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OF PREGNANT AND LACTATING RATS AS STUDIED
BY IMMUNOHISTOCHEMISTRY AND RADIOIMMUNOASSAY.

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PROLACTIN AND LUTEINIZING HORMONE CELLS OF PREGNANT AND LACTATING RATS AS STUDIED BY IMMUNOHISTOCHEMISTRY AND RADIOIMMUNOASSAY

by

Frederick Wayne Merchant

A dissertation submitted in partial fulfillment
of the requirements for the degree of
Doctor of Philosophy
(Anatomy)
in The University of Michigan
1973

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INTRODUCTION

Cytological modifications that may occur in the hypophyseal pars distalis during pregnancy and lactation are poorly understood. This situation is due partly to the inadequacy of histological staining procedures that have been used for differentiating specific cell types.

With respect to pregnancy in the rat, Haterius ('32), Stein ('34), and Desclin ('34) were unable to demonstrate any significant variation in percentages of acidophils during pregnancy. However, during the latter two-thirds of pregnancy Wolfe and Cleveland ('33) observed an enlargement of acidophils (their type I cells) which stained intensely with orange G. Severinghaus ('39) reported that many acidophils have an enlarged Golgi apparatus and abundant mitochondria during pregnancy and he postulated that such alterations are suggestive of an increase in secretory activity. Subsequently, Lacour ('50), applying the histological technique of Romeis (Heidenhain's azan procedure with orange G), reported that orangeophils (a subclass of acidophils) are hypertrophied during gestation and concluded that these cells secrete prolactin. Similarly, Pasteels ('63) with the tetrachrome technique of Herlant observed that during pregnancy erythrosinophils (a subgroup of acidophils believed to be the source of prolactin) are characterized by an increase in number and size, a hypertrophied Golgi complex, and expanded ergastoplasm. Thus, there appears to be some evidence that during pregnancy in

the rat cytological changes occur in acidophils that suggest accelerated secretory activity.

The influence of pregnancy on the basophil class of cells is less clear. Wolfe and Cleveland ('33) reported that basophils enlarge and increase in number during the latter two-thirds of pregnancy in the rat; similarly Pasteels ('63) concluded that γ-cells (a subgroup of basophils) multiply early in pregnancy and accumulate glycoprotein as gestation progresses. Pasteels regarded γ-cells as the source of luteinizing hormone. On the other hand, Everett and Baker ('45), using a less favorable histological procedure, found that the relative percentage of basophils was slightly, and probably insignificantly, lower at the 20th than at the tenth day of pregnancy. Thus, there is a need to determine which type of cell(s) in the basophil class changes during pregnancy.

In hypophyses of <u>lactating</u> rats, Everett and Baker ('45) reported on the basis of differential cell counts that at three to seven days postpartum the relative percentage of acidophils is increased almost 100% when compared to the percentage present in pregnant rats. Subsequently the relative number of acidophils decreased somewhat as lactation progressed. No distinction was made between the acidophils that produce growth hormone and those that secrete prolactin. Later Pasteels ('63) described a similar response in erythrosinophils, which was accompanied by cellular hypertrophy that peaked at six days. Since these changes seemed

to parallel the rise and fall of pituitary prolactin content as determined by Meites and Turner ('48) with bioassay, it was deduced that acidophils are the source of prolactin. With respect to basophils, Stein ('34) and Everett and Baker ('45) found no significant changes during gestation while Pasteels ('63) reported that γ-cells "involute" early in lactation and remain unchanged during the remainder of the period; β basophils were said to continue without modification during lactation.

It is clear that the issue of cytological change in the pars distalis during pregnancy and lactation needs to be re-examined with more specific methods for identification of cell types so that the functional meaning of such modulations can be interpreted in terms of concurrent alterations in pituitary hormone content and secretory activity. To this end two objectives were posed for this investigation. First, the cytology of the rat hypophyseal pars distalis was analyzed during pregnancy and lactation by means of the immunohistochemical technique of Nakane and Pierce ('67). With it most pituitary cell types can be identified accurately, including the growth hormone cell, prolactin cell, corticotropic cell, thyrotropic cell, and gonadotropic cell (luteinizing hormone cell, LH-cell). In this study, however, only prolactin and LH-cells were considered. Second, the levels of prolactin and luteinizing hormone in the hypophysis and blood were quantified during pregnancy and lactation at the same times when the cytological exam-

inations were made. Radioimmunoassay procedures for luteinizing hormone (Niswender et al., '68) and prolactin (Niswender et al.,'69) were used for this purpose.

LITERATURE REVIEW

Introduction

This review will deal with pertinent contributions in the field of pituitary research which have assisted in clarifying hypophyseal structure and function during pregnancy and lactation. It encompasses only matters not otherwise considered in the Introduction or Discussion.

Pregnancy

Morphological Changes in the Hypophysis

The initial observation that the pituitary gland varies in size and histological structure during pregnancy was made by Comte (1898) who described hypertrophy and hyperplasia of the human hypophysis. These observations were confirmed by many early workers including Launois and Mulon ('04), Erdheim and Stumme ('09), and Mayer ('10).

Among the investigators working on lower forms, however, there is considerable difference of opinion concerning weight variation of the hypophysis during pregnancy. Guerrini ('05) and Wolfe et al. ('33) described an increase in pituitary weight during pregnancy for the dog; however, Holst and Turner ('39) reported no significant weight change in the hypophysis of the rabbit.

In regard to the ungulates, neither Rosta ('33), nor Nalbandov and Casida ('40) observed changes in weight of the hypophysis during pregnancy in the cow. In contrast, however, Kostner ('33) found a 100% rise in the weight of the

sow's hypophysis during late pregnancy. In addition, in the pregnant mustang (Catchpole and Lyons, '34) and in sheep (Warbritton and McKenzie, '37) a greater pituitary weight was noted.

Hypertrophy of the mouse hypophysis during gestation was observed by Urasov ('28) and by Hurst and Turner ('42). In addition, Urasov ('28) postulated that the greater pituitary weight was due to enlargement and development of a new basophilic cell type. Ladman and Runner ('53; '59) also observed that the mouse hypophysis became heavier during the second half of gestation. Furthermore, Ladman ('54) attributed the increased weight of the mouse hypophysis to an acceleration in cellular division of all three cell types, the most significant change being among the acidophils.

In contrast to the greater weight of the mouse hypophysis during pregnancy, relatively insignificant change was observed by most investigators for the rat (Haterius, '32; Stein, '34; Desclin, '34; Reece, '37; Everett and Baker '45; Grosvenor and Turner, '60), and the guinea pig (Desclin, '34; Holst and Turner, '39). Herring ('20), however, reported a 24% decline in weight of the pregnant rat hypophysis. In addition to these observations, Everett and Baker ('45) and Hunt ('49) demonstrated that, unlike the mouse hypophysis, mitotic activity in the rat pituitary gland persists at a low level throughout pregnancy.

Thus, at present, our understanding of the influence of pregnancy on weight of the hypophysis is not clear for most

animals studied; however, the evidence indicates that during pregnancy in the rat there is minimal fluctuation.

Cytological Changes

Acidophils. Changes in the number or structure of acidophils during pregnancy have been reported for many species. Erdheim and Stumme ('09) noted that in the human hypophysis during gestation there was a progressive decrease in acidophils; however, subsequently Severinghaus ('39), Romeis ('40), Goluboff and Ezrin ('69), and Pasteels et al. ('72) concluded that acidophils became more numerous in the human hypophysis during pregnancy. These findings were also confirmed for the bat (Herlant, '56), and rhesus monkey (Pasteels et al., '72). In addition, Allanson et al. ('66) found that after mid-pregnancy in the rabbit acidophils increased greatly in number and spread throughout the pars distalis proper.

As for the guinea pig, Weis ('35) observed that acidophils were more numerous during pregnancy; however, this work was not confirmed by Kirkman ('37) who stated that the number of acidophils was greater only at term and that pregnancy, in general, was characterized by degranulation of chromophils. Baniecki ('28), on the other hand, noted fewer acidophils during pregnancy for the guinea pig.

It is probable that for the species investigated, acidophils increase in number toward the end of pregnancy.

Pregnancy cells. Erdheim and Stumme ('09) first

demonstrated that associated with the pituitary hypertrophy and hyperplasia described by Comte (1898) for man, there also appeared an unusual cell type that was constant in its occurrence and dissimilar to any type found in the non-pregnant human hypophysis. This specific cell type, which was characterized by an intense eosinophilic staining reaction of the cytoplasm, was designated the "pregnancy cell." Such cells were observed in the later pars distalis and became prominent during the last trimester. Generally believed to originate from the chief or chromophobic cells, the presence of pregnancy cells has been confirmed for man (Launois and Mulon, '04; Mayer, '10; Kolde, '12; Romeis, '40; Floderus, '49; Gropp, '71), rabbit (Kolde, '12), sow (Kostner, '33), Guinea pig (Kolde, '12; Baniecki, '28), and claimed for the rat (Lehmann, '28; Haterius, '32; Lacour, '50). Romeis ('40) described the pregnancy cells as being large and containing an accumulation of granules which he termed "η." Romeis considered the η-granules to be specific for this cell and absent from the non-pregnant hypophysis. Floderus ('49) found that pregnancy cells of man often were located centrally in cell cords with other cell types being located peripherally. Pregnancy cells in the human hypophysis are presumably the source of prolactin secretion (Purves, '61). In rats, Haterius ('32) reported that the pregnancy cell is sparsely scattered in early pregnancy, increases in number until the twelfth day, after which it does not change further until after parturition. It should

also be noted that certain investigators were unable to identify specialized pregnancy cells in man (Rasmussen,'33), guinea pig (Desclin, '34; Kirkman, '37), rat (Wolfe and Cleveland, '33; Severinghaus, '33, '39; Desclin, '34; Stein, '34), or sheep (Warbritton and McKenzie, '37).

Thus, at present the evidence for the existence of a prolactin-secreting cell unique to pregnancy is inconclusive.

Hormone Content and Secretory Activity

Pituitary gonadotropin. Reports of pituitary gonadotropic hormone activity in many species are rather inconsistent; thus, increases, decreases, and little or no change in potency of the gland have been described. These discrepancies are probably attributable to species differences, and to varying procedures for collecting, storing, and particularly for assaying the glands (Cowie and Folley, '55).

