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The Pituitary Glands of Ewes in Various Phases of Reproduction

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Animal Husbandry Department, Missouri Agricultural Experiment Station, and the United States Department of Agriculture Cooperating

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ABSTRACT

Pituitary bodies from sixty ewes slaughtered at different phases of reproduction have been studied by numerous techniques. The pituitary of the ewe resembles that of other species in general structure but has a more complex cell pattern than has been described in other forms. Nine morphologically different types of cells from the glandular lobe have been identified and placed in six fundamentally different groups. Two of these six types show changes, which can be interpreted as secretory, correlated with the phase of reproduction. The tuberal lobe contains chiefly non-granular cells and colloid, but all the cells found in the glandular lobe occur there. Glandular cells have not been observed in the neural and intermediate lobes, and the secretions of these lobes are attributed to neural elements.

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INTRODUCTION

The pituitary glands of sheep have been used as the sources of gonadotropic and lactogenic hormones because they have a relatively high content of these substances. Ewes show ovarian and mammary responses similar to those induced in smaller animals injected with the various hormones. It seems reasonable to suppose that these reactions are induced by the release of hormones from the pituitaries of the ewes. A difference in the amounts of hormones should, therefore, exist in pituitaries of animals in various phases of the estrual cycle and pregnancy. This difference might be shown by physiological tests of the potency through extraction or transplantation of the gland, by microchemical analyses if techniques were sufficiently refined, or by microscopic examination of the cells of the pituitary. Microscopic methods, in spite of numerous defects, seem best suited for the demonstration of variations in individual glands.

Five problems are involved in a microscopic analysis of the pituitary gland:

(1) The types of cells in the ewe.
(2) The relationship of different kinds of cells to each other.
(3) Changes in the cells during the reproductive cycle.
(4) The resemblances to and differences from the cells in other species.
(5) The relationship of structures in the pituitary to its products.

The study of the pituitary gland is part of a larger project on the physiology of reproduction in farm animals. Previous reports of the studies include the outline of the project by McKenzie, Allen, Guthrie, Warbritton, Terrill, Casida, Nahm, and Kennedy (1933); the histology of the genital tract by Casida and McKenzie (1932); the cytology of corpora lutea during the estrual cycle by Warbritton (1934); a preliminary report on the duration of the estrual cycle by Terrill (1936); and the structure of the adrenal gland by Nahm

and McKenzie (1937). Other studies completed except for publication or in progress include one on the estrual cycle and several on the structure of the endocrine systems as found in follicles, corpora lutea of pregnancy, pineal bodies, thyroid glands, and pituitary glands of castrate and anestrual ewes.

MATERIAL AND METHODS

Pituitaries from sixty ewes have been studied. Thirty-three of the animals were slaughtered during pregnancy; twenty-three during the breeding season for estrual changes, three during anestrum, and one after castration. The ewes were killed at intervals after the onset of their last estrus. Records were kept of the age, breeding, weight, finish at time of slaughter, date slaughtered, and previous estrual cycles for each of the ewes. This information is presented in Table 1. The ewes varied in age from two to eight years with most of the group being five or six. They were chiefly grade Hampshires. Weights ranged from seventy to two hundred fourteen pounds; but only one animal weighed less than one hundred, and one, more than one hundred seventy. Most of the animals were studied originally, and most intensively, when grouped upon the basis of phases of reproduction; but they have been restudied when grouped according to age, to breeding, to weight, to finish, and to date of slaughtering.

The removal of a pituitary gland required two to three minutes; but the head was dissected after the genital tract, thyroid gland, and adrenal gland were removed. The head was severed from the body and opened by sawing through the skull just behind the pineal body. After the pineal had been taken out, the brain was loosened and removed by cuts through the infundibulum or stalk and larger nerves. This exposed the diaphragm covering the sella turcica. A cut around the outer margin of the diaphragm permitted the pituitary to be lifted from the sella. The gland was dissected from the diaphragm and cut into the pieces desired for preservation. This usually involved a median sagittal cut so that one-half of the gland could be preserved intact and so that the other half furnished small pieces for other methods of fixation. The glands were usually in the fixing fluids within twenty minutes after the animal was slaughtered (by sticking or bleeding).

In the preservation of the pituitary gland several methods of fixation have been employed. For the half glands and large pieces the methods of Bouin, Zenker, or Regaud (Lee) or a solution of

TABLE 1.—THE HISTORY OF THE EWES STUDIED.

A. NON-PREGNANT SERIES

Ewe	Stage (days)	Age (years)	Breeding	Wt. (lbs.)	Finish	Date killed	Length of cycle (days)
1251a	0.0	3	Shropshire	170	good	12/14/31	17.4; 16 4; 16.7
7b	0.5	8	grade Hampshire	148	good	11/29/32	17.0; 17.0; 16.7
2a	0.7	4	Shropshire	---	medium	11/17/31	15.4; 16.0; 32.0; 16.2
45a	0.9	2	unknown	110	medium	11/ 9/31	16.0; 16.0; 16.0
22a	1.8	5	unknown (Hampshire)	116	medium	11/20/31	15.4; 17.7; 17.0
7a	2.0	7	Hampshire	170	medium	12/ 5/31	14.8; 77.8; 4.0
20b	3.3	6	grade Hampshire	150	good	10/29/32	16.0; 16.7; 17.0
25a	3.8	5	unknown	164	medium	12/ 1/31	34.5; 16.0; 16.5; 14.7; 15.8
22b	5.3	7	grade Hampshire	134	good	11/ 1/32	16.0; 16.5; 18.2; 17.0
53a	5.3	3	unknown	130	medium	11/ 1/31	11.0; 10.6; 19.8; 32.5; 7.0
14b	7.1	6	grade Hampshire	120	good	10/20/32	15.9; 16.7
23a	7.4	6	unknown (Hampshire)	130	medium	12/ 9/31	14.6; 14.0; 15.3; 10.7
16a	7.6	6	unknown (Hampshire)	162	good	12/18/31	16.0; 17.4; 17.0; 16.7; 16.3; 18.1
24b	7.9	5	grade Hampshire	120	poor	12/ 8/32	16.5; 16.0; 15.4; 16.7; 17.3
44a	7.9	6	unknown (Hampshire)	---	----------	12/30/31	16.1; 16.1; 17.9; 15.5; 17.1; 16.1; 16.3
28a	9.1	6	unknown (Hampshire)	120	medium	12/31/31	16.5; 17.5; 17.0; 16.2; 16.3; 17.0
3b	9.9	7	grade Hampshire	120	medium	10/ 4/32	17.0
38a	11.0	2	unknown (Hampshire)	120	medium	11/30/31	16.4; 16.1; 16.3; 15.0; 16.8
12b	11.9	5	grade Hampshire	148	good	10/29/32	16.0; 18.0; 16.5
21b	12.3	8	grade Hampshire	107	medium	12/ 9/32	16.4; 15.5
35a	14.0	2	unknown (Hampshire)	138	medium	12/ 5/31	20.1; 15.7; 15.9; 51.2
10b	14.3	6	grade Hampshire	111	medium	10/31/32	16.0; 15.0; 15.0
8c	15.0	3	grade Hampshire	111	----------	12/19/34	15.7; 16.0; 16.2; 15.5; 16.5
34a	----	5	unknown (Hampshire)	148	good	1/26/32	Castrate
17a	40.4	7	unknown	100	poor	1/18/32	16.0; 16.7; 36.4; 15.4; 16.0 Anestrual
46a	52.3	6	unknown	132	poor	1/26/32	15.2; 34.8; 35.7; 16.8 Anestrual
26a	110.8	5	unknown (Hampshire)	165	good	2/15/32	18; 4. 6; 36; 17.0 Anestrual

B PREGNANT SERIES

50a	1.8	-	unknown	106	medium	12/ 1/31	16.3
47a	2.3	7	unknown	126	medium	11/10/31	16.3; 17.6; 16.3; 16.0
6a	4.8	5	Southdown	138	medium	11/16/31	17.1; 15.9; 16.5
6b	7.3	6	grade Hampshire	114	medium	11/ 8/32	16.0; 16.4; 16.6
5b	9.3	5	grade Hampshire	112	medium	11/ 4/32	30.6; 21.0
43a	11.3	6	unknown (Hampshire)	112	medium	11/17/31	17.0; 16.1; 16.7; 17.2; 16.6
17b	12.1	5	grade Hampshire	140	good	12/31/32	16.3; 17.6; 17.5; 16.6; 17.0
31a	13.8	5	unknown (Hampshire)	146	----------	12/ 4/31	29.0; 37.0; 15.5

TABLE 1.—THE HISTORY OF THE EWES STUDIED (Continued)

