone, in this study there were only a few possible occurence of such a cell type (See Table 1 for staining protocol). (350X)

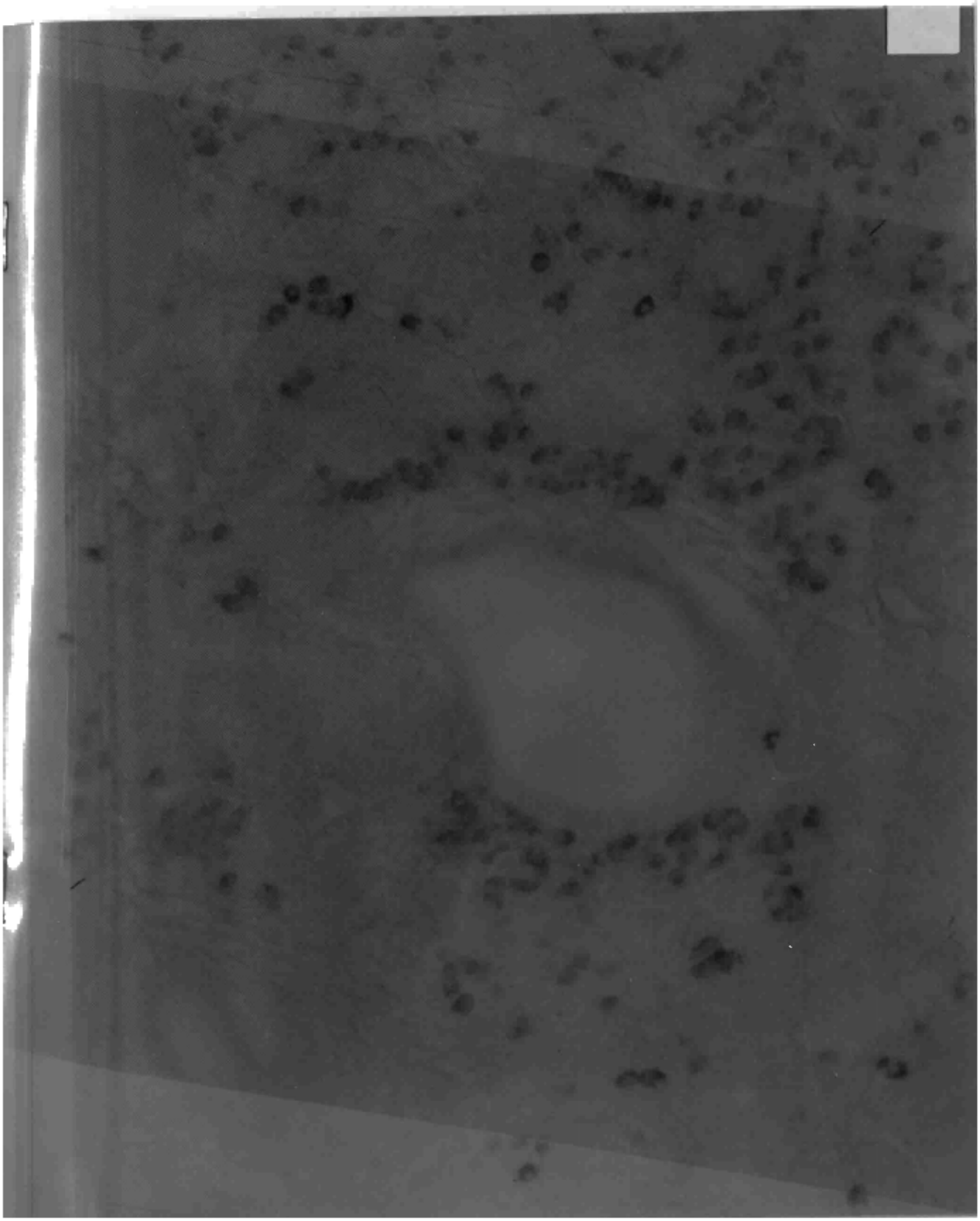
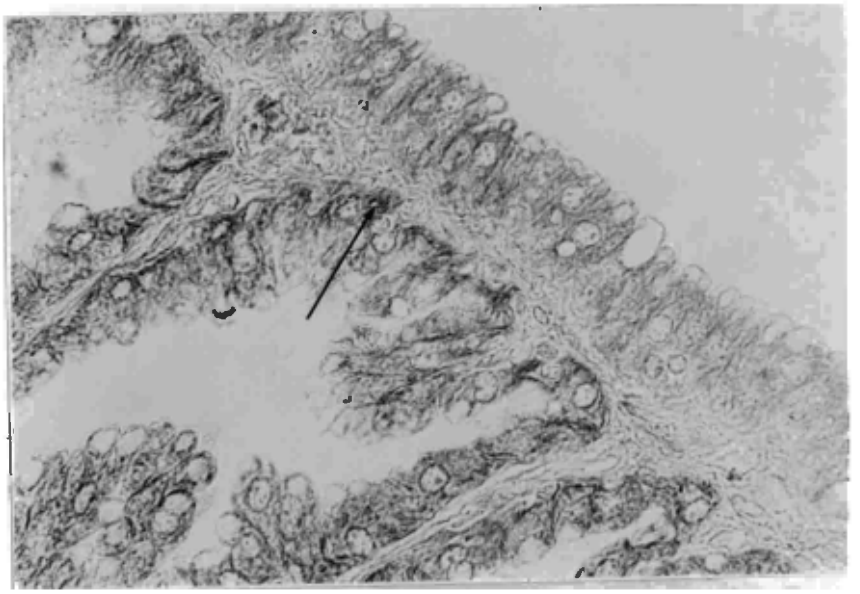