In the hypophyses of pregnant cattle Evans and Simpson ('29) and Bates et al. ('35) reported an elevated gonadotropic content of the hypophysis during pregnancy when compared to pituitary glands of non-pregnant animals. The former investigators implanted fresh pituitary tissue of cattle into immature rats and mice and utilized qualitative reactions in the genital tract as an end-point, while the latter workers employed extracts of cattle hypophyses and observed the percentage increase in testicular weight of immature ring doves after injection of the extract.

In contrast, Nalbandov and Casida ('40) found a gradual

decline in gonadotropic potency throughout gestation. They concluded that estrogen secreted by the placenta inhibits the production of gonadotropic hormones by the hypophysis.

In man, a number of investigators have tested the activity of the hypophysis for gonadotropin during pregnancy. Zondek and Aschheim ('27), Witschi and Riley ('40), and Bettendorf et al. ('62) observed reduced potency during pregnancy when compared to non-pregnant, castrated or menopausal women. Thus, there is general agreement that in man the pituitary gonadotropic activity falls during the second month of gestation and remains barely detectable until a few days postpartum (Bruner, '51).

In the pregnant mare, Catchpole and Lyons ('34) and Hellbaum ('35) observed that in comparison with the average potencies of pituitary glands from non-pregnant mares, the hypophyses of pregnant mares showed considerable decline in activity at the time of implantation. As pregnancy progressed the gonad-stimulating potency of the gland rose again, reaching a maximum around three months; then it declined until just before parturition when the activity approximated the average for the non-pregnant mare.

Fluctuations in pituitary gonadotropic activity during gestation have been reported for both rabbits and mice. In rabbits (Hill, '34) there was an initial decline in pituitary gonadotropic potency followed by a rise and during late pregnancy, a terminal fall. In mice, Ladman and Runner ('53) likewise observed that gonadotropic potency was low

during early pregnancy, rose to a maximum by midpregnancy, and then declined during late pregnancy. This low activity during late pregnancy was correlated with rapid release of the hormone with no synthesis. Thus, these workers questioned the view that high glandular content paralleled output (Cowie and Folley, '55).

Lactation

Cytological Changes

Acidophils. A greater number of acidophils has been noted in the rat hypophysis during lactation (Pasteels,'61). Purves ('61) postulated that the increased number of acidophils at the time of parturition could be accounted for by the accumulation of secretory granules in the cells of the acidophilic class which were related to prolactin secretion. As lactation progressed, however, the number of acidophils declined (Everett and Baker, '45).

In rabbits, Allanson et al. ('66) demonstrated that erythrosinophils (presumptive prolactin cells) underwent a massive build-up in number just prior to parturition. These cells showed widespread degranulation within 24 hours after parturition and during the first two weeks of lactation. Most of these degranulated forms revealed signs of intense secretory activity: the nucleus, nucleolus, and Golgi apparatus were hypertrophied; the cytoplasm contained dense masses of RNA; and vacuolization of the cell periphery suggested rapid extrusion of secretory material. As

lactation progressed the number of granular forms rose, while degranulated cells declined.

Pregnancy cells. Haterius ('32) reported that pregnancy cells persisted in the rat hypophysis throughout lactation and for at least two days after weaning. Furthermore, he observed that premature withdrawal of the litter resulted in the disappearance of pregnancy cells within two or three days. No information, however, has been forthcoming to either confirm or dispute these observations.

MATERIALS AND METHODS

Experimental animals. Mature male and female rats (Sprague-Dawley Co., Madison, Wisconsin) were maintained at 23° C with 14 hours of illumination daily and given water and Purina rat chow ad libitum. Females were placed with mature males on the evening of proestrus, and mating was confirmed the next morning (day 1 of pregnancy) by the presence of spermatozoa in the vaginal smears. Animals were killed on each day of gestation and on days 1-5, 7, 9, 11, 13, 15, 17, 19, and 21 of lactation (table 1 for distribution of animals). During lactation size of the litter was maintained at six, the young being removed from each mother rat 18 hr before she was killed. In addition, several proestrous females were killed after being observed to experience two successive estrous cycles. Their hypophyses served as a baseline for determination of cytological changes occurring during pregnancy and lactation. Vaginal smears were made on microscopic slides and stained so that the accuracy of staging could be checked. All animals were killed by decapitation between 0800 and 1100 hours.

Procurement of hypophyses and serum. Hypophyses to be stained were fixed in situ in Bouin's fluid for 24 hr, dehydrated, embedded in paraffin, and sectioned at 3 μ on a dorsoventral (transverse) plane. Representative sections from three different zones in the hypophysis were stained for study.

Pituitary glands to be assayed for hormone content were

excised and, after discarding the pars nervosa, the pars distalis was weighed, homogenized, and diluted to a concentration of 1 mg/ml with 0.1% gelatin-phosphate-buffered saline. The homogenates were centrifuged at 8,000 R.P.M. for 30 minutes at 4° C; the supernate was distributed into aliquots and quickly frozen in an ethanol-dry ice bath.

Individual blood samples obtained from all rats after decapitation were centrifuged at 2400 R.P.M. for 30 minutes at 4° C; the serum was distributed into aliquots and quickly frozen. All pituitary and serum samples were stored at -20° C until assayed for luteinizing hormone and prolactin.

Immunohistochemical technique. Immunohistochemical staining was carried out according to the method of Nakane and Pierce ('67) which involves the following essential steps: (1) application to the tissue section of rabbit antiserum to the hormone; (2) application of sheep antiserum to rabbit γ-globulin conjugated with horseradish peroxidase; and (3) histochemical demonstration of peroxidase activity. 3,3'-Diaminobenzidine served as the substrate for the peroxidase reaction. Antisera to rat prolactin (anti-prolactin)[1] and ovine luteinizing hormone (anti-oLH) were obtained from rabbits according to a method described previously (Midgley et al., '71). Sheep anti-rabbit γ-globulin was conjugated with peroxidase (Sigma, Type II) according to the procedure

[1]The prefix "anti-" stands for antiserum to the hormone subsequently named. The small letter preceding the name of the hormone indicates the species of origin, e.g., "o" = ovine.

of Nakane and Pierce ('67).

Several control procedures were carried out to verify the specificity of the staining reaction. No staining of prolactin or LH-cells was obtained under the following conditions: substitution of normal rabbit serum for anti-prolactin or anti-oLH; incubation of tissue sections in peroxidase substrate without prior treatment with the appropriate antiserum or γ-globulin-peroxidase conjugate; or by blockade of immunoreactive sites on the hormone antibody when attached to the prolactin or LH-cell by application of unconjugated anti-rabbit-γ-globulin prior to treatment with conjugated anti-rabbit-γ-globulin. No staining of prolactin or LH-cells occurred if the anti-prolactin and anti-oLH had been previously absorbed with prolactin (NIH-P-S-6) or luteinizing hormone (NIH-LH-B1), respectively. As further proof of specificity of the immunohistochemical procedure for prolactin and LH-cells, Baker et al. ('69) and Baker et al. ('72) showed that prolactin and LH-cells so revealed react in a predictable manner when activity of the cells and pituitary content of the hormone are altered by experimental means. Also, Baker et al. ('72) demonstrated that antiserum to the specific subunit of bLH-β permits immunohistochemical staining of the same cells in the rat as anti-oLH and anti-human chorionic gonadotropin.

Differential cell counts. Differential cell counts were made by the method of Baker and Everett ('44) on hypophyses of nine rats taken at different times during

pregnancy. The purpose was to ascertain if a shift in the relative percentage of prolactin cells might have occurred that was not detected by careful observation of sections taken from three zones of the hypophysis. The mean relative percentages of prolactin cells was 32, 42 and 40% for the first, second and third trimesters of pregnancy. Because of the small differences observed and considerable variation, the procedure was abandoned.

Radioimmunoassay. Serum and pituitary samples were assayed utilizing radioimmunoassay procedures as described previously for luteinizing hormone (Niswender et al., '68) and prolactin (Niswender et al., '69) with the exception that 0.1% gelatin-phosphate-buffered-saline (PBS) was used rather than PBS-1% lyophilized egg-white. The amount of hormone was expressed in terms of a standard curve based on either a reference preparation of prolactin (NIAMD-Rat-Prolactin-RP-1) or luteinizing hormone (NIAMD-Rat-LH-RP-1) both being gifts of the National Institute of Arthritis and Metabolic Diseases to Dr. A. R. Midgley, Jr.

Statistical methods. By utilizing computer programs developed to interpret radioimmunoassay data (Duddleson et al., '72), a weighted, least squares regression analysis was performed on all assay results. In addition, Student *t* tests and analyses of variance were carried out on all radioimmunoassay results using the MIDAS (Michigan Interactive Data Analysis System) statistical computer programs developed at the University of Michigan (Fox and Guire,

'72). A p value equal to or less than 0.05 was considered significant.

TABLE 1

Summary of distribution of animals for immunohistochemistry (IHC) and radioimmunoassay (RIA)

Day of pregnancy	Number of rats IHC	RIA	Day of lactation	Number of rats IHC	RIA
1	5	9	1	6	11
2	-	6	2	1	3
3	-	3	3	2	3
4	1	3	4	-	3
5	2	3	5	-	2
6	1	3	7	6	5
7	-	3	9	2	11
8	1	3	11	-	3
9	3	3	13	2	3
10	4	4	15	-	3
11	3	5	17	2	3
12	5	10	19	2	3
13	3	3	21	5	10
14	3	4	Total	28	63
15	-	3			
16	2	3			
17	-	3			
18	-	3			
19	1	2			
20	3	2			
21	-	4			
22	4	5			
Total	41	87			

OBSERVATIONS

Cytology of Prolactin Cells during Pregnancy and Lactation

During pregnancy, two forms of prolactin cells could be observed in the rat hypophyseal pars distalis. One form was small and polyhedral; it constituted by far the greater proportion of the prolactin cell population in the pregnant and nonpregnant state. For descriptive purposes this form will be designated prolactin cell I (fig. 14). Also, during gestation a second form of prolactin cell that can be found occasionally in pituitary glands of nonpregnant rats became prominent. It was also polyhedral, but many times larger than prolactin cell I. It had a distinctive location within the gland, appearing in a region restricted to the lateral, central and caudal area of each lobe of the pars distalis (figs. 15, 16). This cell will be designated prolactin cell II.