Ewe	Stage (days)	Age (years)	Breeding	Wt. (lbs.)	Finish	Date killed	Length of cycle (days)
16b	14.0	4	grade Hampshire	141	good	10/ 7/32	Bred at first estrus
25b	14.2	5	grade Hampshire	146	good	12/ 1/32	16.4; 15.5; 18.0; 17.1
23b	18.9	6	grade Hampshire	113	medium	10/11/32	Bred at first estrus
21a	20.0	2	unknown (Hampshire)	110	medium	12/ 9/31	None observed
15b	20.6	6	grade Hampshire	117	poor	10/17/32	16.4
13a	21.6	5	Southdown	136	good	12/ 4/31	62.8; 11.5
51a	24.9	-	Shropshire	70	poor	12/ 2/31	51.4; 17.1
9b	27.3	6	grade Hampshire	130	good	12/13/32	17.0; 16.3; 18.6; 17.0
2b	29.9	4	grade Hampshire	124	medium	11/10/32	16.0
8a	30.8	5	Shropshire	122	medium	12/ 7/31	23; 17; 33.0; 17.0
19b	32.3	8	grade Hampshire	105	medium	11/ 1/32	16.0
4b	35.0	5	grade Hampshire	120	good	12/17/32	15.6; 32.0; 5.0; 16.0; 16.1
48a	36.3	5	unknown (Shropshire)	138	medium	12/ 2/31	21.5; 30.0
26b	45.7	5	grade Hampshire	116	good	1/ 2/33	17.6; 48.4
30a	48.2	6	unknown (Hampshire)	130	medium	12/16/31	16.5; 35.6
19a	60.0	5	unknown (Hampshire)	214	good	1/11/32	36.5; 16.0
52a	72.9	3	unknown (Hampshire)	128	medium	1/ 9/32	17.0; 15.0; 30.6
41a	85.1	5	unknown (Hampshire)	138	good	12/ 5/31	18; 16.0
37a	97.1	5	unknown	118	poor	12/17/31	Bred at first estrus
9a	108.3	5	Southdown	104	medium	12/18/31	Bred at first estrus
1161a	120.5	4	Hampshire	161	medium	3/11/32	68.8
20a	133.1	5	unknown	110	medium	1/ 8/32	Bred at first estrus
1a	136.3	5	Shropshire	142	medium	1/15/32	Bred at first estrus
33a	141.0	2	unknown (Hampshire)	170	medium	1/18/32	Bred at first estrus
24a	148.7	-	unknown (Hampshire)	150	poor	1/26/32	Bred at first estrus

four per cent by volume of formaldehyde were used; with small pieces the methods of Helly (Lee), Champy (Lee), or Severinghaus (1932). The pieces fixed in the fluids of Helly and Bouin have shown most differentiation into cell types.

Pituitaries in the *a* series were preserved by only three methods, Bouin's, Champy's, and Helly's. These methods seemed completely inadequate in the preliminary studies[1] and the others were introduced. Uniform penetration was secured with formalin and with the methods of Zenker and Regaud. The constituents of the fixing fluids and the

1 The preliminary study of the pituitary of the ewe was made by Dr. Laura J. Nahm. Most of the slides prepared by the Champy-Kull and Champy-Severinghaus and Helly-Kull methods were made by her, and her records of the fat content of the pituitary have been used for all the animals of the "a" series. We wish to express our gratitude to her for the valuable and orderly records of these observations.

hydrogen ion concentration, as determined with a glass electrode[2], are shown:

Bouin: saturated picric acid, formalin, acetic acid, pH 1.3.

Champy and Severinghaus' modification: potassium bichromate, chromic acid, osmium tetroxide, pH 1.5-1.8; pyroligneous acid, chromic acid, pH 2.91; potassium bichromate, pH 3.8-3.9.

Helly: potassium bichromate, sodium sulfate, mercuric chloride, formalin, pH 4.0; potassium bichromate, sodium sulfate, pH 4.2.

Regaud: potassium bichromate, formalin, pH 3.5; potassium bichromate, pH 3.8-3.9.

Severinghaus' method for Golgi elements: potassium bichromate, chromic acid, osmium tetroxide, pH 1.5-1.8; osmium tetroxide, pH 7.0.

Zenker: potassium bichromate, sodium sulfate, mercuric chloride, acetic acid, pH 2.2.

10% formalin: pH 3.7; neutralized with $CaCO_3$, 7.2; neutralized with $MgCO_3$, 8.3.

The problems of protein and fat fixation are of importance in a study of the pituitary gland. The acidity of the fixing mixtures is so great that the proteins are precipitated on the acid side of their isoelectric points as cations. Some constituent of the nucleus, possibly nucleo-protein, behaves as if it had an isoelectric point between the extremely acid fluids of Champy, Severinghaus, and Bouin and those of Regaud, Helly, and formalin. The fixatives have other effects on proteins but these are not satisfactorily explained. Very poor penetration is secured with the fluids containing osmium tetroxide, although the compound is volatile and should penetrate readily. The fact that osmium tetroxide reacts with almost all the constituents of the cell suggests that it cannot diffuse far into the tissue before it is used, unless the time of fixation is extended. The longer treatment with osmium tetroxide (Severinghaus, 1932) for the demonstration of the Golgi elements gives better general fixation in the center of the pieces. The fats reduce the valence of the osmium and may convert it to a tetravalent ion which forms a hydroxide, dark brownish-black in color and slowly soluble in fats and fat solvents. Some other types of reducing agents convert the osmium tetroxide to a lower oxide which is black in color and is insoluble in fat solvents (Ephraim, 1934). The Golgi elements may contain one of the latter types of reducers.

2 The determinations of the hydrogen ion concentration of fixing fluids and dye solutions were made by F. Kavanagh, to whom we wish to acknowledge our indebtedness and appreciation.

Baley (1937) has pointed out that fixation of cytoplasmic granules in small pieces of material or at the edges of large pieces fixed in chrom-sublimate-formol is very unsatisfactory. Bouin's and Helly's fluids showed this type of variation in fixing blocks of the pituitary.

Several modifications of the paraffin embedding method have been used. The routine alcohol-xylene-paraffin method was tried first but hardened tissues. Aniline followed by wintergreen oil was substituted for the 80% to absolute alcohol steps, and occasionally for xylene. This left tissues softer but sometimes without paraffin in the centers. An embedding mixture of paraffin, bayberry wax, and semi-crude rubber was used during the summer of 1934 when the temperature was very high. Recently dioxan has been used with satisfactory results in place of all the alcoholic solutions and xylene. The object of these variations has been to obtain two to four micra sections of entire pituitaries.

The sections of the pituitary glands have been stained by the techniques described by Kull (Lee), Heidenhain (Lee), Mallory (Lee), Wright (Galigher), Mann (Conn), Maurer and Lewis (1922), Severinghaus (1932), Kindell (1933), and Martins (1933) and by various modifications of these methods. The methods of Kull and Heidenhain were used in the preliminary studies but failed to differentiate consistently, even two types of cells. A study of the vast literature on the pituitary suggested the other methods, and the books by Baker (1933) and Conn (1929) stimulated further modifications. An unpublished modification of Mallory's method by W. O. Nelson, using somewhat stronger solutions of acid fuchsin and aniline blue than ordinarily employed, has been very useful following fixations by Helly's method. The constituents used in the various methods were as follows:

Mallory: acid fuchsin; aniline blue, orange G, phosphomolybdic acid.
Kull: acid fuchsin; toluidine blue or thionin; aurantia.
Severinghaus: acid fuchsin; alcoholic picric acid; phosphomolybdic acid; acid violet, methyl green.
Cleveland and Wolfe: Ehrlich's hematoxylin; potassium dichromate; erythrosin; orange G, phosphomolybdic acid; aniline blue.
Martins: hematoxylin; acid fuchsin; phosphomolybdic acid, methylene blue.
Wright: methylene blue, eosin.
Mann: methyl blue; eosin.
Kindell: acid fuchsin; methyl blue.

Heidenhain: iron alum; hematoxylin; eosin.
Maurer and Lewis: safranin, acid violet.

The nature of the dyes used is shown in Table 2. Hematin, the dye principle of hematoxylin, is a weakly acid dye (Baker, 1933) which requires a mordant. The mordants are usually trivalent aluminum, chromium, or iron or bivalent copper salts which hydrolize

TABLE 2.—DYES USED IN STAINING THE PITUITARY GLANDS OF THE EWE.

Dye	Source	Staining type	Theoretical chemical nature
Acid fuchsin, aq. ---- anilin pH 5.6	Gruebler, Will Corp. -----	weakly acid	sulfonated rosanilins and pararosanilins
Acid violet---------- alcoholic pH 7.5	Gruebler, Coleman Bell---	moderately or weakly acid	sulfonated, methylated rosanilins and pararosanalin
Anilin blue---------- aqueous pH 7.0	Gruebler water soluble----	moderately acid	sulfonated phenylated rosanilins and pararosanilin
Eosin---------- aqueous pH 5.7	Gruebler, Coleman Bell---	moderately acid	brom-fluorescein
Erythrosin----------	Coleman Bell----------	moderately acid	iodo-fluorescein
Hematin---------- alcoholic pH 3.1	Gruebler hematoxylin-----	uncertain acidity	complex phenolic acid
Methyl blue--------	Gruebler----------	moderately acid	sulfonated triphenyl rosanalin
Methyl green------- aqueous pH 4.5	uncertain ----------	basic	methylated crystal violet
Methylene blue----- Loeffler aq. pH 6.6	Gruebler-Loeffler---------	basic	methylated thionin and its oxidation products
Orange G---------- aqueous pH 8.2	Gruebler, Nat. Anil. cert. NO-1, Schults No. 38. dye 86%----------	strongly acid	sulfonated azo
Safranin O--------- aqueous pH 8.6	Nat. Anil. cert. NS-9 Schults No. 679, dye, 97%	basic	methylated phenosafranin
Thionin---------- aqueous pH 6.5	Gruebler----------	basic	diamino thiazin
Toluidine blue------ aqueous pH 6.7	Gruebler----------	basic	tri-methyl thionin

and are precipitated as hydroxides in the tissues, the metals behaving as cations. It has been assumed that they continue to function as cations when the hydroxyl group is replaced by the colored anion of the phenolic acid, hematin, and that the result is a basic dye. The phenolic acids in vitro form complex, deeply colored anions with the metalic ions employed as mordants. It seems plausible to assume that the same thing occurs in tissues, and that the staining substance is an anion and a relatively strong acid dye. The staining of chromatin with hematin may be due to the presence of one of the metals in the nucleus, for mordanting is often not necessary.