Figure 5a. Mammary gland tissue section stained for immunoglobulin-like molecules (IGLM) (arrow) using the direct application of goat anti-rabbit IgG-peroxidase, followed by the chromogenic substrate, 2,3-diaminobenzidine-peroxide. IGLM are stained intensely inside the active, columnar, milk secretory cells, demonstrating their high concentration inside these cells. This staining of IGLM inside the milk secretory cells was prevented by preincubating the tissue sections with goat anti-bovine IgG (Figure 5b) (See Table 2 for staining protocol). (600X)

Figure 5b. Mammary gland tissue section incubated with goat anti-bovine IgG prior to the application of goat anti-rabbit IgG-peroxidase, and 2,3-diaminobenzidine-peroxide. Preincubation of the mammary gland tissue section with goat anti-bovine IgG clearly prevents the staining of IGLM (arrow) seen in Figure 9a (See Table 2 for staining protocol). (600X)



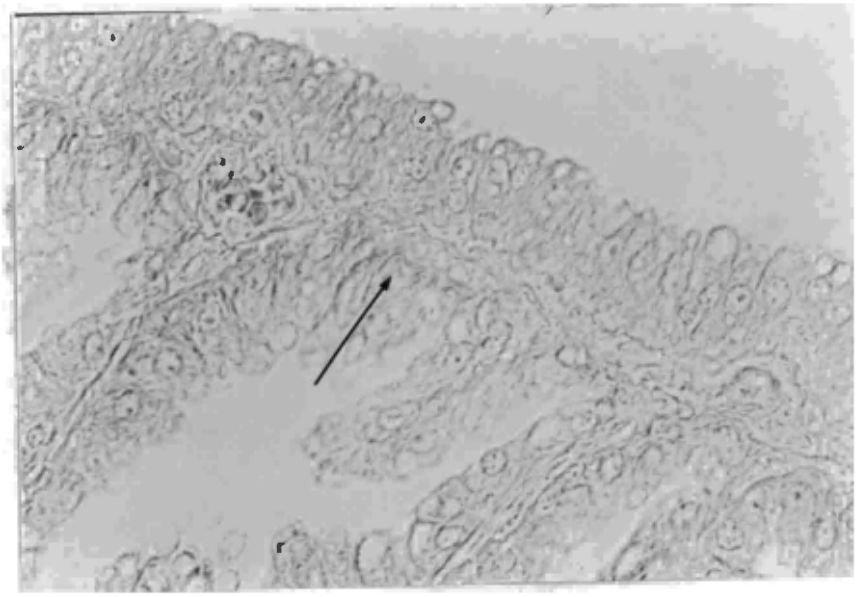
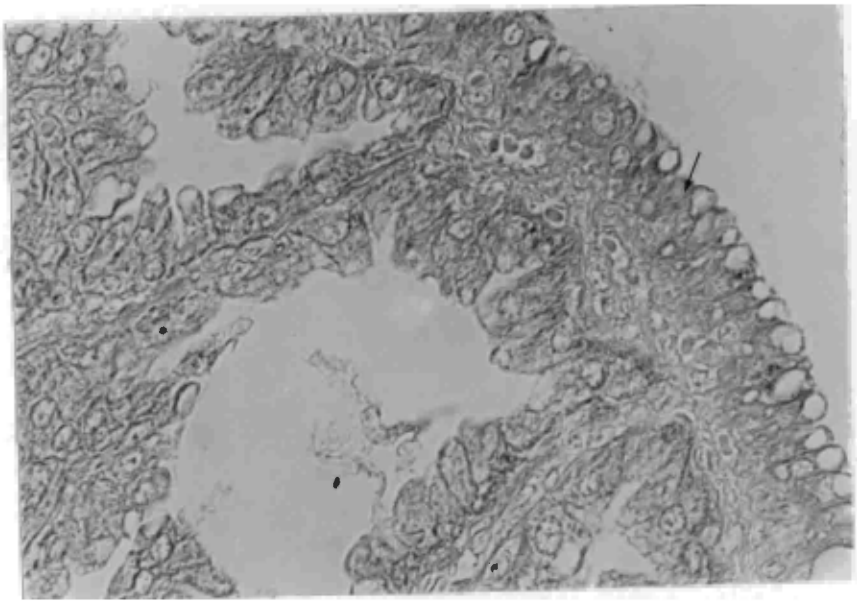