Prolactin cells I and II differed somewhat in their response to pregnancy. During the first days of pregnancy prolactin cells I were large, had rather dispersed cytoplasmic granules (figs. 11, 12). and resembled prolactin cells of the proestrous female hypophysis (figs. 9, 10). During later periods it was difficult to ascertain whether any significant change had occurred because of variation between animals and between prolactin cells in different areas of a single section. In some rats, as illustrated by a specimen on day 14 (figs. 13, 14) prolactin cells I appeared smaller

and more intensely stained due to compaction of the population of secretory granules as compared with those on the first day of pregnancy (figs. 11, 12). Such a change was not true of all specimens. On the other hand, prolactin cells II were unobtrusive during early pregnancy because of their weak staining and thus, only rarely could they be identified (fig. 12). By day 14, however, prolactin cells II became more evident due to their increased staining reaction (fig. 14). These cells continued to increase in staining capacity as pregnancy progressed so that by day 22 they were quite prominent (figs. 15, 16).

With respect to lactation, there was little change in staining capacity, size, or number of the prolactin cells I when compared to day 22 of pregnancy; however, prolactin cells II were rarely found after the first day of lactation (fig. 20). Thus, on day 1 of lactation (figs. 17, 18) the appearance of the prolactin cells I was similar to that on day 22 of pregnancy (figs. 15, 16) and for the remainder of lactation they showed little variation (fig. 20). The prolactin cells II, that were so deeply stained during late pregnancy, declined significantly in staining capacity; as a result few such cells could be found on day 1 of lactation (figs. 17, 18) and only an occasional, weakly stained cell could be identified thereafter (figs. 19, 20).

Prolactin Concentration in the Hypophysis and Serum during Pregnancy and Lactation

During the first half of pregnancy, there was no significant variation in pituitary prolactin concentration; however, greater modulation in pituitary concentration was evident during the latter half of pregnancy. Thus, the average pituitary prolactin concentration between days 1 and 11 was 383.5±74.3 ng/mg (fig. 1) with none of the intervening day-to-day variations being significant. However, from days 12 to 16, pituitary prolactin concentration gradually declined, reaching its lowest level on day 16 (157.8±65.1 ng/mg) ($p<0.03$). By day 18 an increase ($p<0.03$) had occurred in prolactin concentration over day 16. However, by day 21 another decline had occurred ($p<0.03$). The mean value for days 12-22 was significantly lower than the mean value for days 1-11 ($p<0.05$).

As for lactation, pituitary prolactin concentration varied little except that a somewhat lower prolactin concentration was observed during the last few days. Thus, no significant change occurred in pituitary prolactin concentration from day 1 to day 15 (average 420.2±57.2 ng/mg) (fig. 2). By day 17, however, a decrease ($p<0.03$) in prolactin concentration below the level on day 15 was evident. This lowered concentration of prolactin was not altered significantly during the subsequent days of lactation.

Serum prolactin concentration was higher during early and late pregnancy than in the middle. Thus, for the first

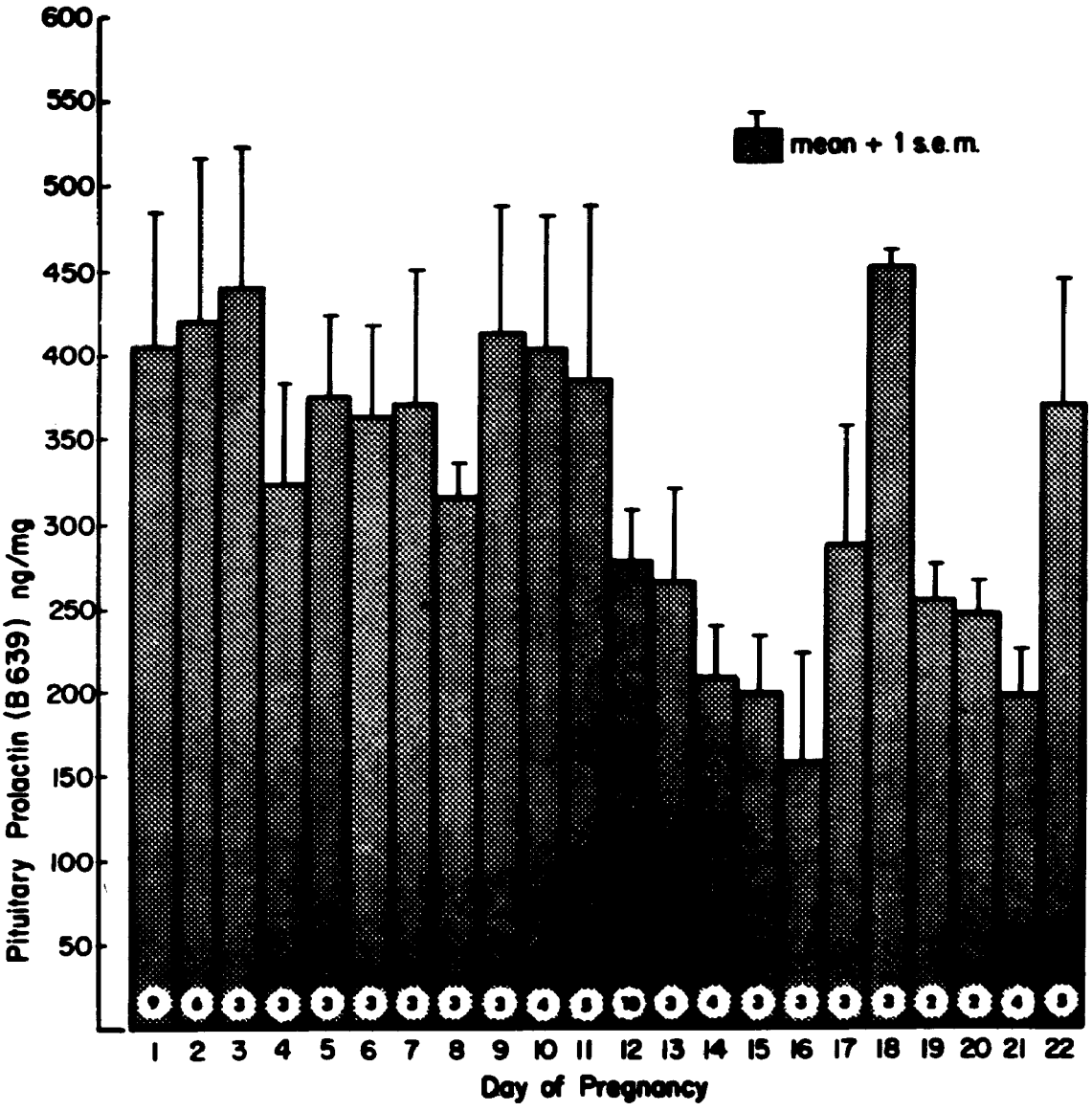

Fig. 1 Pituitary prolactin concentration at various times during pregnancy as determined by radioimmunoassay. B639 represents the standard reference preparation for prolactin (NIAMD-Rat-Prolactin-RP-1). The number of rats used to obtain the specified mean concentrations is given at the bottom of each bar.

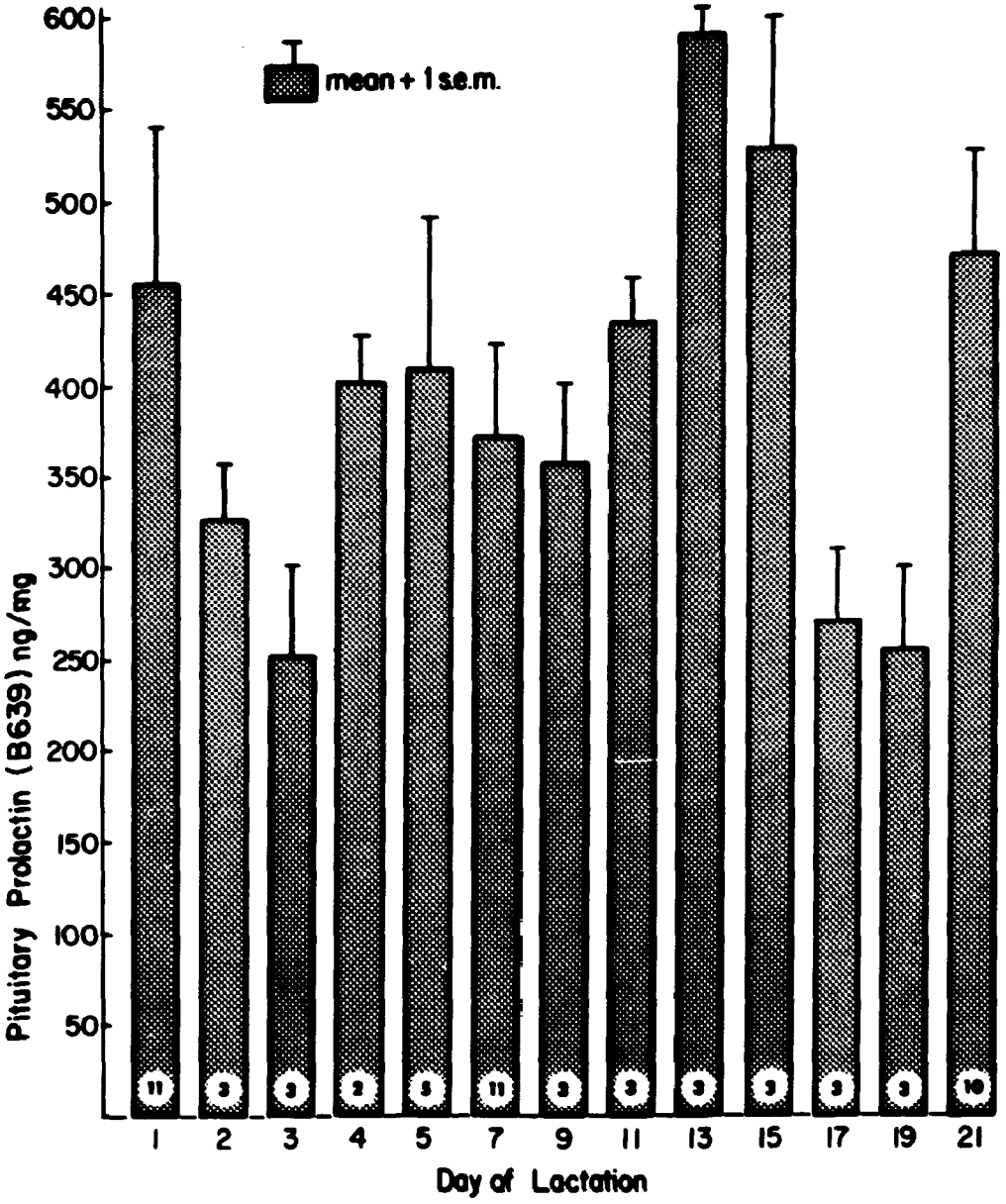

Fig. 2 Pituitary prolactin concentration at various times during lactation as determined by radioimmunoassay. B639 is the standard reference preparation. The number of rats used to obtain the specified mean concentrations is given at the bottom of each bar.

eight days the serum level (fig. 3) was highest on days 3 (96.2±21.6 ng/ml), 7 (86.5±36.8 ng/ml), and 8 (82.5±18.7 ng/ml). However, on day 9 serum prolactin had fallen abruptly ($p<0.005$) to a mean concentration of 21.3±1.6 ng/ml, this level remaining unchanged until day 22 (93.5±29.6 ng/ml) when the concentration was higher than on day 21 ($p<0.002$).