Three major difficulties are met in applying the various staining combinations to the pituitary: (1) many of the combinations consist entirely of acid dyes; (2) frequently the color combinations do not furnish sufficient contrast; and (3) the tissue color produced is not the one anticipated. The methods of Kull, Severinghaus, Martins, Maurer and Lewis, and Wright include basic dyes. Kull's method rarely shows even the nucleus stained with the basic dye due to the tendency of the strong acid fuchsin to overstain. Methyl green frequently breaks down into crystal violet giving a purple color interfering with its effective combination with acid violet. Safranin, according to the observations of Maurer and Lewis, stained red the cells usually stained by acid dyes; and the acid violet stained the basophilic or beta cells green. Wright's stain is theoretically the most valuable of these five dye combinations, but its specificity is easily disturbed by an improper pH or staining time. The methods of staining in which only acid dyes are used have given better cytoplasmic differentiation than have the combinations of acid and basic dyes either in sequence or as neutral dyes. Ordinarily Mallory's triple stain for connective tissue does not show good contrast between cells stained by orange G and by acid fuchsin. Methyl blue and eosin tend to stain the same cells in the pituitary and to give poor contrast. Hematoxylin demonstrates nuclei well, but reduces the selectivity of the cells for other dyes. Methyl green and acid violet give colors other than the ones expected.

The method of staining which has been used most in this study is as follows:

1. Bring the slide to water.
2. Flood it with 10% aniline-acid fuchsin (Grübler), heat to steaming, cool for 5-10 minutes.
3. Rinse in distilled water.
4. Place the slide in Mallory's aniline blue, orange G, phosphomolybdic acid mixture or a modification of it for 2-10 minutes.
5. Rinse in water and in 95% alcohol quickly.
6. Dehydrate, clear, and mount in gum damar or balsam.

Cells stained with orange G do not appear, but a purple color is formed with aniline blue and either fuchsin or orange G.

Microscopic observations have been made with Leitz and Nachet instruments with a maximum magnification, when projected by a prism to table level, of 3400. Cell counts were made with the Nachet (highest magnification without oil) but were discontinued because the original hypothesis of three cell types on which the counts were

based proved inadequate, and because the method of sectioning gave slides from areas not strictly comparable. Figures of cells have been drawn with camera lucida at a magnification of 2000, increased to 4000 by the method of squares, and reduced to 2000 in reproduction. Photographs were taken through a Leitz photomicroscope and reproduced without reduction.

OBSERVATIONS

General Structure.—The pituitary body of the ewe lies in a rather deep sella turcica separated from the cranial cavity by a tough cartilaginous diaphragm. At the anterior end of the diaphragm is the opening through which the stalk of the neural lobe of the pituitary joins the infundibulum. The neural lobe extends posteriorly next the diaphragm as the mid-dorsal part of the gland (Fig. 1). The anterior end of the stalk is cut in dissecting the pituitary from the brain. The intermediate lobe is closely attached to the neural lobe along its ventral surface and blends into the tuberal part at the anterior end of the pituitary. A thickened region of the intermediate lobe extends along the neural lobe to its posterior end. Between the intermediate lobe and the glandular lobe at the posterior end of the pituitary is the hypophyseal cleft which is about as wide as the intermediate lobe and is usually open at the posterior end in the median plane (Fig. 2). The glandular lobe occupies the ventral portion of the sella and rarely extends anterior to the attachment of the neural lobe at the stalk. Lateral wings extend dorsally to the diaphragm. The glandular lobe is much larger in the ewe than the other divisions. A very large unidentified lobe extended anterior to the glandular lobe in one sheep pituitary obtained from a commercial firm.

The gland which has been called the "pituitary", because the English term seemed appropriate for sheep, is equivalent to the "hypophysis" or "hypophysis cerebri" of many investigators. The names used for the lobes of the pituitary body of the sheep and their synonyms are as follows:

glandular lobe = anterior lobe = pars distalis = pars anterior
tuberal lobe or part = pars tuberalis
intermediate lobe = pars intermedia
neural lobe = posterior lobe = pars nervosa.

The terms "glandular" and "neural" have been substituted for "anterior" and "posterior" respectively because the position occupied by these divisions in the ewe make the latter names misnomers.

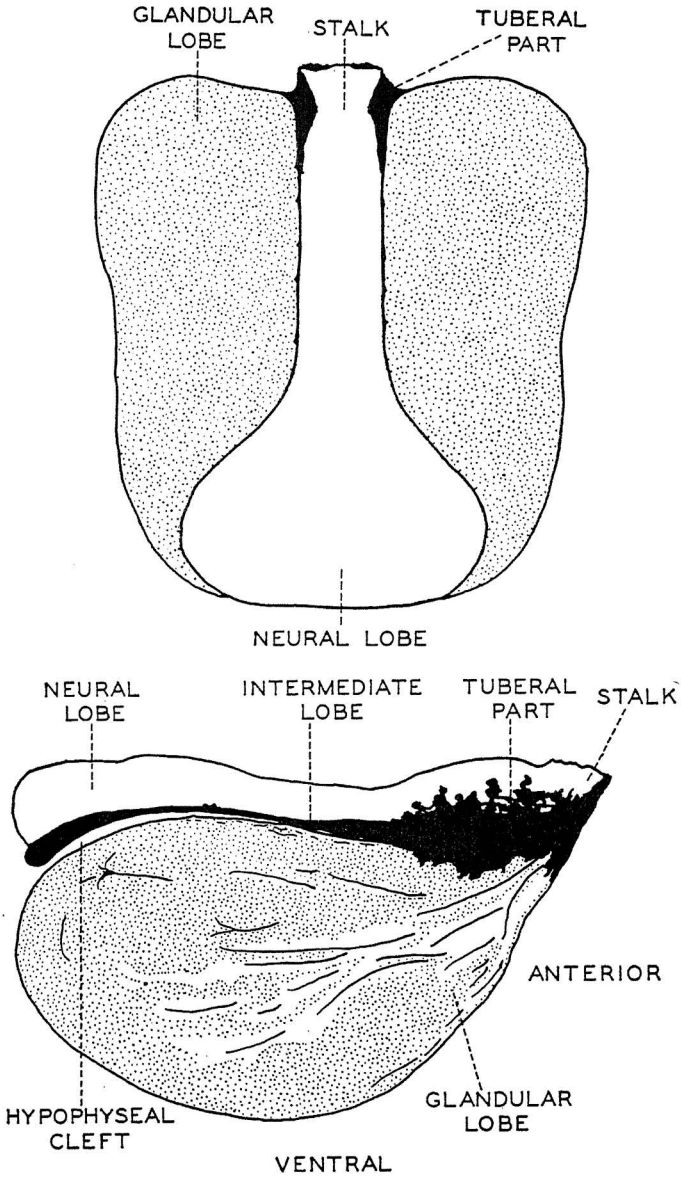

Figure 1.—The general structure of the pituitary body of the ewe. The upper figure is a diagram of the dorsal surface of the gland; the lower, a diagram of a median sagital section showing the central core in white and the blood vessels as black lines. X 7.

A median sagittal section of a pituitary before fixation shows a vascular area radiating from the anterior tuberal part throughout

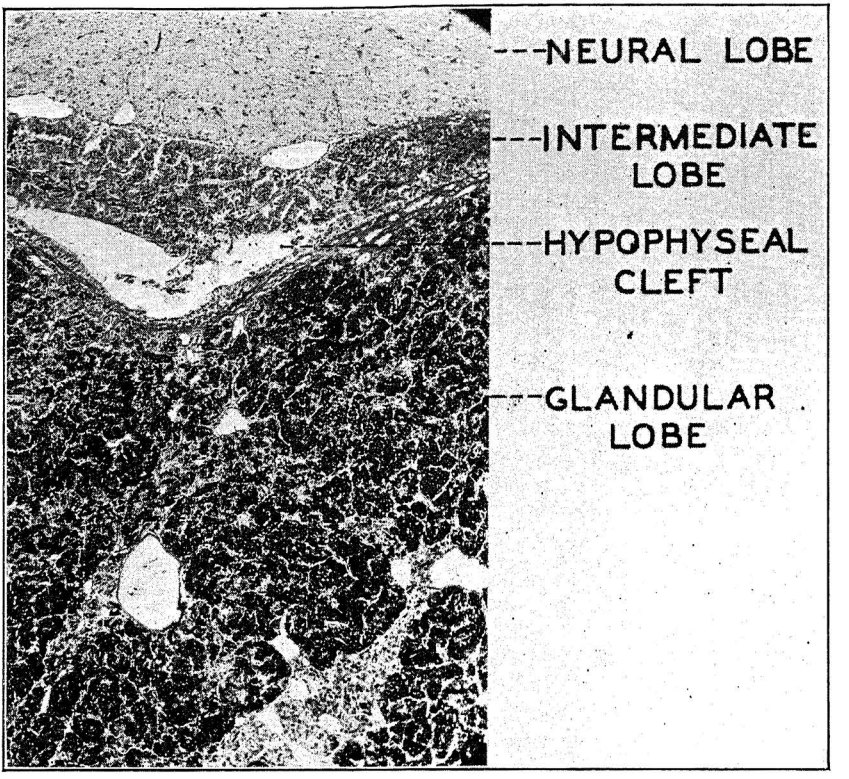

Figure 2.—A portion of a cross section through the pituitary showing the width of the intermediate lobe and hypophyseal cleft. In the lower right hand corner of the glandular lobe the core substance is shown. Ewe 52a, Bouin-Mallory (strong), green filter. X 75.

the central part of the gland. Frequently, the neural and intermediate lobes macroscopically appear more vascular than the glandular lobe. After fixation and staining, the vessels in the anterior lobe are shown as very large sinusoids from which a rich net of small sinusoids branch among the cells. Small arteries and veins occur in the intermediate and neural lobes and in the glandular lobe adjacent to the cleft or intermediate lobe. . Large vessels run through a connective tissue band which parallels the hypophyseal cleft about three cell layers ventral to it. These are the only arteries and veins that have been observed in the glandular lobe of the ewe. Lightly stained pituitary cells lie along these blood vessels. Arteries and veins only were observed in the neural, but capillaries are abundant in the intermediate lobe.