Figure 6a. This is a mammary gland tissue section stained for prolactin (arrow). The milk secretory cells are stained darkly where prolactin is located. Figures 6a, b, c, and d are all adjacent serial sections. Compare the staining seen in this figure with Figure 6b, the PBS-EDTA treated control (See Table 2 for staining protocol). (600X)

Figure 6b. In this mammary gland tissue section PBS-EDTA was substituted for rabbit anti-ovine prolactin. Note the absence of staining (arrow) here compared with Figures 6a, c, and d. Figures 6a, b, c, and d, are all adjacent serial tissue sections (See Table 2 for staining protocol). (600X)



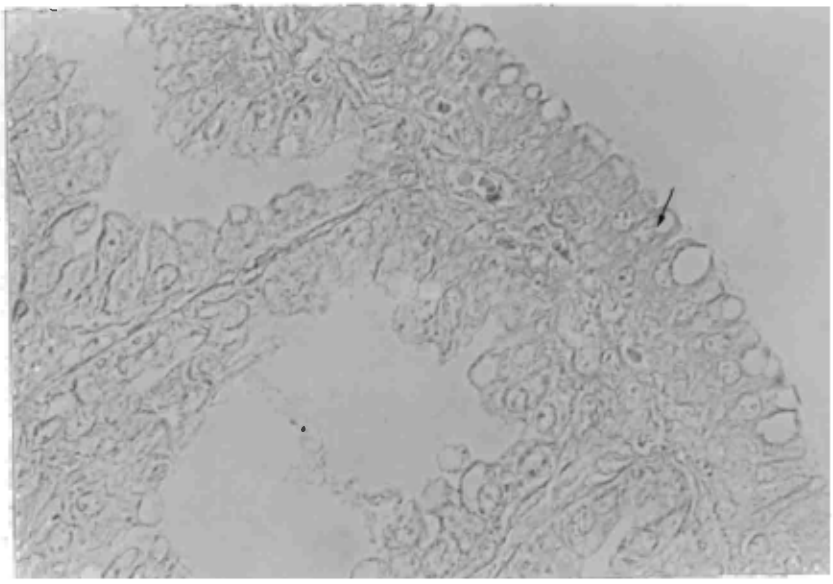
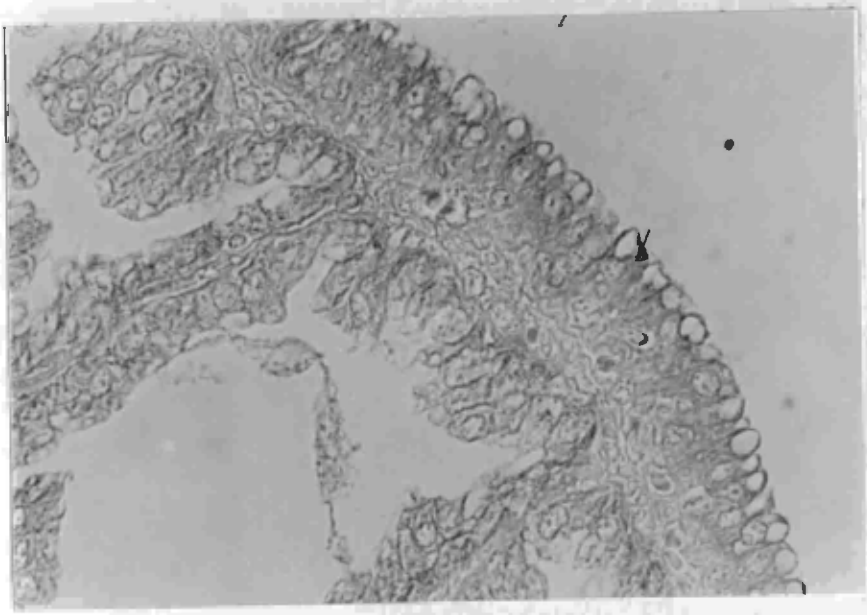