With respect to lactation, serum prolactin concentration gradually decreased from day 1 (41.2±7.1 ng/ml) to day 3 18.8±0.7 ng/ml) ($p<0.01$) and continued unchanged during the subsequent postpartum period (days 4-21) (fig. 4).

Cytology of the LH-cells during Pregnancy and Lactation

LH-cells as observed by immunohistochemical staining revealed profound alterations during pregnancy. On day 1 (figs. 23, 24) LH-cells were small, lightly stained, and the number identifiable was decreased when compared to LH-cells in hypophyses of proestrous females (figs. 21, 22). The cytological picture observed on day 1 was maintained for approximately 3 to 4 days; however, by day 5 LH-cells began to increase in staining capacity and apparent number so that on day 12 (figs. 25, 26) LH-cells were much more prominent than on day 1 of pregnancy. As gestation progressed, LH-cells continued to increase in staining capacity and size so that by day 22 (figs. 27, 28) a striking change had occurred in their status as compared with day 1 (figs. 23, 24).

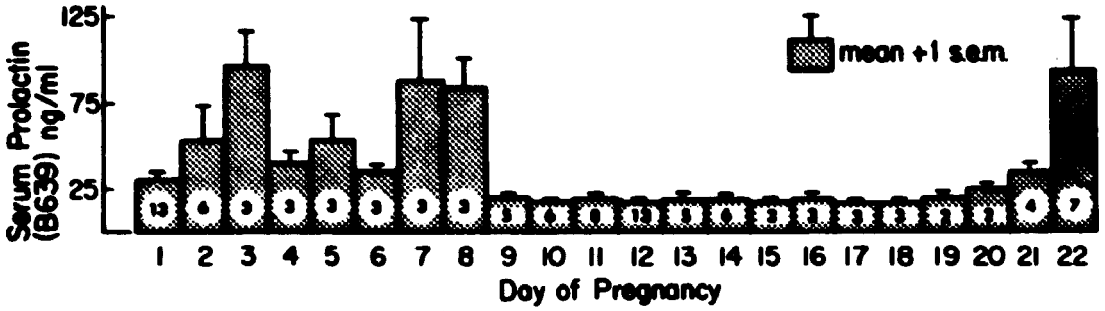

Fig. 3 Serum prolactin at various times during pregnancy as determined by radioimmunoassay. B639 is the standard reference preparation. The number of rats used to obtain the specified mean concentrations are given at the bottom of each bar.

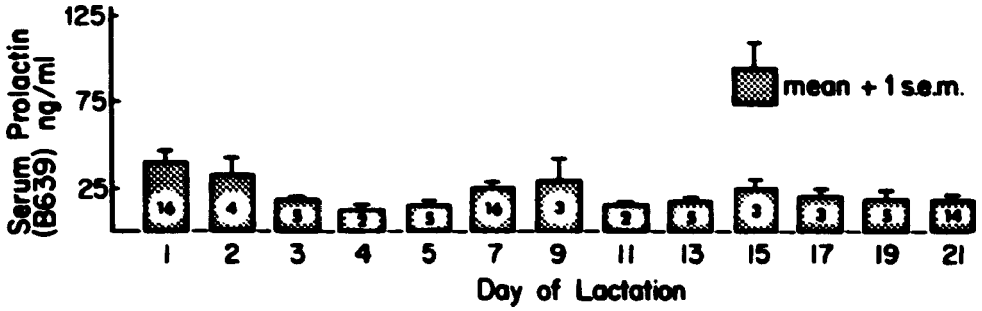

Fig. 4 Serum prolactin at various times during lactation as determined by radioimmunoassay. B639 is the standard reference preparation. The number of rats used to obtain the specified mean concentrations are given at the bottom of each bar.

As compared with late pregnancy, on day 1 of lactation a drastic decrease had occurred in staining capacity, size, and number of identifiable LH-cells (figs. 29, 30). This condition of the LH-cells was maintained for three to four days of lactation, but by day 5 their staining capacity had increased and their status then remained unchanged through day 21 of lactation (figs. 31, 32). The staining capacity of LH-cells on day 21 of lactation, however, did not equal that observed on day 22 of pregnancy.

LH Concentration in the Hypophysis and Serum during Pregnancy and Lactation

Pituitary LH concentration during pregnancy (fig. 5) was lowest during the first four days (mean, 5.7±1.2 µg/mg); however, the level had risen ($p<0.008$) by day 5 (15.3±0.7 µg/mg) and, thereafter, continued upward to a peak on day 14 (42.6±3.3 µg/mg). From day 14 to day 17 the LH concentration declined ($p<0.007$) to 29.5±0.7 µg/mg and underwent only insignificant variation during the remainder of pregnancy (days 18-22).

As for lactation, a marked reduction in pituitary LH concentration (fig. 6) occurred on day 1 (2.1±0.4 µg/mg) when compared to the high mean concentration on day 22 of pregnancy. This low concentration was maintained for the first nine days of lactation ($p>0.24$). On day 13 (26.5±7.4 µg/mg) a peak in luteinizing hormone concentration had been reached. On days 15 to 21 a somewhat lower level of LH

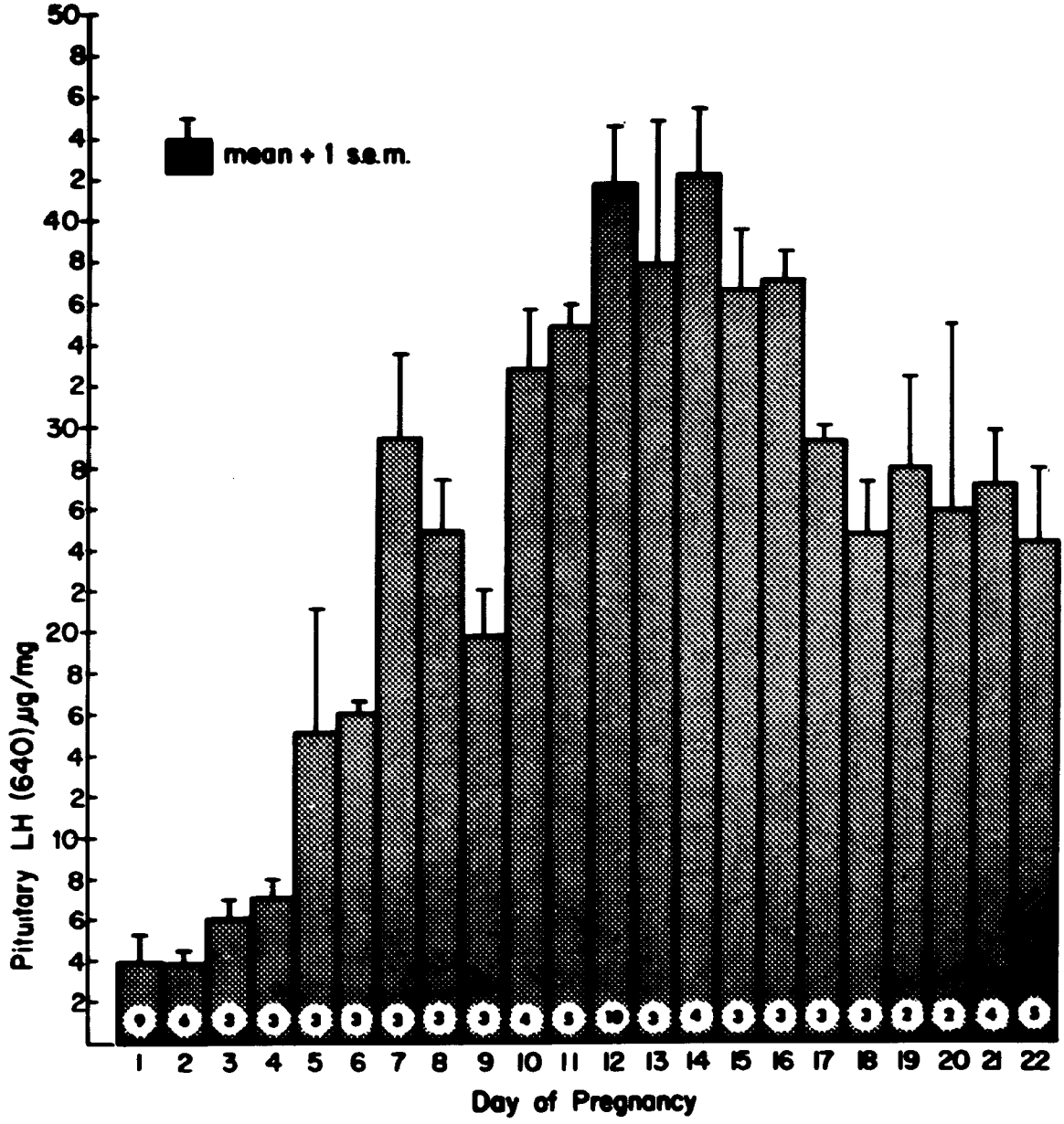

Fig. 5 Pituitary LH concentration at various times during pregnancy as determined by radioimmunoassay. B640 represents the standard reference preparation for LH (NIAMD-Rat-LH-RP-1). The number of rats used to obtain the specified mean concentration is given at the bottom of each bar.