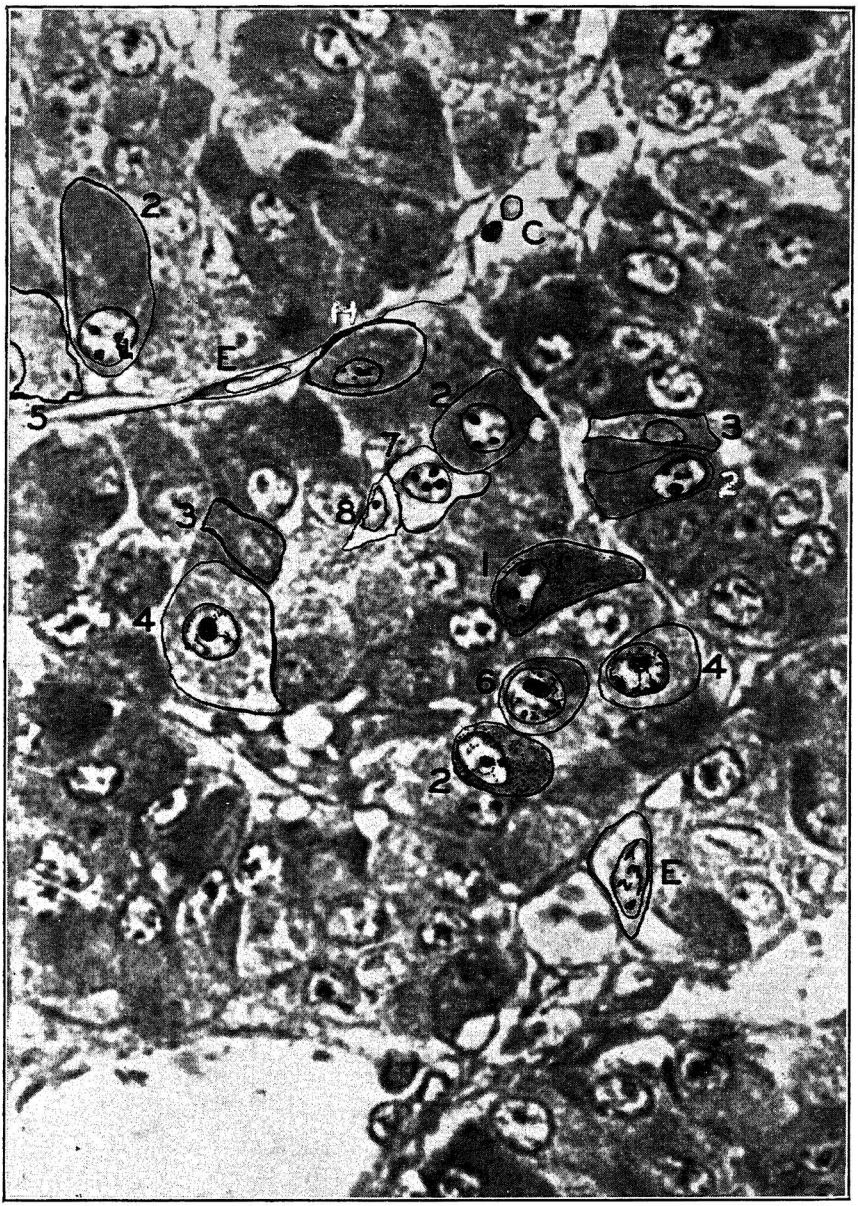

Figure 3.—The glandular lobe showing cells of type 1, 2, 3, 4, 5, 6, 7, and 8. The cell of type 1 and one of type 2 have been stippled in an attempt to demonstrate the maculae. *H*, histiocyte; *E*, endothelial cell; *C*, erythrocyte. Ewe 31a, Bouin-Mallory (strong), green filter. X 1600.

The Glandular Lobe.—*Structure.*—The cells of the glandular lobe appear singly, in follicles, or in columns separated by sinusoids. The various types of cells are somewhat localized in different regions. An area of faintly stained cells designated the central core is found in the anterior region of the glandular lobe and spreads in the shape of a fan in the lobe almost to the posterior end. Cells with conspicuous chromophilic granules in the cytoplasm are numerous near the periphery of the gland.

The cells which make up the anterior lobe of the pituitary are of several kinds (Fig. 3). Their classification into the conventional acidophils, basophils, and chromophobes has not proved easy or profitable. The pituitary body of the ewe is very complex. The following factors have been considered in deciding which cells represent different morphological types: (1) size of cell, (2) shape of cell, (3) reaction to fixatives, (4) reaction to stains, (5) cytoplasmic inclusions, (6) relative number and position of cells, (7) arrangement of cells in relation to other cells, (8) nucleocytoplasmic relations, (9) nucleoli, (10) chromatin. Nine cell types have been distinguished. In order to make any desired piece of information more easily available, the types are described in outline form.

TYPE 1. (FIG. 4 SKETCHES, *1* IN PHOTOGRAPHS IN FIGS. 3, AND 15-21, INCL.)

Size: 8 to 20 micra in greatest dimension.
Shape: oval, round, or quite angular.
Reaction to fixatives:
Bouin: fine to medium closely packed granules in center, hyaline at periphery.

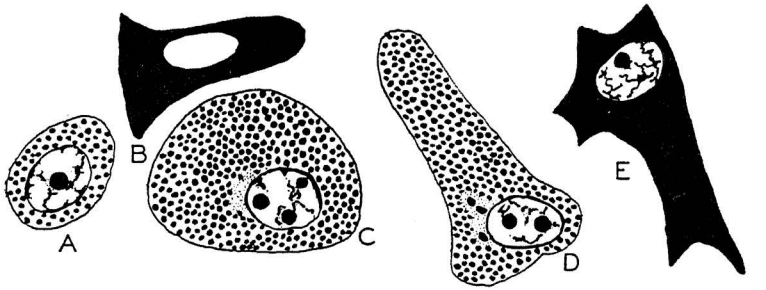

Figure 4.—Cells of type 1. *A*, *C*, and *D*, Bouin-Mallory (strong), granules red, macula bluish. *B* and *E*, Champy-Kull, cytosome red, hyaline, nucleus in *B* yellow-orange. X 2000.

Champy: hyaline cytoplasm and nucleus; nucleus more heavily chromatic than cytoplasm.
Severinghaus 1: hyaline cytoplasm and nucleus.
Severinghaus 2: hyaline, osmicated or black lined cytoplasm, nucleus dark.
Helly: fine to medium closely packed granules.
Regaud: medium granules.
Zenker: fine granules, closely packed.
Formalin: medium granules.

Reactions to stains:	Cytosome	Nucleus
Mallory (Nelson)	orange and red	purple or blue
Mallory (strong)	red, fuchsin	red
Kull	red	red or yellow
Severinghaus	red-orange	purple or green
Cleveland and Wolfe	red	blue
Martins	red	blue
Wright and methylene blue	pink	blue
Mann	pink	blue
methylene blue-eosin	pink	blue
Heidenhain hematoxylin-eosin	blue-black	bluish black
Kindell	blue	red
safranin-acid violet	blue	red

Cytoplasmic inclusions:
fat: few to several drops.
chondriosomes: mitochondria and short rods.
Golgi elements: netted throughout cell or as nuclear cap.
macula: a nuclear cap.

Relative number and position: not numerous, 0 to 35 in a 0.1mm square; more numerous in the peripheral zone but larger in the core of glandular lobe.

Arrangement of cells in relation to others: usually isolated from others of type 1, may be in columns or alveoli with types 1 or 3, or separated by connective tissue and blood vessels.

Nucleo-cytoplasmic ratio: nucleus about seven micra in diameter, cytosome variable.

Nucleoli: 3 to 7 small, 1 to 2 micra, scattered, acidophilic.

Chromatin: clumped at nuclear membrane and a few scattered strands or granules.

TYPE 2 (FIGS. 5 AND 14 SKETCHES, 2 IN PHOTOGRAPHS, FIGS. 3, 13, AND 15-20 INCL.)

Size: 8 to 20 micra in greatest dimension.
Shape: cuboidal, oval, round, or columnar.
Reaction to fixatives:

Bouin: fine to medium, closely packed granules in center of piece; smooth cytoplasm with few sharply defined granules at edge.

Champy and Severinghaus: fine to medium granules, closely packed, or non-granular.

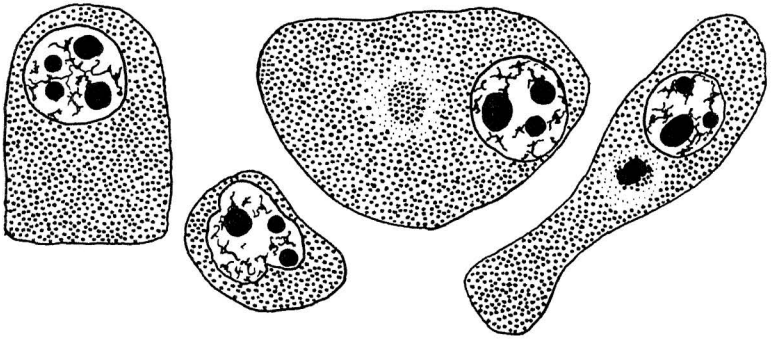

Fig. 5.—Cells of type 2. Bouin-Mallory (strong), two with maculae, non-granular blue areas with granular or smooth, dark purple area in center; granules purple. X 2000.