Figure 6c. This is a mammary gland tissue section stained for prolactin (arrow). The milk secretory cells are stained darkly where prolactin is located. Figures 6a, b, c, and d, are all adjacent serial tissue sections. Compare the staining seen in this figure with Figure 6b, the PBS-EDTA control (See Table 2 for staining protocol). (600X)

Figure 6d. This mammary gland tissue section is stained for prolactin (arrow). The milk secretory cells are stained darkly where prolactin is located. Figures 6a, b, c, and d, are all adjacent serial tissue sections. Compare the staining seen in this figure with Figure 6b, the PBS-EDTA treated control (See Table 2 for staining protocol). (600X)



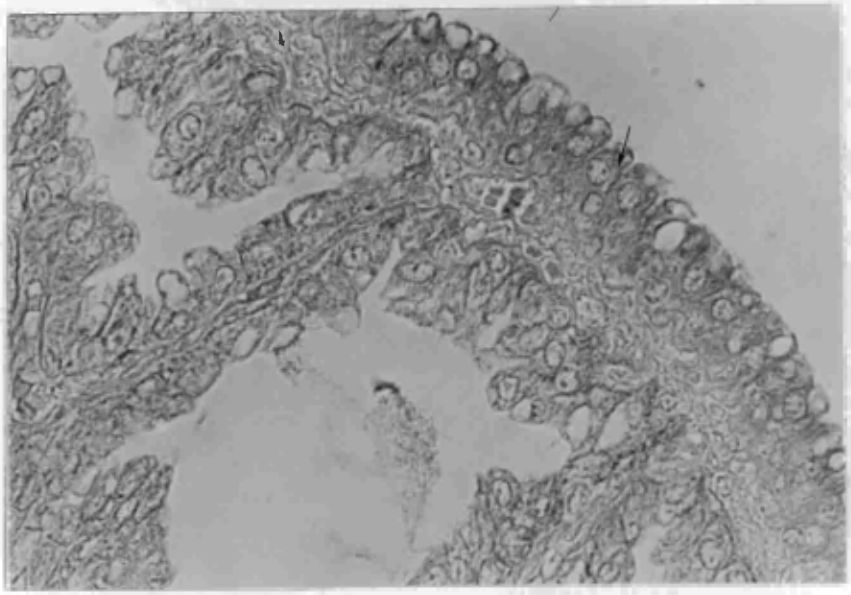


Figure 7. This is a small portion of mammary gland tissue stained for PRL. The milk secretory cells are stained on their nuclear membranes (n), fat globule membranes (f), endoplasmic reticular (e) and Golgi apparatus (G) regions of the cells. (2400X)