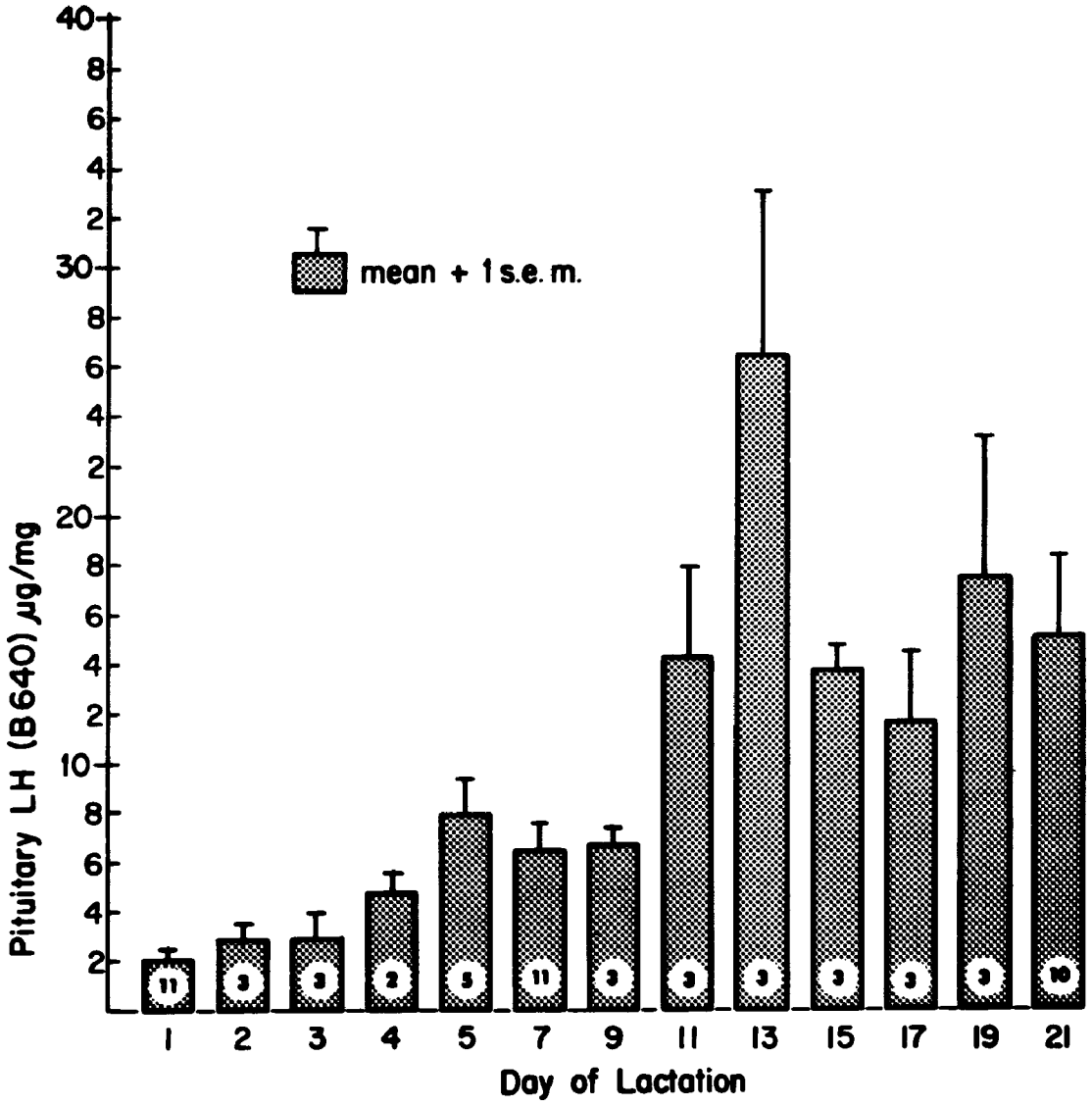

Fig. 6 Pituitary LH concentration at various times during lactation as determined by radioimmunoassay. B640 is the standard reference preparation. The number of rats used to obtain the specified mean concentrations is given at the bottom of each bar.

concentration was observed (average, 15.1±2.6 µg/mg) ($p<0.01$).

Serum LH concentration during pregnancy (fig. 7) gradually decreased from day 1 (22.0±4.4 ng/ml) to day 15 (1.8±1.1 ng/ml) ($p<0.008$). This low level remained unchanged through day 21, but an abrupt increase ($p<0.008$) occurred on day 22 (25.0±5.5 ng/ml). In general, the curve for serum LH concentration was roughly reciprocal to the curve for pituitary LH concentration during pregnancy.

During lactation, serum LH concentration (fig. 8) for the first seven days was equivalent to that on day 22 of pregnancy, but by day 9 (33.6±9.6 ng/ml) a rise in serum LH had occurred ($p<0.01$). From days 11 to 21 of lactation serum LH was maintained at a somewhat lower concentration (average, 12.8±1.2 ng/ml) ($p<0.007$).

Weight and Hormone Content of the Pars Distalis during Pregnancy and Lactation

During pregnancy and lactation, minimal variations were observed in the weight of the pituitary glands (pars distalis) either within the samples of a group studied at a specified time or between the mean values for groups obtained at different times (table 2,3).In addition, the mean weights of the glands for pregnancy (9.09 mg) and lactation (9.13 mg) were not significantly different. Hence, as would be expected the mean values calculated for the total glandular content of prolactin and LH varied with the time of

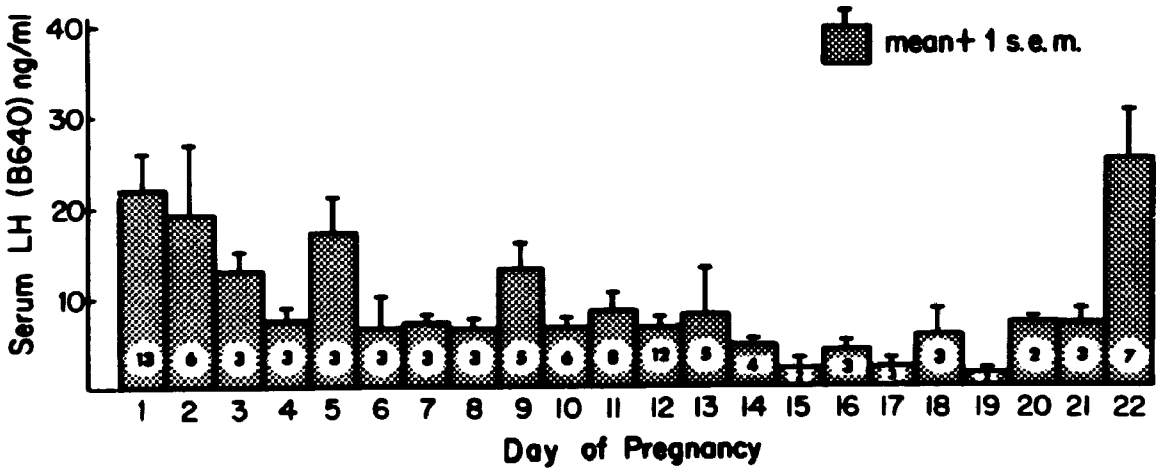

Fig. 7 Serum LH concentration at various times during pregnancy as determined by radioimmunoassay. B640 is the standard reference preparation. The number of rats used to obtain the specified mean concentration is given at the bottom of each bar.

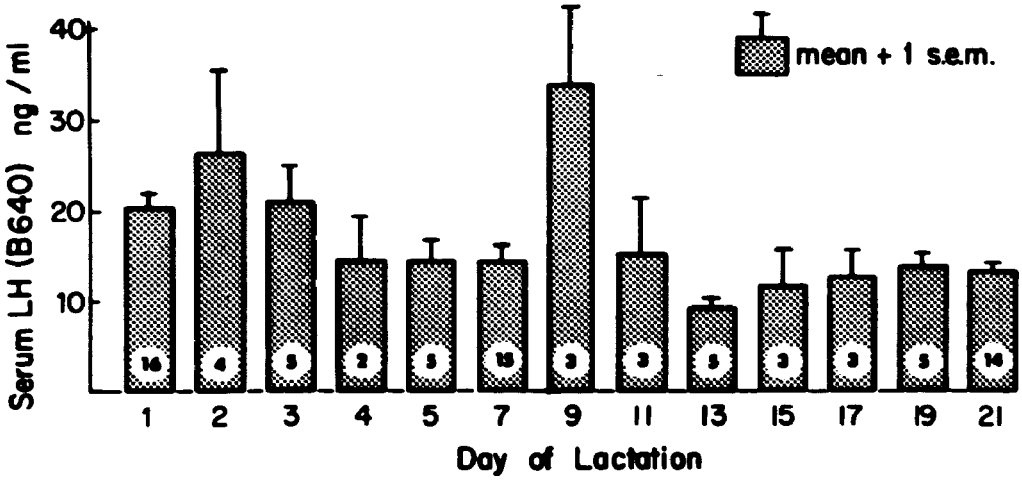

Fig. 8 Serum LH concentration at various times during lactation as determined by radioimmunoassay. B640 is the standard reference preparation. The number of rats used to obtain the specified mean concentration is given at the bottom of each bar.

gestation and lactation in a manner similar to the hormone concentrations.