Helly: rarely granular, dense cytoplasm.

Regaud: non-granular or granules very small, dense.

Zenker: dense non-granular cytoplasm.

10% formalin: medium granules not distinguishable from type 1.

Reactions to stains:	Cytosome	Nucleus
Mallory (Nelson)	orange over red or blue	purple
Mallory (strong fuchsin)	purple or red-purple	red
Kull	red	red
Severinghaus	violet	purple or green
Cleveland and Wolfe	yellow orange	blue
Martins	red	blue
Wright and methylene blue	pink	blue
Mann	pink or blue	pink or blue
Heidenhain hematoxylin-eosin	blue or black	black
Kindell	red	red

safranin-acid violet	after Bouin, red; otherwise blue	red
methylene blue-eosin	pink	blue

Cytoplasmic inclusions:

fat: very few drops.

chondriosomes: usually not preserved, several mitochondria and short rods.

Golgi elements: circle with occasionally a net within it.

macula: oval or spherical mass, or a clear ring with denser center more or less like the cytosome.

Number of cells and position in pituitary: most numerous type in the periphery in many animals, several scattered in the core, 0 to 80 per 0.1 mm square.

Nucleo-cytoplasmic ratio: nuclei about 7 micra in diameter, cytoplasm quite variable.

Nucleoli: 3 to 10, usually 4, small, less than 2 micra in diameter, scattered.

Chromatin: never abundant, clumps, associated with nucleoli, and strands.

TYPE 3 (FIGS. 6 AND 14, SKETCHES, *3* IN PHOTOGRAPHS, FIGS. 3, 15, 16, 20, AND 21)

Size: 8 to 20 micra in greatest dimension.

Shape: cuboidal, oval, round, or columnar.

Reaction to fixation:

Bouin: smooth, finely granular cytoplasm.

Champy and Severinghaus: smooth or finely granular cytoplasm, very difficult to distinguish from type 2.

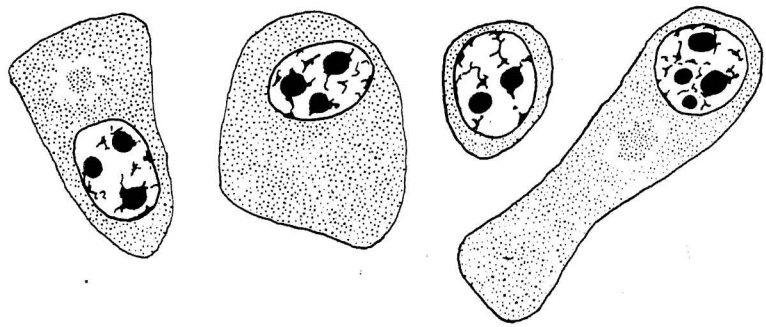

Figure 6.—Cells of type 3. Bouin-Mallory (strong), non-granular purplish cytoplasm and mass in center of macula, a somewhat bluer area surrounding it. X 2000.

Helly: smooth and somewhat lighter cytoplasm.
Regaud: non-granular.
Zenker: non-granular.
10% formalin: doubtful identification.

Reaction to stains:	Cytosome	Nucleus
Mallory (Nelson)	grayish pink or blue	purple
Mallory (strong)	mauve to pink	red
Kull	red or light red	red
Severinghaus	red or purple	purple or green
Cleveland and Wolfe	orange	blue
Martins	red	blue
Wright and methylene blue	pink	blue
Mann	pink	blue
methylene blue-eosin	pink	blue
Heidenhain hematoxylin-eosin	graysish	black
Kindell	fuchsin red	red
safranin-acid violet	blue; after Bouin's, pink	red

Cytoplasmic inclusions:
fat: scarce.
chondriosomes: mitochondria and short rods, numerous enough to be confused with granules of other types after fixation in Champy or Helly.
Golgi elements: ring or ring-shaped net beside nucleus.
macula: not easily seen but present in most cells.
Number of cells and position in pituitary: at times the most numerous type in the perphery of the pituitary, 0 to 80 per 0.1 mm square.
Arrangement of cells: in columns or alveoli of similar cells.
Nucleo-cytoplasmic ratio: nuclei about 7 micra in diameter, cytoplasm variable.
Nucleoli: 3 to 8, small, less than 2 micra in diameter, scattered.
Chromatin: never abundant, small clumps about nucleoli and in fine strands.

TYPE 4 (FIG. 7, SKETCHES, *4* IN PHOTOGRAPHS, FIGS. 3 AND 15-21 INCL.)
Size: 8 to 18 micra in greatest dimension.
Shape: oval or somewhat irregular.
Reaction to fixation:
Bouin: medium to large irregular masses.
Champy and Severinghaus: smooth cytoplasm or irregular masses.

Helly: medium granules.
Regaud: medium to large granules.
Zenker: medium granules of variable size within cell.
10% formalin: medium to large irregular masses.

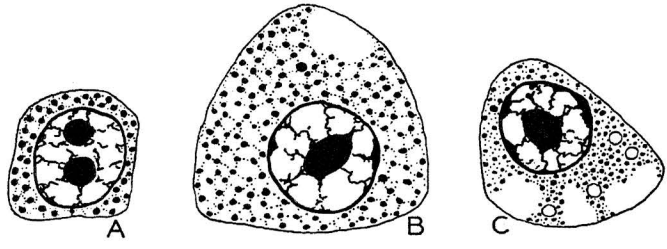

Fig. 7.—Cells of type 4. *A* and *B*, Bouin-Mallory (strong), blue granules and cytoplasmic strings. *C*. Champy-Kull, granules red, circles represent fuchsinophilic spheres, clear areas represent vacuoles. X 2000.

Reaction to stains:	Cytosome	Nucleus
Mallory (Nelson)	blue (aniline blue)	purple
Mallory (strong fuchsin)	blue (aniline blue)	red
Kull	red (fuchsin)	red
Severinghaus	blue, purple, or green	purple or green
Cleveland and Wolfe	blue with red granules (aniline blue-erythrosin) or red	blue
Martins	red	blue
Wright and methylene blue	pale diffuse blue	blue
Mann	blue	blue
methylene blue-eosin	pale blue	blue
Heidenhain hematoxylin-eosin	pink	black
Kindell	blue	red
safranin-acid violet	pale violet	red

Cytoplasmic inclusions:
fat: a few drops.
chondriosomes: mitochondria, some large, not numerous.
Golgi elements: ring beside nucleus.
macula: usually not visible, spherical mass when seen.

Number and position of cells: somewhat less numerous than type 1, never reaching more than 5 to 8 per 0.1 mm square and limited chiefly to the peripheral portion.

Arrangement of cells: on the outer rim of alveoli or along columns of cells of types 2 and 3.

Nucleo-cytoplasmic ratio: nuclei about 7 micra in diameter, cytoplasm quite variable.

Nucleoli: usually one large, 3 to 4 micra in length, centrally located, occasionally a few small ones in addition.

Chromatin: a ring at the nuclear membrane, fine strands running to the nucleolus giving a wheel-shaped arrangement.

TYPE 5 (FIG 8, SKETCHES, *5* IN PHOTOGRAPHS, FIGS. 3 AND 19)

Size: 10 to 22 micra in greatest dimension.

Shape: oval or somewhat irregular.

Reaction to fixatives:

Bouin: smooth scant cytoplasm, small vacuoles.

Champy and Severinghaus: scant faintly granular cytoplasm.

Helly: smooth scant cytoplasm.

Regaud: smooth cytoplasm with some vacuoles.

Zenker: medium sized scattered granules, like type 4.

10% formalin: granular masses of cytoplasm, like type 4.

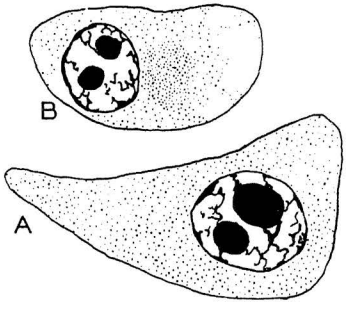

Figure 8.—Cells of type 5. Bouin-Mallory (strong), *A*, smooth, finely granular cytoplasm; *B*, vacuoles and a mass of condensed cytoplasm which may represent a macula. X 2000.

Reaction to stains:	Cytosome	Nucleus
Mallory (Nelson)	blue, very pale violet, or gray	purple
Mallory (strong)	blue, very pale violet, or gray	red
Kull	red	red
Severinghaus	purple, blue, or green	purple or green
Cleveland and Wolfe	pale blue	blue

Martins	red, occasionally bluish	blue
Wright with methylene blue	blue	blue
Mann	blue	blue
methylene blue-eosin	pale blue	blue
Kindell	blue	red
Heidenhain hematoxylin-eosin	pink	black
safranin-acid violet	blue	red

Cytoplasmic inclusions:
fat: a few drops.
chondriosomes: several mitochondria.
Golgi elements: a ring, more or less distorted or broken.
macula: rarely seen, an irregular mass of closely packed, fine granules.
fuchsinophilic spheres: a few in most cells fixed by osmium tetroxide.
Number and position of cells: not very abundant in the periphery but rather numerous in the central core.
Arrangement of cells: infrequently beside alveoli or columns of cells in peripheral zone; in clumps or alveoli in central region.
Nucleo-cytoplasmic ratio: nuclei relatively constant in size, cytoplasm variable.
Nucleoli: usually one large, about 3 micra in length, and one to three smaller.
Chromatin: clumped at nuclear membrane and fine strands elsewhere.