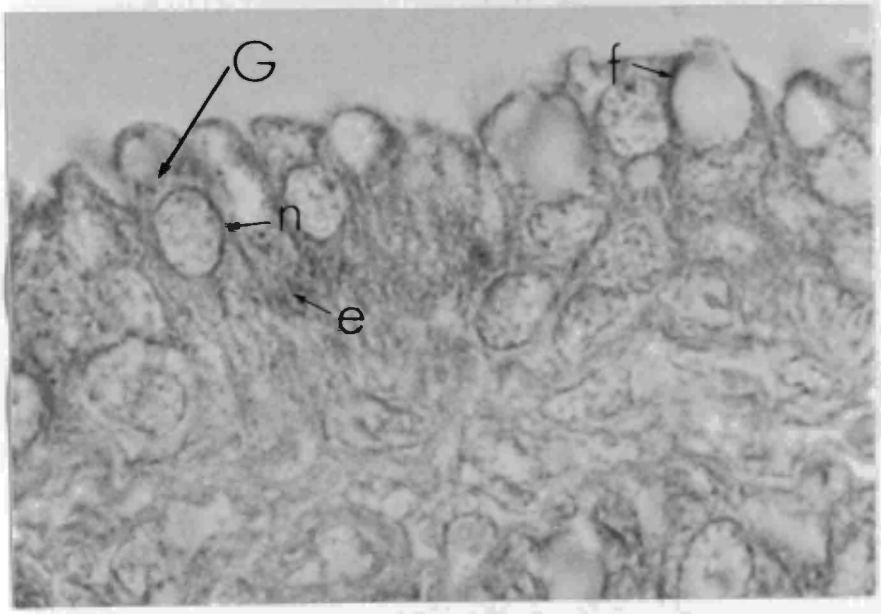
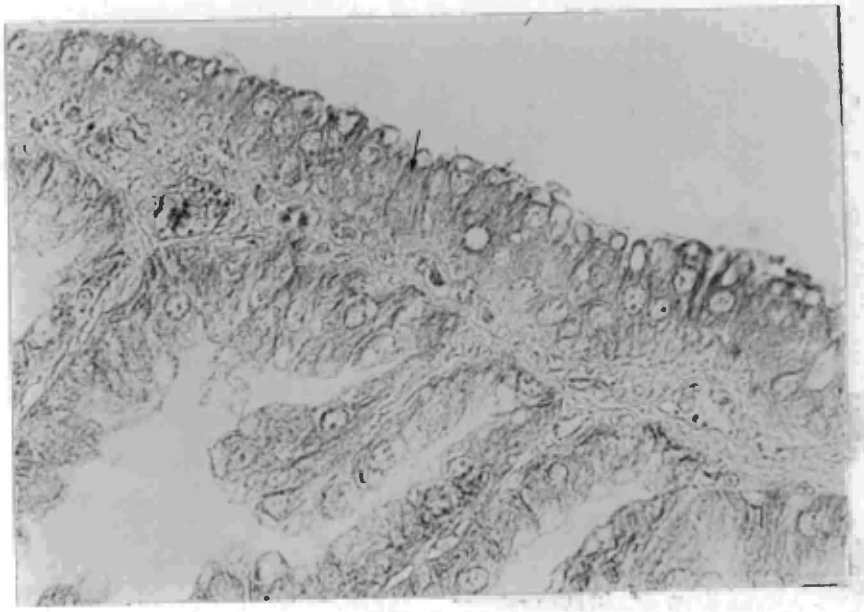

G
f
n
e

Figure 8a. This mammary gland tissue section was stained for growth hormone (arrow). Note the darkly stained portions of the milk secretory cells, and compare them to the PBS-EDTA treated (control) mammary gland tissue section seen in Figure 8b. Clearly, growth hormone is present inside the milk secretory cells of the mid-lactational Holstein cow. Figures 8a, b, c, and d are all adjacent serial tissue sections (See Table 2 for staining protocol). (600X)

Figure 8b. In this mammary gland tissue section PBS-EDTA buffer was substituted for AOGH, and served as a negative control for Figures 8a, c, and d (arrow). Figures 8a, b, c, and d are all adjacent serial tissue sections. Note the absence of staining when comparing this figure with figures 8a, c, and d (See Table 2 for staining protocol). (600X)