TABLE 2

Prolactin and luteinizing hormone content
of the pars distalis during pregnancy

Day of pregnancy	No. of rats	Mean weight of pars distalis (mg)	Mean total hormone content	
			Prolactin (µg)	Luteinizing hormone (µg)
1	9	9.2±0.3*	3.9±0.8	35.6±13.7
2	6	9.7±0.4	2.8±1.1	41.2± 8.3
3	3	9.5±0.7	4.3±1.1	60.2± 9.7
4	3	8.3±1.2	2.8±0.8	62.3±14.8
5	3	9.7±0.3	3.6±0.4	143.5±57.4
6	3	9.7±0.3	3.5±0.6	155.6±10.6
7	3	9.0±0.5	3.4±0.9	263.0±20.9
8	3	8.7±0.3	2.7±0.2	219.0±28.4
9	3	8.7±0.3	3.6±0.7	170.7±16.1
10	4	8.0±0.7	3.2±0.7	261.4±12.4
11	5	8.8±0.3	3.5±1.1	307.9±10.3
12	10	8.6±0.2	2.4±0.3	359.5±21.2
13	3	9.3±0.6	2.4±0.4	346.2±42.1
14	4	8.0±0.4	1.6±0.2	337.1±12.9
15	3	9.3±0.3	1.8±0.2	344.2±32.0
16	3	9.3±0.3	1.4±0.5	347.6± 2.9
17	3	9.5±0.2	2.8±0.7	279.8± 5.1
18	3	9.7±0.3	4.3±0.1	240.3±29.5
19	2	9.5±0.5	2.4±0.1	265.2±29.0
20	2	10.0±0.0	2.5±0.1	260.7±97.6
21	4	9.3±0.3	1.8±0.2	250.7±17.1
22	5	10.1±0.3	3.8±0.8	242.5±29.2

*Standard error of the mean

TABLE 3

Prolactin and luteinizing hormone content of the pars distalis during lactation

Day of lactation	No. of rats	Mean weight of pars distalis (mg)	Mean total hormone content	
			Prolactin (µg)	Luteinizing hormone (µg)
1	11	8.9±0.3*	4.2±0.8	19.4± 4.2
2	3	9.0±0.0	2.9±0.2	26.1± 6.8
3	3	9.3±0.3	2.3±0.4	26.5±10.4
4	2	8.0±0.0	3.2±0.2	37.5± 7.3
5	5	8.7±0.2	3.5±0.8	67.7±12.2
7	11	9.5±0.1	3.5±0.5	61.5± 9.9
9	3	9.3±0.3	3.3±0.4	62.8± 8.8
11	3	10.0±0.0	4.3±0.2	141.6±36.9
13	3	8.3±0.8	4.8±0.3	217.8±57.2
15	3	9.6±0.3	5.9±1.4	130.5± 9.5
17	3	9.3±0.6	2.5±0.5	108.8±30.6
19	3	9.2±0.8	2.4±0.5	151.1±49.5
21	10	9.4±0.2	4.4±0.6	142.2±29.7

*Standard error of the mean

DISCUSSION

In order to evaluate the functional significance of changes that occur in prolactin and LH-cells during pregnancy and lactation, it is first necessary to summarize evidence pertaining to secretory activity that is available in the literature, as well as that obtained from my study. The conclusions drawn from this survey will then be used to interpret the cytological alterations observed, considering first the prolactin cell and, secondly, the LH-cell.

Prolactin Cells

Pregnancy

Prolactin levels in the hypophysis and serum. Earlier observations on pituitary prolactin concentration, based on bioassay of glands from the rat, are quite contradictory. Some workers observed little change during gestation (Meites and Turner, '48) while Reece ('37) reported a slightly higher level at 21 days than at 12 days. In contrast, Grosvenor and Turner ('60) observed that early in pregnancy, pituitary prolactin concentration increased rapidly to a peak by day 16. Subsequently a decline occurred during the last third of pregnancy. The latter observation seems to agree with the more recent radioimmunoassay data. In my study little alteration in pituitary prolactin concentration was observed from days 1 to 11; however, after this time prolactin concentration, although variable, fell to a lower level. Similarly, Bast and Melampy ('72) also demonstrated

a slight decrease in pituitary prolactin concentration during the latter half of pregnancy in the rat. Thus, most evidence indicates that pituitary prolactin concentration tends to decrease in the rat during the latter half of pregnancy.

With respect to prolactin concentration in the serum, my findings are in general agreement with those of Amenomori et al. ('70), Linkie and Niswender ('72), and Bast and Melampy ('72). In all of these studies prolactin concentration was higher at the beginning and end of pregnancy than in the middle.

Thus, based on both pituitary and serum concentrations of prolactin the synthesis and release of prolactin by the hypophysis seem to continue rather rapidly and at a fairly high level during the first portion of pregnancy; they are then less active during the latter half of pregnancy but finally accelerate again on day 22.

Correlation of pituitary secretion of prolactin with prolactin cell cytology. In the rat hypophysis the number of acidophils has been variously reported to decrease (Herring, '20; Lehmann, '28), increase (Wolfe and Cleveland, '33), or to remain unchanged (Haterius, '32; Desclin, '34; Stein, '34). Subsequently, Lacour ('50) and Pasteels ('61) reported that the number of prolactin cells (a sub-group of acidophils) showed a marked rise during gestation.

I found that during the first half of pregnancy prolactin cells I, which are the major prolactin cell form in

both pregnant and nonpregnant rats, were unchanged in size, number, and staining capacity. This was paralleled by minimal alteration in the concentration of pituitary prolactin. However, the generally depressed secretory activity of the pars distalis during the latter part of pregnancy, which is represented by a decline in pituitary and serum prolactin concentrations, was not sufficiently great to be reflected in a significant structural change in prolactin cells I. In general, there is not a close correlation between the cytology of prolactin cells I and secretory activity of the hypophysis during the later period of gestation. The striking increase in staining capacity of prolactin cells II seems to have no counterpart in the radioimmunoassay data, unless they have a special association with the elevated prolactin secretion at parturition.

Pregnancy cells. In the rat hypophysis, Lehmann ('28), Haterius ('32), and Lacour ('50) described a large, atypical acidophil that appears during pregnancy, which they believed might be identical to the unusual acidophil demonstrated in the human hypophysis during gestation that Erdheim and Stumme ('09) designated as the "pregnancy cell" or "Schwangershaftzelle." Of particular interest is the possibility that prolactin cells II are the "pregnancy cells." In addition to their greater size as compared with prolactin cells I, prolactin cells II attained maximal prominence during late pregnancy. Based on their immunohistochemical staining they appear to be involved in the production of

prolactin, even though radioimmunoassay data did not show this to occur except on the last day of pregnancy. However, on several counts they do not seem equivalent to Lacour's ('50) orangeophils of pregnancy which were described as being abundant and also limited to the periphery of the gland. The significance of prolactin cell II during pregnancy is not clear.

Lactation

Prolactin levels in the hypophysis and serum. During lactation as observed with radioimmunoassay the only significant change in pituitary prolactin concentration was a fall from a peak on day 15 to a lower level on day 17. This observation differs from the results of Meites and Turner ('48) who found with bioassay that pituitary prolactin content increased significantly by day 3 and then declined.

Serum prolactin concentration in the postpartum rat of my study was higher during the first two days than any other time during lactation. The concurrent high pituitary prolactin level indicates rapid secretion of prolactin at delivery. Midgley (unpublished) has shown that prolactin secretion is accelerated prior to the ovulation that occurs within hours after parturition; thus, the higher serum concentration of prolactin on the first two days of lactation, as observed in this study, may have reflected this postparturient surge.

Amenomori et al. ('70) found with radioimmunoassay that

that serum prolactin remained high in the postpartum rat for at least eight days after which it declined to about half the level observed on day 1 of lactation. Further, the serum level was higher in the experiments of Amenomori et al. than in mine. The major reason for the latter difference lies in the presence or absence of suckling prior to death of the lactating rat. Amenomori et al. ('70) permitted their rats to nurse the young until death and noted further that removal of litters and, therefore, cessation of suckling for only 3 hr was sufficient to lower the serum prolactin level and increase pituitary prolactin content. Thus, without the stimulation of suckling to cause prolactin release, Meites and Turner ('48),Grosvenor and Turner ('58), Amenomori and Nellor ('69), and Amenomori et al. ('70) found little prolactin in the serum during the postpartum period. In my rats, which were not suckled for 18 hr prior to death, prolactin concentration was higher in the hypophysis and lower in the serum.

The available information does not permit broad generalizations regarding prolactin secretion during lactation. The evidence indicates that there is an outpouring of prolactin after delivery of the young and that formation of prolactin by the hypophysis, and its secretion, may fall toward the end of lactation.

Correlation of pituitary prolactin concentration with prolactin cell cytology. During lactation, minimal change in the staining capacity, size, or number of prolactin cells

I was evident when compared to the condition in late pregnancy. However, prolactin cells II lost most of their staining capacity early in lactation. These results are contrary to previously reported observations of several other workers. Thus, Everett and Baker ('45) reported a 100% increase in number of acidophils during the first three days of lactation and Pasteels ('61; '63) demonstrated that certain erythrosinophilic cells (presumptive prolactin cells) increase in size and number during early lactation. There may be several reasons for the discrepancies in these observations. Growth hormone cells (also a subgroup of acidophils) were included by Everett and Baker in their counts, since a method for accurate differentiation between prolactin and growth hormone cells was not available at that time. Therefore, their reported increase in number of acidophils three days after parturition may have been due, in part, to changes in growth hormone cells; the cytology of these cells during lactation has not been elucidated. Variation in results may have been influenced also by whether or not the newborn rats were nursed before termination of the experiment as well as by the length of this period. Thus, Pasteels ('63) demonstrated that suckling up to the time of killing the rat results in degranulation of prolactin cells in the mother's hypophysis and,in addition, prolactin content in the gland decreases (Reece, '37; Grosvenor and Turner, '60; Sar and Meites, '67). In my study degranulation of prolactin cells was avoided by

removal of the litter 18 hr before the mother rat was killed. This period of non-suckling was presumably sufficient to allow regranulation of the prolactin cells, since no apparent decrease in staining intensity or pituitary prolactin concentration occurred during lactation.

As for correlation with pituitary secretory activity, it is suggested that during early lactation prolactin cells II are stimulated to release their stored hormone, perhaps as a result of suckling stimulation or by a changing estrogen-progesterone ratio. They persist as lightly stained, rare cells throughout the postpartum period. Their number is probably too low for degranulation to be accompanied by a significant early fall in overall prolactin concentration of the gland, although such a decline is indicated. The late decline in pituitary prolactin concentration is not reflected by a significant change in prolactin cells I.

Luteinizing Hormone Cells

Pregnancy

LH levels in the hypophysis and serum. Pituitary LH concentration was extremely low the first four days following mating, but increased significantly during the remainder of pregnancy, reaching its peak around day 14 and declining slightly thereafter. These results are almost identical to the radioimmunoassay data of Brown-Grant et al. ('72) and Bast and Melampy ('72). Similarly with bioassay Zeiner ('52) and Contopoulos and Simpson ('56) reported a two- to three-

fold increase in pituitary LH potency, as compared with the level in nonpregnancy.