TYPE 6 (FIG. 9, SKETCHES, *6* IN PHOTOGRAPHS, FIGS. 3, 17, 18, AND 19)
Size: 8 to 9 micra in greatest dimension.
Shape: round or nearly so.
Reaction to fixatives: smooth, dense cytoplasm in all.

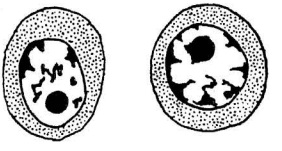

Figure 9.—Cells of type 6. Bouin-Mallory (strong), cytoplasm, bright blue. X 2000.

Reaction to stains:	Cytosome	Nucleus
Mallory	bright blue	purple
Kull	red	red
Severinghaus	purple, blue, green	green
Cleveland and Wolfe	blue	blue

Martins	red	blue
Wright with methylene blue	pale blue	blue
Mann	blue	blue or pink
methylene blue-eosin	pale blue	blue
Heidenhain hematoxylin-eosin	pink	bluish black
Kindell	blue	red
safranin-acid violet	pale violet	red

Cytoplasmic inclusions:
fat: none.
chondriosomes: small mitochondria numerous.
Golgi elements: loose net.
macula: not seen.

Number and position of cells: never numerous, often very scarce in both central and peripheral zones.

Arrangement of cells: beside alveoli or columns of cells of types 2 and 3 in clumps with types 5 and 7.

Nucleo-cytoplasmic ratio: large and relatively constant.

Nucleoli: usually one or two small, about 1 micron in diameter, occasionally a larger centrally located one.

Chromatin: clumps of moderate size near the nuclear membrane.

TYPE 7 (FIG. 10, SKETCHES, *7* IN PHOTOGRAPHS, FIGS. 3 AND 19)

Size: 11 to 28 micra in greatest dimension.

Shape: round, oval, or somewhat irregular.

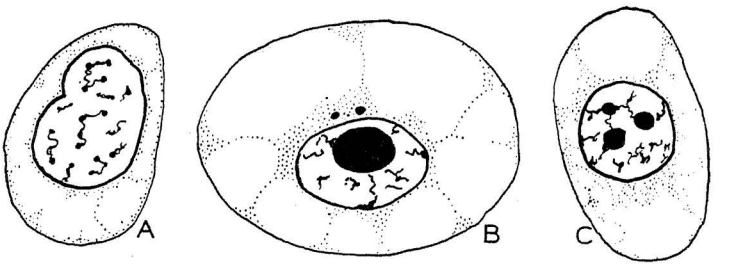

Figure 10.—Cells of type 7. *A* and *C*, Bouin-Mallory (strong), cytoplasm pale blue, *A* with giant type nucleus; *B*, Champy-Severinghaus, cytoplasmic strands green; black spheres, fat. X 2000.

Reaction to fixatives:
Bouin: stringy cytoplasm with large vacuoles.
Champy and Severinghaus: very little cytoplasm, faintly granular.
Helly and Regaud: scant cytoplasm.
Zenker: little granular cytoplasm.
10% formalin: little stainable cytoplasm, finely granular.

Reaction to stains:	Cytosome	Nucleus
Mallory	blue	red
Kull	red	red
Severinghaus	blue, purple, or green	same
Cleveland and Wolfe	yellow-orange or blue	blue
Martins	red	blue
Wright with methylene blue	pale blue	blue
Mann	blue	blue
methylene blue-eosin	pale blue	blue
Heidenhain hematoxylin-eosin	pink	black
Kindell	blue	red
safranin-acid violet	pale violet	red

Cytoplasmic inclusions:
fat drops: very few.
chondriosomes: a few mitochondria near nucleus.
Golgi elements: broken ring-shaped, near nucleus.
macula: rare, occasionally a spherical mass near the nucleus.
fuchsinophilic spheres: few.

Number and position of cells: few in the periphery but the predominant cell type in core (the ones in the core are smaller than at the periphery).

Arrangement of cells: beside alveoli or columns or in relatively large clumps.

Nucleo-cytoplasmic ratio: small and quite variable.

Nucleoli: usually one large about 3 micra in length and two or three small without definite arrangement, several cells without nucleoli.

Chromatin: ring at nuclear membrane and light strands or more or less chromosome-like bodies within a nuclear membrane.

TYPE 8 (FIG. 11, SKETCHES *8* IN FIGS. 3 AND 19, AND IN FIG. 13)

Size: 6 to 12 micra in greatest dimension.

Shape: round, oval, or very irregular.

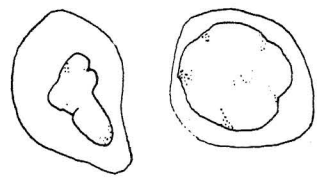

Figure 11.—Cells of type 8. Bouin-Mallory (strong), usually red. X 2000.

Reaction to fixatives: only a few strands of cytoplasm ever preserved.
Reaction to stains: membranes and strands stained like cytoplasm of cells of types 4 and 7.
Cytoplasmic inclusions: none.
Number and position of cells: not numerous but more in core than at periphery.
Arrangement of cells: in the center of alveoli surrounding the colloid, in alveoli in which no colloid is present.
Nucleo-cytoplasmic ratio: large.
Nucleoli: usually none, occasionally some very small.
Chromatin: scant, the nuclear membrane usually precipitated in a very irregular form.

TYPE 9 (FIG. 12, SKETCHES *9* IN FIG. 18)

Size: 7 to 9 micra in greatest dimension.
Shape: Round or oval.
Reaction to fixatives: cytoplasm not fixed or very finely granular.
Reaction to stains: faintly fuchsinophilic, if fixed.
Number and position of cells: scattered throughout pituitary but very few.
Arrangement of cells: scattered or in the center of clumps of cells.

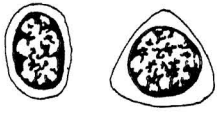

Figure 12.—Cells of type 9. Bouin-Mallory (strong) red or purple chromatin. X 2000.

Nucleo-cytoplasmic ratio: very large and constant.
Nucleoli: none or one to three very small, less than 1 micron in diameter.
Chromatin: granules and clumps scattered throughout the nucleus.

COLLOID MASSES (FIG. 13)

Size: 20 to 150 micra in diameter.
Shape: usually spherical or oval, occasionally irregular with arms extending between cells.
Reaction to fixatives:
Bouin: smooth, hyaline or finely granular.
Champy and Severinghaus: smooth hyaline.
Helly: finely granular or not fixed.
Regaud: hyaline.
10% formalin: not fixed.

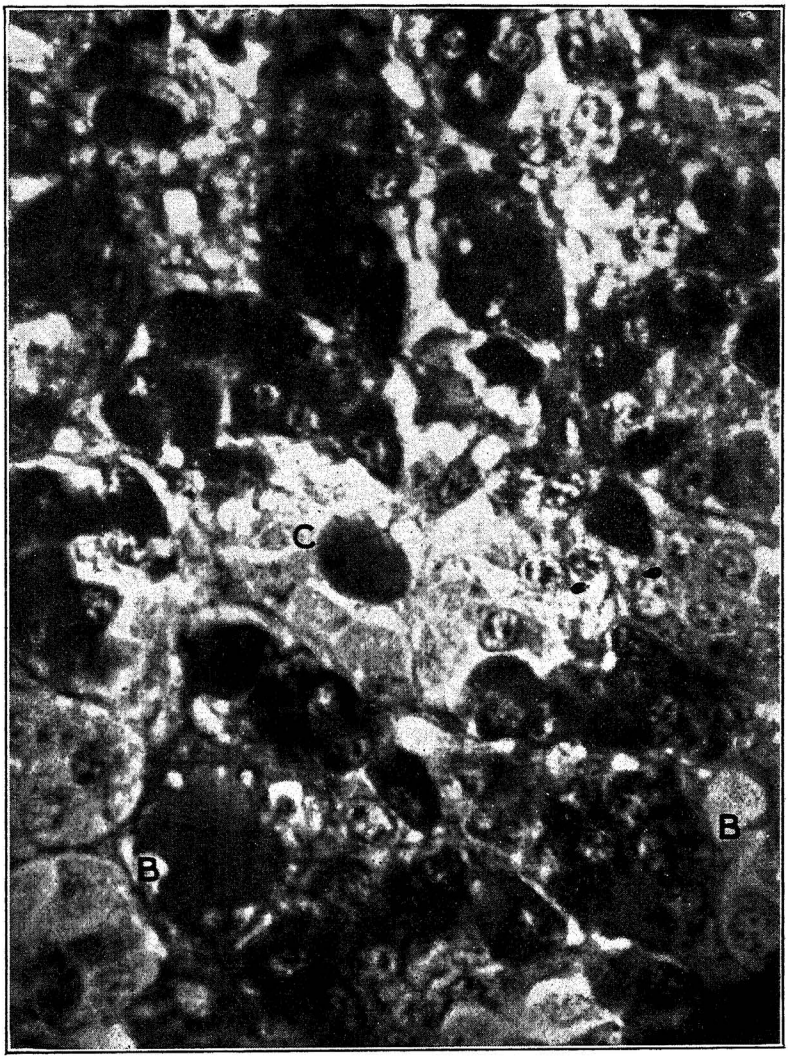

Figure 13.—A colloid mass *C* surrounded by cells of type 8. The dark cells are of type 2. Blood sinusoids, *B*. Ewe 52a, Bouin-Mallory (strong), green filter. X 1000.

Reaction to stains:

Mallory	blue, very bright
Kull	red, orange, or yellow

Severinghaus	green, purple, or orange
Wright with methylene blue	blue
Mann	blue
Kindell	blue
Heidenhain hematoxylin-eosin	pink
safranin-acid violet	red

Position: most abundant in or near central zone in center of alveoli in the region of the tuberal part.