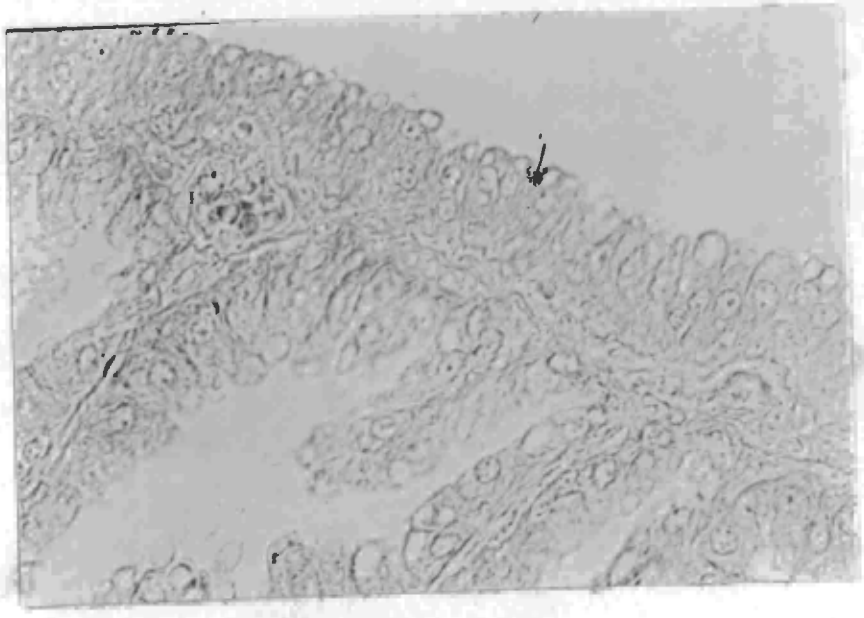
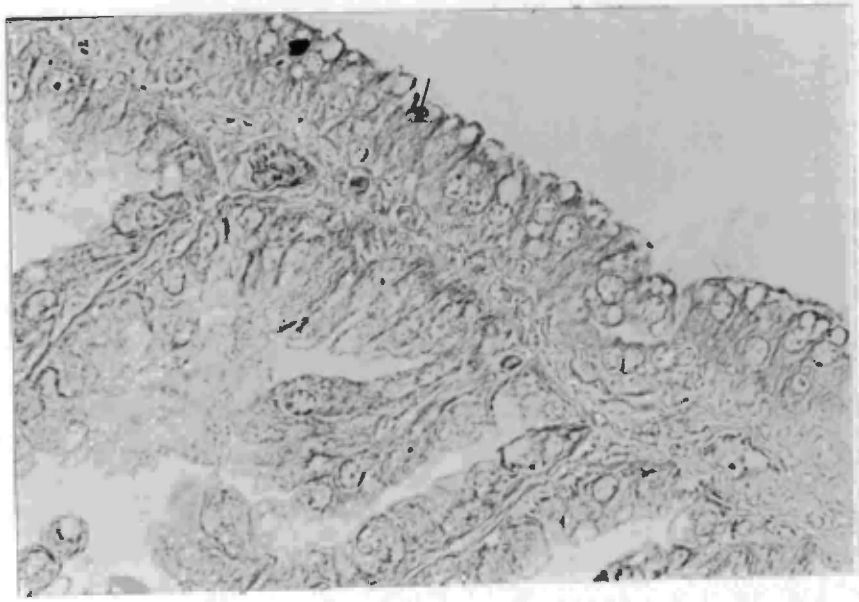


Figure 8c. This mammary gland tissue section was stained for growth hormone (arrow). Note the darkly stained portions of the milk secretory cells, and compare them to the PBS-EDTA treated (control) mammary gland tissue section seen in Figure 8b. Clearly, growth hormone is present inside the milk secretory cells. Figures 8a, b, c, and d are all adjacent serial tissue sections (See Table 2 for staining protocol). (600X)

Figure 8d. This mammary gland tissue section is stained for growth hormone (arrow). Note the darkly stained portions of the milk secretory cells, and compare them to the PBS-EDTA treated (control) mammary gland tissue section seen in Figure 8b. Clearly, growth hormone is present inside the milk secretory cells. Figures 10a, b, c, and d are all adjacent serial tissue sections (See Table 2 for staining protocol). (600X)