The curve for LH concentration in the serum was roughly the reciprocal of that for pituitary LH. Thus, serum LH concentration was higher during early and late pregnancy than in the middle. Similar results have also been observed by Brown-Grant et al. ('72), Bast and Melampy ('72), and Linkie and Niswender ('72). These reciprocal curves for serum and pituitary LH concentration indicate that luteinizing hormone is depleted from the pituitary gland and released into the blood in greater amounts during early and late pregnancy, but stored with little release during midpregnancy.

Correlation of pituitary LH secretory activity with LH-cell cytology. Basophils (presumptive LH-cells) have been reported to increase in number from early to late pregnancy in the mouse (Urasov, '28), guinea-pig (Weis, '35; Kirkman, '37), rabbit (Cameron et al., '66), bat (Herlant, '56), cat (Herlant and Racadot, '57), and rat (Wolfe and Cleveland, '33). In the rat the latter workers were unable to identify accurately the basophilic cell type that was involved. This study of the rat hypophysis, when stained immunohistochemically with anti-oLH, revealed that the LH-cells are involved since they increase progressively in staining capacity, size, and number from early to late pregnancy. These changes were paralleled by an increase of almost ten-fold in pituitary LH concentration from day 1 to

day 14 of pregnancy. On the other hand, the fall in pituitary LH concentration during the latter half of pregnancy did not result in detectable morphological changes in the LH-cells. Thus, both cytological and physiological data affirm that LH is secreted early in pregnancy and that storage rather than secretion takes place for most of the remainder of pregnancy.

Lactation

LH levels in the hypophysis and serum. Our data show that pituitary LH concentration was low at days 1 to 3, rose to a maximum by day 13, and maintained a somewhat lower concentration between days 15 and 21. As during pregnancy there was an overall rough reciprocal relationship between pituitary and serum concentrations of LH, secretion of LH being indicated soon after parturition with storage occurring as lactation progressed. Midgley (unpublished) measured pituitary LH concentration within hours after parturition and found that it fell from a high level to a nadir 18 hr later; the concentration obtained at this time was approximately equivalent to that which I observed on day 1 of lactation. In the same study, serum LH concentration increased sharply by 5 hr after parturition but declined thereafter so that by 18 hr the serum LH concentration approximated the level observed for serum LH at day 1 of lactation in my study.

It appears that at parturition the increasing estrogen

levels, concurrently with the decreasing progesterone levels (Meites and Turner, '48; Fajer and Barraclough, '67; Shaikh, '71; Brown-Grant et al., '72; Sanyal and Villee, '73) trigger the release of LH from the hypophysis which causes postpartum ovulation in the rat. Therefore, the extremely low pituitary LH concentration and the high serum LH concentration which were observed during early lactation result from several hormonal interactions leading up to ovulation which occurs during the first day of lactation.

Correlation of pituitary LH secretory activity with LH-cell cytology. LH-cells underwent a drastic decrease in staining capacity and in number of identifiable cells on day 1 of lactation. This change was correlated with the postparturient outpouring of LH by the pituitary gland. As lactation progressed both the staining intensity and size of LH-cells, as well as the pituitary LH concentration, increased significantly. These findings for LH-cells agree with those of Everett and Baker ('45) who reported that the number of basophils was low early in lactation and gradually increased as lactation progressed. Observations in other species also support this conclusion. Kirkman ('37) and Cameron et al. ('66) found that the percentage of basophils drops following parturition in hypophyses of the guinea pig and cat, respectively; thereafter, the percentage was said to remain at approximately the level found in the nonpregnant state. Further, Cameron et al. ('66) postulated that this sharp decline in number of basophils was due to a loss of

specific mucoid granules and was associated with the termination of pregnancy and the initiation of estrus. This is supported by investigations of Hill ('34) who reported that in the rabbit gonadotropic content of the hypophysis declined prior to parturition and remained low throughout lactation. Thus, these changes in the cytology and secretory activity in LH-cells during pregnancy appear to characterize other mammals as well as the rat.

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Illustrations

PLATE 1

Explanation of Figures

All photographs on this plate are of sections stained immunohistochemically for prolactin cells. The sections were cut on a frontal plane. Figures 9, 11, and 13 represent one-half of a section through the hypophysis. X 31. The areas illustrated at higher magnification (figs. 16, 18, 20) were located in the central region of the lateral lobe of the pars distalis. X 250. D, pars distalis; I, pars intermedia; N, pars nervosa; P_1, prolactin cells I; P_2, prolactin cells II.

9 Proestrous female. Prolactin cells are represented by the dark spots in the pars distalis. Prolactin cells I are distributed uniformly in the pars distalis.

10 Proestrous female. Prolactin cells I are shown. They are of fairly uniform size.

11 Day 1 of pregnancy. There is little change in appearance of prolactin cells I when compared to those in the proestrous female (fig. 9). Prolactin cells II are not evident.

12 Day 1 of pregnancy. Prolactin cells I reveal little significant alteration when compared to those in the proestrous female hypophysis (fig. 10). Only a few weakly stained prolactin cells II can be seen (arrows).

13 Day 14 of pregnancy. At this magnification little

PLATE 1
(continued)

Explanation of Figures

change in prolactin cells is observed when compared to those on day 1 of pregnancy (fig. 11).

14 Day 14 of pregnancy. Prolactin cells I stain more intensely than at day 1 of pregnancy (fig. 12). Prolactin cells II are more numerous and also show increasing staining capacity (arrows). Inset: typical prolactin cell I and prolactin cell II in this section. X 1000.

PLATE 1

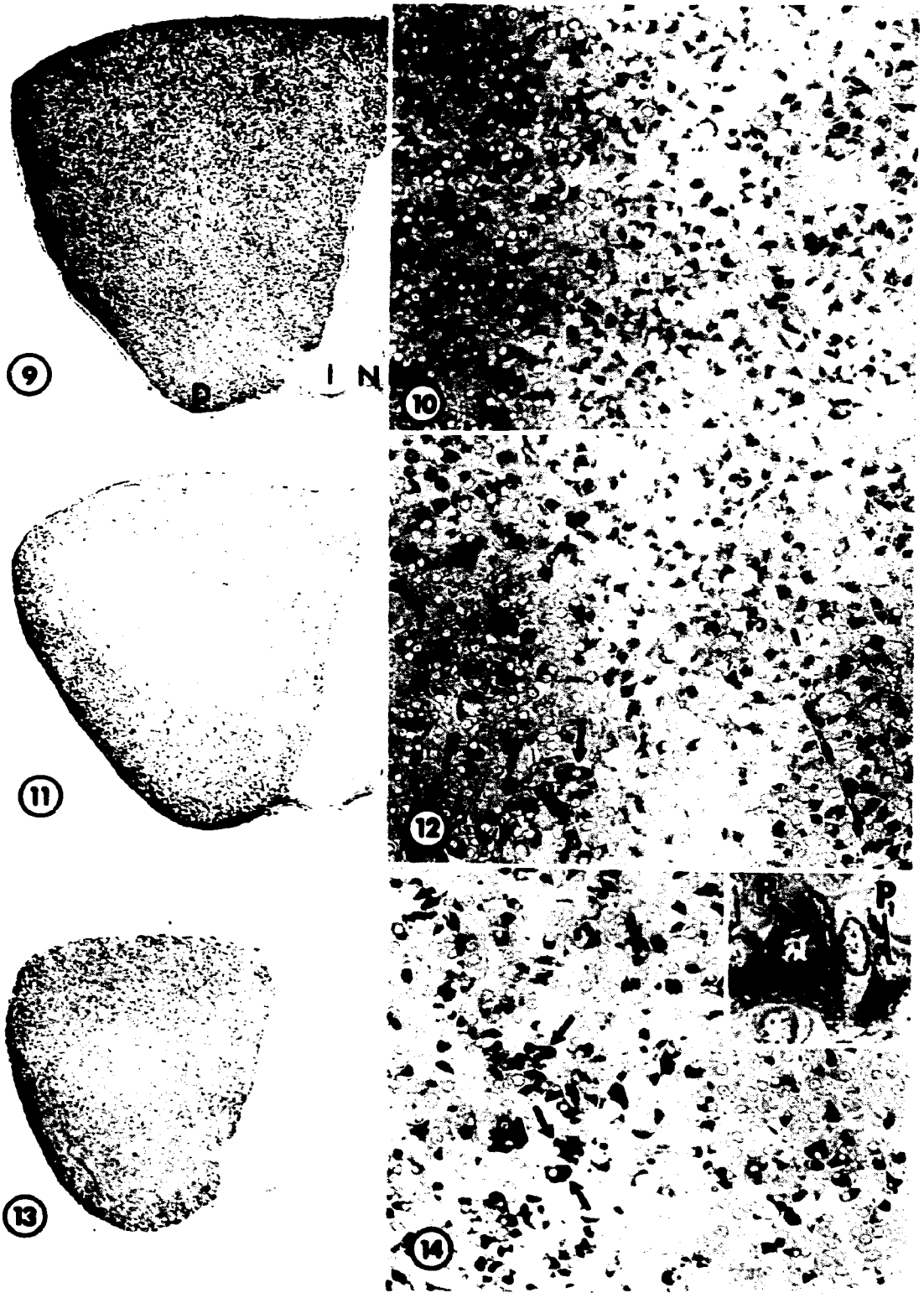

PLATE 2

Explanation of Figures

All photographs are of preparations stained for prolactin cells. The sections were cut on a frontal plane. Figures 15, 17, and 19 represent one-half of a section through the hypophysis. X 31. The areas illustrated at higher magnification (figs. 16, 18, 20) were located in the central region of the lateral lobe of the pars distalis. X 250.

15 Day 22 of pregnancy. Prolactin cells I appear the same as on day 14 of pregnancy (fig. 13). Prolactin cells II are visible in the central and lateral areas of the pars distalis (arrows).

16 Day 22 of pregnancy. The staining capacity of prolactin cells I is not altered over that at 14 days (fig. 14), but prolactin cells II are stained more intensely (arrows). Inset: a typical prolactin cell II in this section. X 1000.

17 Day 1 of lactation. Prolactin cells I show little change in staining intensity when compared to those on day 22 of pregnancy (fig. 15). Prolactin cells II are rare.

18 Day 1 of lactation. Prolactin cells I appear unchanged when compared to day 22 of pregnancy (fig. 16). However, prolactin cells II are fewer (arrows).

PLATE 2

(continued)

Explanation of Figures

19 Day 21 of lactation. No change is apparent in prolactin cells I when compared to day 1 of lactation (fig. 17). Prolactin cells II are not visible.