Most fixing and staining combinations permit the identification of at least five types of cells, but the distinctive features of the nine types are shown best in the center of rather large pieces of pituitary after fixation by the techniques of Bouin and Mallory or a modification of Mallory's method. These features are summarized in Table 3. Chondriosomes and fat drops are not preserved in such cells. The cells of types 1 and 2 are often so closely packed with granules that individual granules can scarcely be seen. If the staining time is not properly adjusted, all these cells may be stained red with the acid fuchsin or purple with excess aniline blue. The over-stained cells seem to consti-

TABLE 3.—A COMPARISON OF CELL TYPES IN THE CENTER OF LARGE PIECES AFTER FIXATION IN BOUIN'S FLUID AND STAINING BY A STRONG ACID FUCHSIN MODIFICATION OF MALLORY'S TRIPLE CONNECTIVE TISSUE STAIN.

Type	Size in micra	Cytoplasmic		Macula	Number and position	Arrangement	Nucleoli	Chromatin
		Fixation	Stain					
1	8–20	medium-sized granules	red	nuclear cap	less than 5% in any region	singly	3-7 small (1 micron)	loose strands red
2	8–20	fine granules	purple	oval mass	1-90% at periphery, less than 10% in core	clumps columns alveoli	3-10 small (1 micron)	loose strands red
3	8–20	non-granular	mauve	oval	10-90% at periphery, less than 10% in core	clumps alveoli columns	3-8 small (1 micron)	loos strands red
4	8–18	large granules	bright blue	oval, rarely seen	less than 5% in any region	singly	1 large (3 micra)	radial strands red
5	10–22	non-granular	pale blue	oval, rarely seen	less than 1% at periphery, 30-60% in core	singly clumps	1 large (3 micra)	loose strands red
6	8–9	non-granular	bright blue	not seen	less than 3% in any region	singly	1-2, (1-2 micra)	granules strands red
7	11–28	scant stringy	pale blue	oval, rarely seen	less than 1% in periphery, 30-60% in core	singly clumps	1 large (3 micra) 2-3 small (1 micron)	loose strands red
8	6–12	scant stringy	pale blue	not seen	5-10% near tuberal part, less than 1% elsewhere	around colloid	usually none	scant red
9	7–9	scant	pink	not seen	less than 1% in any region	singly or center of clumps	none or small	abundant granular red

tute a single type which is more numerous than the acidophils are usually thought to be in other species. A few of the cells of the larger types have giant nuclei which may be twice the diameter of an average nucleus (Fig. 14B and C). A single fuchsinophilic, hyaline nucleus or cell has been seen in one or two alveoli containing chiefly cells of type 2 in a few pituitaries (Fig. 14A). Two vacuolated nucleoli staining with aniline blue instead of acid fuchsin were found (Fig. 14B).

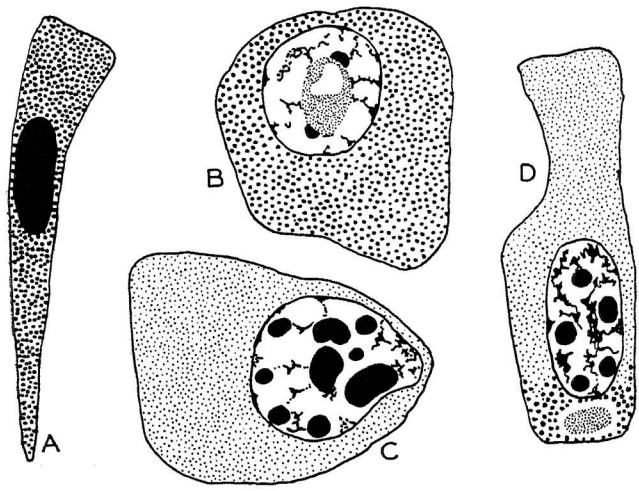

Figure 14.—Unusual types of cells in the glandular lobe. Bouin-Mallory (strong). *A* is type 2 with a hyaline fuchsinophilic nucleus. *B* is type 2 with a large nucleus and a vacuolated nucleolus stained with aniline blue in addition to the usual red nucleoli. *C* is type 3 with a very large nucleus. *D* is a transitional stage between type 2 and type 3 with an unusual location of the macula at the nuclear end of the cell. X 2000.

At the edge of many of such large pieces, the cytoplasm of the peripheral zone is precipitated as a uniformly fine granulation staining with aniline blue. Chondriosomes are preserved as rather large fuchsinophilic spheres or short rods, perhaps ten or fifteen per cell. Cell membranes are indistinct and maculae are not visible. Hyaline bluish-fuchsinophilic nuclei or cytosomes or both are found occasionally in this region.

Other methods by which all these cell types can be identified rather easily are combinations of Helly's method of fixation and Mallory's method of staining for connective tissue, and Regaud's method of fixation and staining with acid fuchsin and aniline blue.

After fixation in Champy's fluid and staining by any method which employs a 10-20% solution of acid fuchsin in aniline water, hyaline masses, which retained the yellow color of the dichromate in fixation

and are stained with varying amounts of fuchsin, are observed. Several of those staining bright red contain more or less normal nuclei and are classed as cells of type 1 (Fig. 4). Irregular vesicular cells with intensely fuchsinophilic or indistinct nuclei seem to be histiocytes and are often quite numerous. The colloid masses in the core of the glandular lobe and in the tuberal part react in the same way as these cells to such fixative and staining combinations.

After formalin fixation with a methylene blue and eosin stain, it is possible to distinguish only two pituitary cell types, one with eosinophilic granules and the other without granules. A few cells with purple granules resembling mast cells have been seen in such preparations.

Cells intermediate in character between certain of the nine types of cells have been found. Those between types 2 and 3 are quite common in many pituitaries (Figs. 14D; 16 and 20, *2-3*). The granular area usually is adjacent to the macula, but nucleus and macula rarely occur at the same end of an elongated cell. The dividing line between cells of types 5 and 7 is difficult to fix. The degree of vacuolation varies from none to almost complete. Cells of type 5 without vacuoles are chromophilic; those of type 7 with numerous large vacuoles, chromophobic. A few large vacuolated cells contain granules like those of type 4. Other large vacuolated cells in the castrate, ewe *34*, contain granules like type 2 or take cytoplasmic stains like type 3.

The lack of orientation of the cytosomal bodies and the nucleus in the pituitary is of considerable interest. The columns of cells in the peripheral zone shows this most clearly. A capillary or sinusoid with definite endothelium passes along one or both sides of the column. The nuclei of cells of types 2 and 3 are found at either end in about equal numbers. Usually the macula is near the center of the cell or at the end away from the nucleus. Nuclei in the center of columnar cells of types 2 and 3 are rare. The cells of other types show no more nuclear orientation in the columns than do types 2 and 3. In the alveoli the nuclei are most often at the end away from the sinusoids; but if the follicle contains colloid, the nuclei of the row or rows of chromophilic cells, surrounding those of type 8, may be at either end of the cell. Granules may be clumped at either end of cells intermediate between types 2 and 3.

The blood channels of the anterior lobe are chiefly capillaries and sinusoids. The capillaries show a well defined endothelium surrounded by a few connective tissue elements. Pericytes have not been identified. The sinusoids are lined with endothelium and a few other cells with nuclei like those of histiocytes. Techniques for demonstrating histiocytes have not been employed; but cells with small, chromatic, irregularly oval or kidney-shaped nuclei are quite numerous near blood chan-

nels. Most of these cells are chromophilic (Figs. 3, and 15-21 incl.) A little loose connective tissue surrounds the sinusoids. Cells with granules stainable with methylene blue occur infrequently in this connective tissue.

A macula or Golgi apparatus is found in many cells (Figs. 3, 4, 5, 6, 8, 14, 15, 17, and 19). The cells of type 1 show a cap-shaped structure as a granule-free area beside the nucleus after fixatives that do not contain osmium tetroxide and a paranuclear net of variable size after osmication. A cell of type 2, 3, 4, 5, or 7 may have a spherical macula, which appears as a granule-free ring, in section, after fixatives without osmium tetroxide, and as a black ring with a clear center with the reduced osmium. Maculae are rarely seen in types 4, 5, and 7 and have not been observed in types 6, 8, and 9.

The term "colloid" has been applied to the material which stains like the colloid in the thyroid glands having high columnar epithelium. It is most abundant in the tuberal part but is present in the glandular and intermediate lobes. The plasma in blood vessels and the exudate in the hypophyseal cleft appear more granular and less intensely stained than the colloid. The function of the colloid, if any, is unknown.

Mitotic figures have not been observed in the pitutiary. The time from slaughter to fixation may have been too long for cells dividing at the time of slaughter to be recognizable, but the process of mitosis requires less time than is generally believed if this is the explanation.

Variations in pituitaries from different ewes.—The pituitaries from different ewes are similar in general structure and arrangement; they contain the same types of cells; but certain variations do occur. In some animals cells of type 2 are most numerous; in others, cells of type 3; often cells intermediate between the two types occur. The size and degree of vacuolation in cells of type 4 also vary in different pituitaries. The relative numbers and kinds of these cells are shown in Table 4.

The normal estrual cycle, which Terrill (1935) has shown to be of 14 to 19 days duration with an average length of 16.6 days, has been divided into five periods: (1) estrus, during which the ewe will permit copulation, (2) early lutein phase, during which the corpus luteum is developing, (3) mid-lutein phase, during which a mature and functional corpus luteum is present, (4) late lutein phase, during which the corpus luteum shows the first traces of retrogression, and (5) proestrum, during which the follicular phase is resumed.