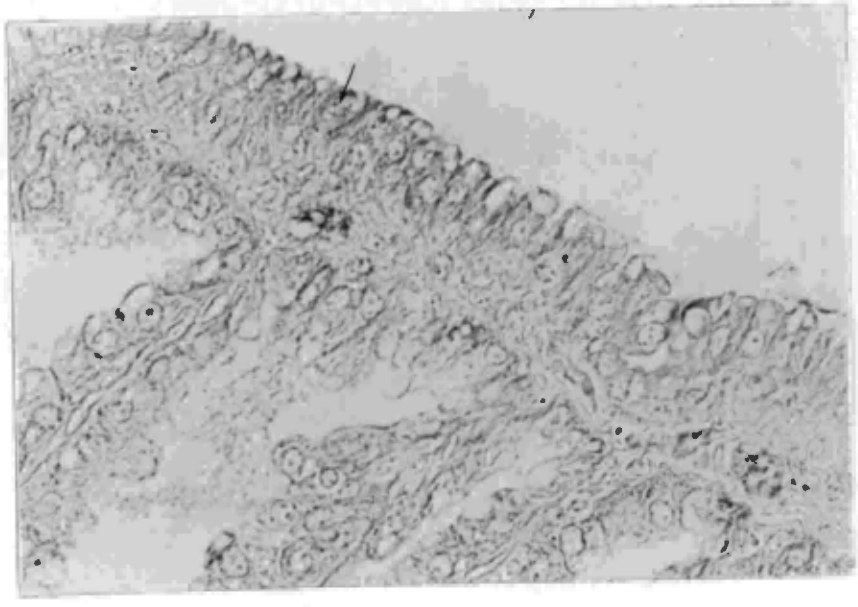


Figure 9. Growth hormone localization is shown at higher magnification on nuclear membranes (n), fat globular membranes (f), in Golgi apparatus (G) and endoplasmic reticular (e) areas of the milk secretory cells (See Table 2 for staining protocol). (2400X)