20 Day 21 of lactation. Prolactin cells I appear the same as for day 1 of lactation (fig. 18). Prolactin cells II are not evident in this field.

PLATE 2

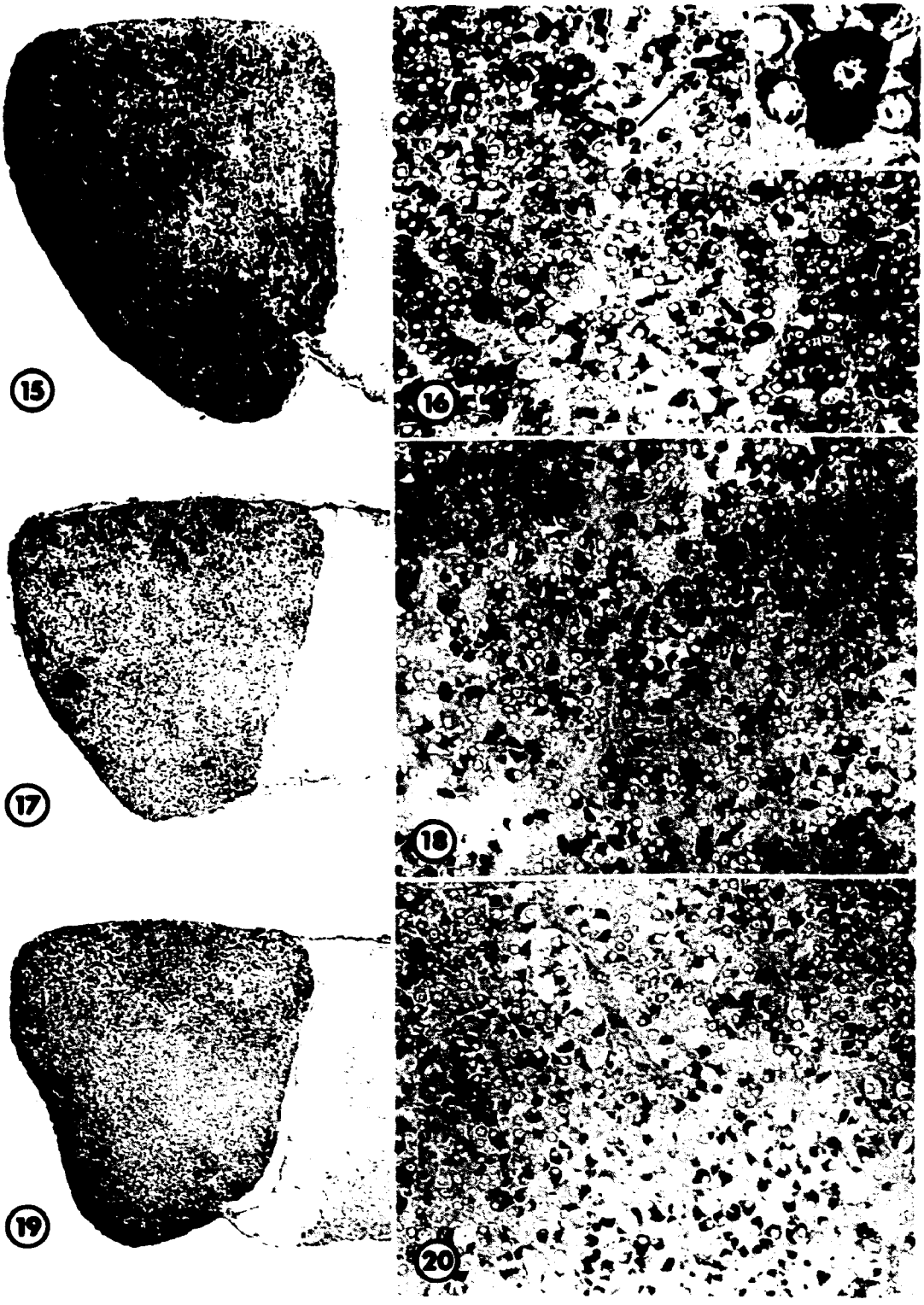

PLATE 3

Explanation of Figures

All photographs are of preparations stained immunohistochemically for LH-cells. The sections were cut on a frontal plane. Figures 21, 23, and 25 represent one-half of a section through the hypophysis. X 31. The areas illustrated at higher magnification (figs. 22, 24, 26) were located in the central region of the lateral lobe of the pars distalis. X 250.

21 Proestrous female. LH-cells are represented by the dark spots in the pars distalis. Note the staining intensity and number of identifiable cells.

22 Proestrous female. LH-cells are generally ovoid with some being polyhedral. Staining intensity is quite uniform among LH-cells. Inset: a typical LH-cell from this section. X 1000.

23 Day 1 of pregnancy. There is a significant reduction in number of identifiable LH-cells, and in their staining capacity throughout the pars distalis as compared to those in the proestrous female hypophysis (fig. 21).

24 Day 1 of pregnancy. LH-cells are small, lightly stained, and apparently fewer when compared to LH-cells prior to mating (fig. 22). Inset: a typical LH-cell from this section. X 1000.

25 Day 12 of pregnancy. LH-cells have increased in stain-

PLATE 3

(continued)

Explanation of Figures

ing intensity as compared with their condition on Day 1 of pregnancy.

26 Day 12 of pregnancy. LH-cells reveal a significant increase in staining capacity and number when contrasted to LH-cells on day 1 of pregnancy. Inset: a typical LH-cell from this section. X 1000.

PLATE 3

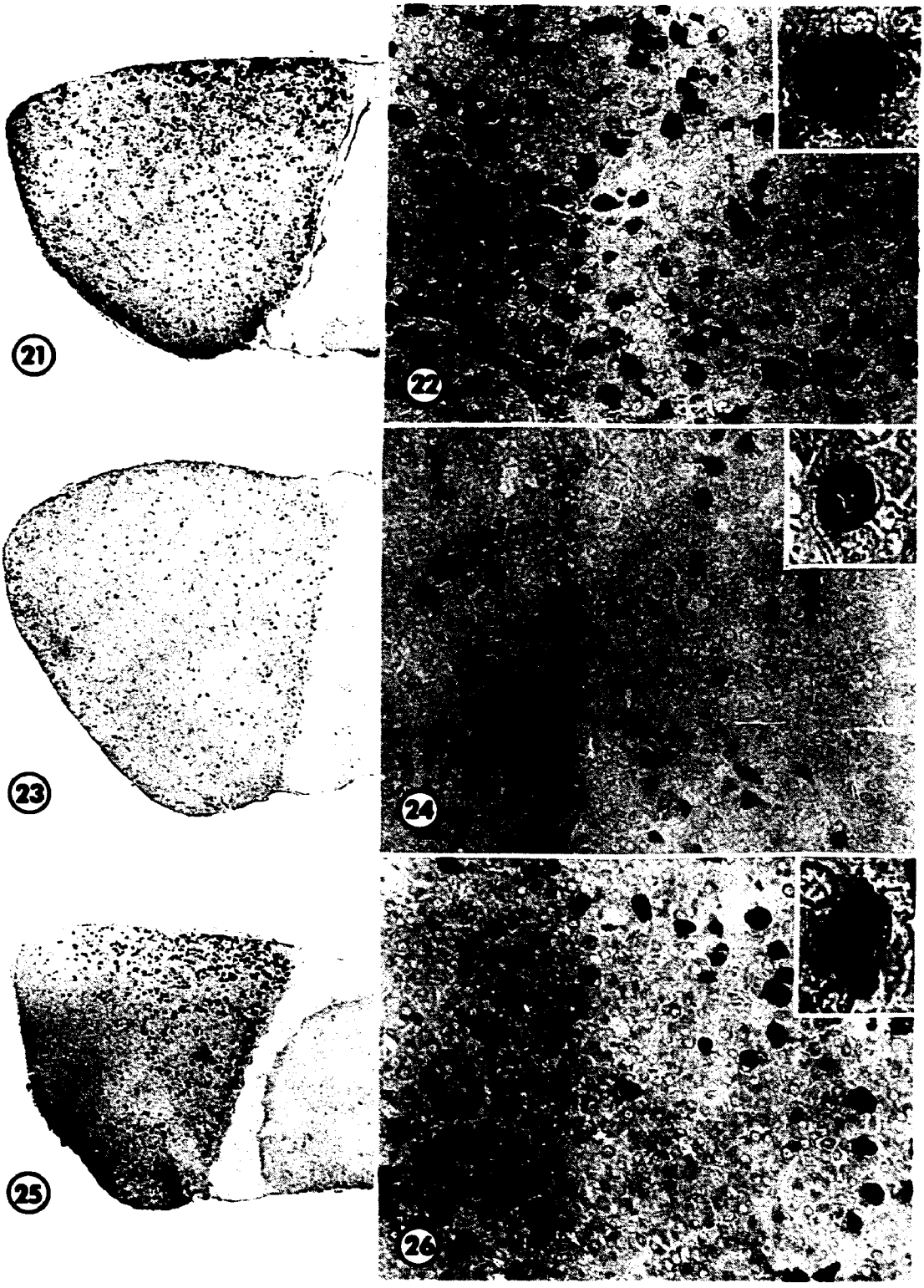

PLATE 4

Explanation of Figures

All photographs are of preparations for LH-cells. They were sectioned on a frontal plane. Figures 27, 29, and 31 represent one-half of a section through the hypophysis. X 31. The areas illustrated at higher magnification were located in the central region of the lateral lobe of the pars distalis. X 250.

27 Day 22 of pregnancy. The distribution of LH-cells is illustrated.

28 Day 22 of pregnancy. Note the increased intensity of staining, size, and number of LH-cells as compared to day 12 of pregnancy (fig. 26). X 250. Inset: a typical LH-cell from this section. X 1000.

29 Day 1 of lactation. LH-cells are less numerous than in late pregnancy (fig. 27).

30 Day 1 of lactation. LH-cells are small, lightly stained, and decreased in number after parturition. Compare with day 22 of pregnancy (fig. 28). Inset: a typical LH-cell from this section. X 1000.

31 Day 21 of lactation. LH-cells are more numerous than on day 1 of lactation (fig. 29).

32 Day 21 of lactation. LH-cells are increased in staining capacity, size, and number over day 1 of lactation (fig. 30). Inset: a typical LH-cell from this section. X 1000.

PLATE 4