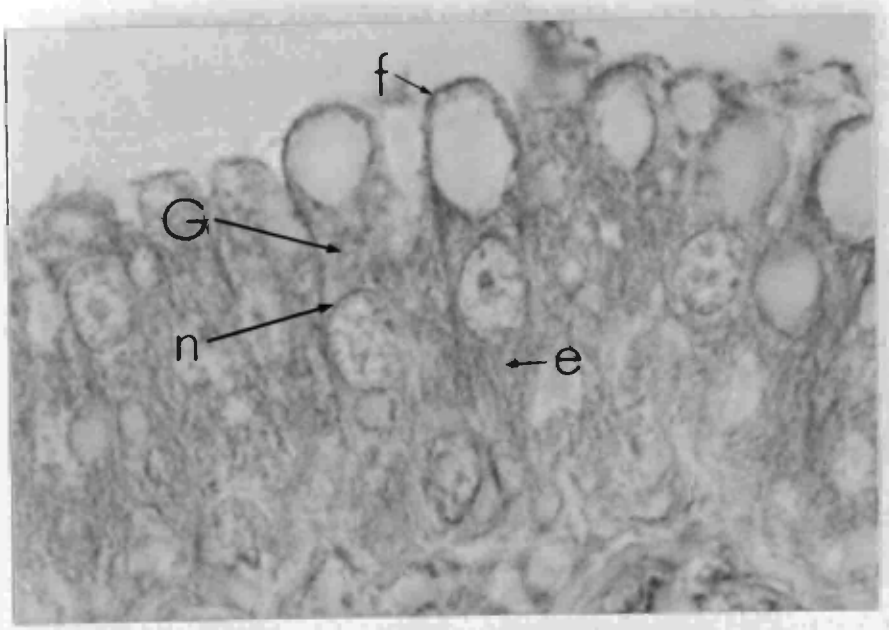
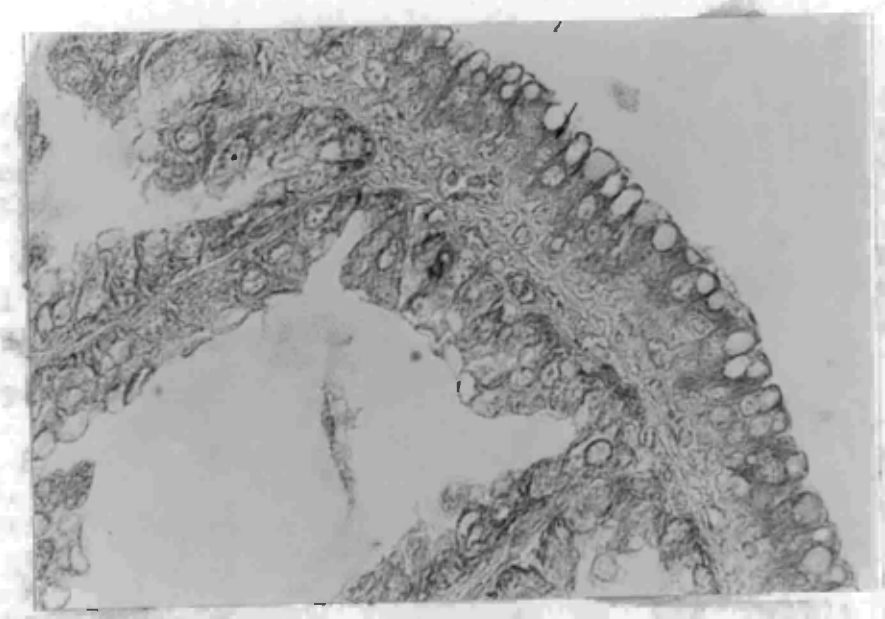
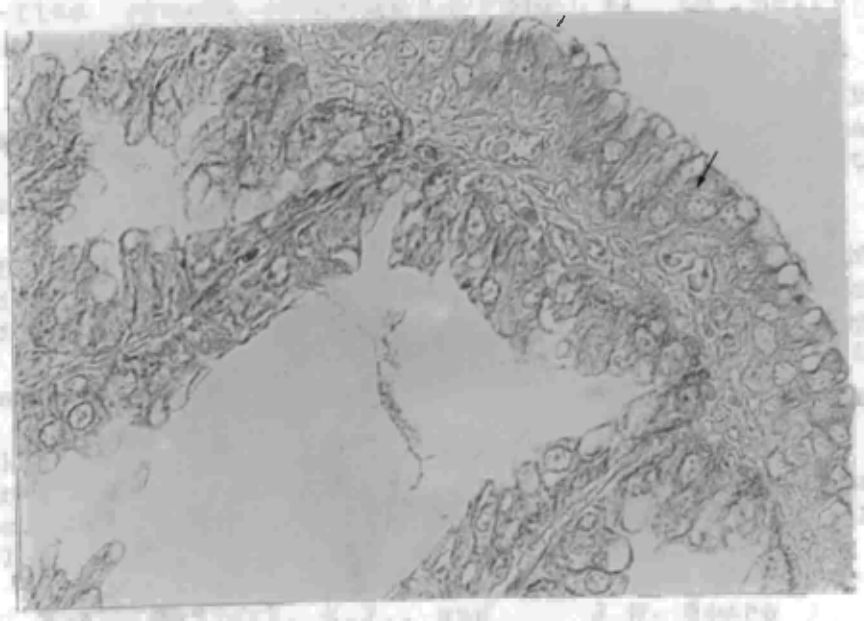

f
G
n
e

Figure 10a. This mammary gland tissue section was stained for growth hormone (arrow) using rabbit anti-ovine growth hormone (AOGH) as the primary antibody. The milk secretory cells are stained darkly where growth hormone is located. This figure and Figure 10b are adjacent serial tissue sections (See Table 3 for staining protocol). (600X)

Figure 10b. This mammary gland tissue section was incubated with rabbit anti-ovine growth hormone that had been mixed with 6.25 ug/ml of bovine pituitary growth hormone. Note the reduction in staining seen in this tissue section (arrow), compared to Figure 10a (See Table 3 for staining protocol). (600X)





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Vita

David Andrew Walkup is the son of retired military parents Mr. and Mrs. David F. Walkup. He was born in Tripoli, Lybia, Africa on Wheelis Air Force Base in 1960. His family resided in Michagan, Ice Land, Texas, and Virginia. He went to Tabb High School in Tabb, Virginia and graduated in 1978. He then went to Ferrum College in Ferrum, Virginia, and graduated with honors an Associate of Science in Biology in 1980. He then went to the University of Richmond in Richmond, Virginia, and graduated a Bachelor of Science in Biology in 1982. He was married to Jamie L. Moore in August of 1988. Currently, he is employed by the Medical College of Virginia in the department of surgery as a Laboratory Specialist. After completion of the Master of Science in Biology program at the University of Richmond in 1989, he plans to go into pharmaceutical research, pharmaceutical sales, or a PhD program at the Medical College of Virginia.