TABLE 4.—VARIATION IN CELLS OF TYPES 2, 3 AND 4 IN THE PITUITARIES OF ANIMALS WITH KNOWN REPRODUCTION HISTORIES.

Ewe	Stage (days)[1]	Type 2[2]	Type 3[2]	Type 4
1251a	0.0 estrus	++++	++++	large, few vacuoles
7b	0.5 estrus	++++	++++	large, few vacuoles
2a	0.7 estrus	++++	++++	large and medium, few vacuoles
45a	0.9 estrus	+++	+++++	large and medium, some vacuoles
22a	1.8 early lutein	+++	+++++	large and medium, some vacuoles
7a	2.0 early lutein	+++	+++++	large, medium, and small
20b	3.3	++++	++++	large, medium, and small
25a	3.8 early lutein	++++	+++	small, medium, and large
22b	5.3	++++++	++	medium and small
53a	5.3 late lutein	+++++	+++	large and medium
14b	7.1	++++++	++	medium and small
23a	7.4 late lutein	++++	+++	large and medium
16a	7.6 mid-lutein	+++++	++	medium and small
24b	7.9	++++++	++	medium and small
44a	7.9	+++++	+++	medium and small
28a	9.1 late lutein	++++	+++	medium and large
3b	9.9 mid-lutein	+++++	++	medium
38a	11.0 lutein	+++++	++	medium
12b	11.9 mid-lutein	++++++	++	medium
21b	12.3 late lutein	+++++	+++	medium
35a	14.0 pro-estrum	++++	+++	large
10b	14.3 late lutein	+++++	+++	medium and large
8c	15.0 pro-estrum	++++	++++	large
34a	castrate	+++	+++++ very large and vacuolated	medium and large
17a	40.0 anestrum	+++++	++	small and medium
46a	52.0 anestrum	+++ small	+++++ small	small, medium, and large
26a	111.0 anestrum	+++ very small	+++++ very small	small, medium, and large
50a	1.8 pregnant	+++	++++	large vacuoles
47a	2.3 pregnant	++++	++++	large vacuoles
6a	4.8 pregnant	+++	+++++	medium and small
6b	7.3 pregnant	+++++	+++	small and medium
5b	9.3 pregnant	++++++	++	medium and small
43a	11.3 pregnant	+++++	+++	small and medium
17b	12.1 pregnant	+++++	+++	small and medium
31a	13.8 pregnant	++++	++++	medium and large
16b	14.0 pregnant	++++	++++	medium and large
25b	14.2 pregnant	++++	++++	small, medium, and large
23b	18.9 pregnant	+++++	+++	small with degran. area
21a	20.0 pregnant	++++++	++	large with vacuoles
15b	20.6 pregnant	++++++	++	medium
13a	21.6 pregnant	+++++	+++	large with vacuoles
51a	24.9 pregnant	++++++	++	small and medium
9b	27.3 pregnant	++++++	++	small but dark
2b	29.9 pregnant	++++	++++	large
8a	30.8 pregnant	++++	++++	large
19b	32.8 pregnant	++++	++++	large
4b	35.0 pregnant	+++++	+++	medium
48a	36.3 pregnant	+++	++++++	large
26b	45.7 pregnant	+++++	+++	small to large
30a	48.2 pregnant	+++++	+++	small and medium
19a	60.0 pregnant	++++	++++	medium and large
52a	72.9 pregnant	+++++++	++	medium and large
41a	85.1 pregnant	++++++++	+	medium
37a	97.1 pregnant	+++++++	++	small
9a	108.3 pregnant	+++++	+++	small to large
1161a	120.5 pregnant	+++++	+++	small
20a	133.1 pregnant	+++	++++++	small and irregular
1a	136.3 pregnant	++++	++++	small to large
33a	141.0 pregnant	++	++++++	small
24a	148.7 pregnant		+++++++++	small

1 The interval since the beginning of the last estrus, and the stage of the cycle as estimated from the study of the corpus luteum (Warbritton, 1934) or from recovery of the fetus.

2 Each "+" represents approximately ten per cent of the total number of cells in the peripheral zone of the pituitary. Partially degranulated cells are recorded with type 3.

Certain changes occur in the pituitary during the estrual cycle. At the beginning of estrus, cells of types 2 and 3 are about equally numerous; and cells of type 4 are large and well filled with granules (Fig. 15). By the end of estrus, the cells of type 3 predominate; and vacuoles are rather numerous in large cells of type 4, and small cells are plentiful. In the early lutein phase, the cells of type 2 and type 3 are about equally numerous (Fig. 16). The cells of type 4 are smaller or more vacuolated. During the mid-lutein phase cells of type 2 are quite numerous and those of type 3, relatively scarce. The cells of type 4 are smallest before the middle of the phase, then appear to increase in granulation (Fig. 17). In the late lutein phase and in proestrum, cells of types 2 and 3 again are approximately equal in number and those of type 4 are large and well filled with granules. In an animal in which a corpus luteum shows premature retrogressive changes, the pituitary resembles that of an animal in the late lutein or proestrual condition as shown by ewes 53, 23, and 28 (Table 4).

During pregnancy, with a duration of 140 to 150 days, the pituitary shows numerous fluctuations in the cells of types 2, 3, and 4 which are hard to describe in general terms. The formative period for the corpus luteum is characterized by an increase in the number of cells of type 2 until the quantity of these cells equals that of type 3 and by a decrease in the size of cells of type 4. Cells of type 2 are much more numerous than those of type 3 by the ninth day, but both types are about equal in quantity at the fourteenth day. Cells of type 4 are smaller at each successive stage until the ninth day and progressively larger until the fourteenth. This cycle of events is repeated at intervals of about fifteen to eighteen days until the sixtieth day (Fig. 18). The stages taken between the seventy-third day and the one hundred eighth day show cells of type 2 predominating, a few cells of type 3, and small or medium sized cells of type 4 (Fig. 19). A gradual but somewhat fluctuating decrease in the number of cells of type 2 occurs until at parturition they are almost absent (Fig. 20). Cells of type 3 show a simultaneous increase (Fig. 21). The cells of type 4 are rather small and in some animals are quite irregular in shape during the latter third of pregnancy. No cell peculiar to pregnancy was observed, and no phase of an ordinary cell could be considered characteristic of more than a short period of pregnancy.

Of the three ewes slaughtered for anestrual stages, the pituitary of one, ewe 17a, shows all the characteristics of a mid-lutein phase in the estrual cycle. The other two have very small cells of types 2 and 3 with many of them in the form of type 3; the cells of type 4 are variable in size, some small and some large.

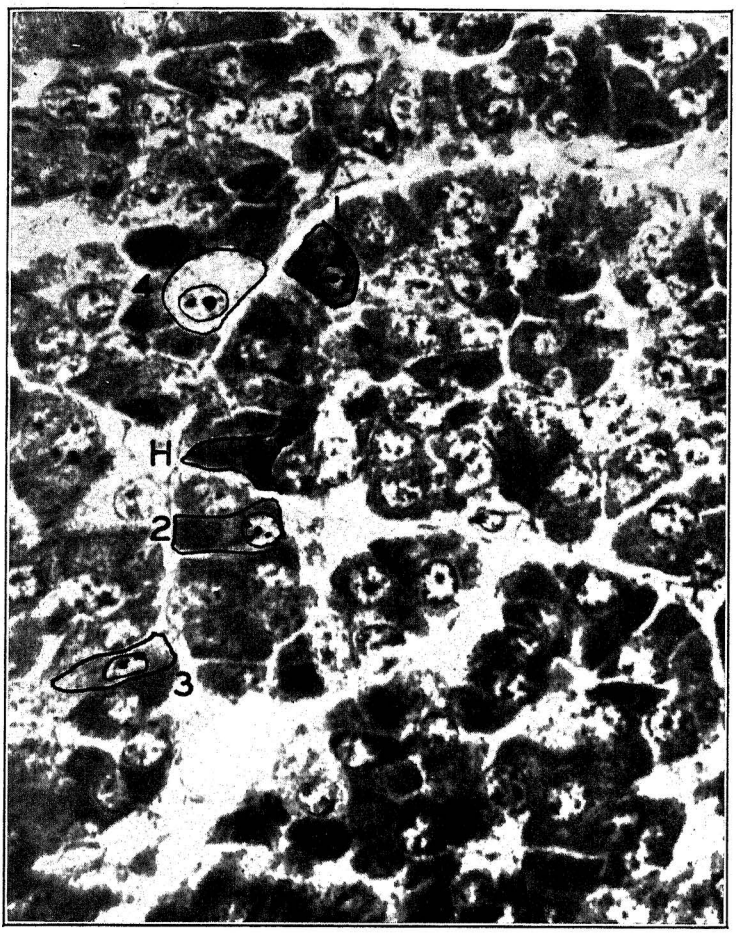

Figure 15.—The glandular lobe of the pituitary during early estrus to show large cells of type 4 and the relatively lightly granulated cells of type 2. Cells of types 1, 2, 3 and 4 and *H*, histiocytes are circled. The cell labeled type 1 is the only one of this kind in the field. Histiocytes are relatively numerous. Ewe 1251a, 1 hour after the beginning of estrus, Bouin-Mallory (strong), green filter. X 1000.

The pituitary of the castrate ewe has very few normal cells of types 2 and 3. Most of the cells in the region where such cells would usually occur are quite large and vacuolated. The cytoplasm resembles that of type 3 more than that of type 2. There is more stainable material in the cytosomes of these cells than ordinarily occurs in cells of type 7, and some granules of the kind characteristic of type 2 are found. The cells of type 4 are relatively large.

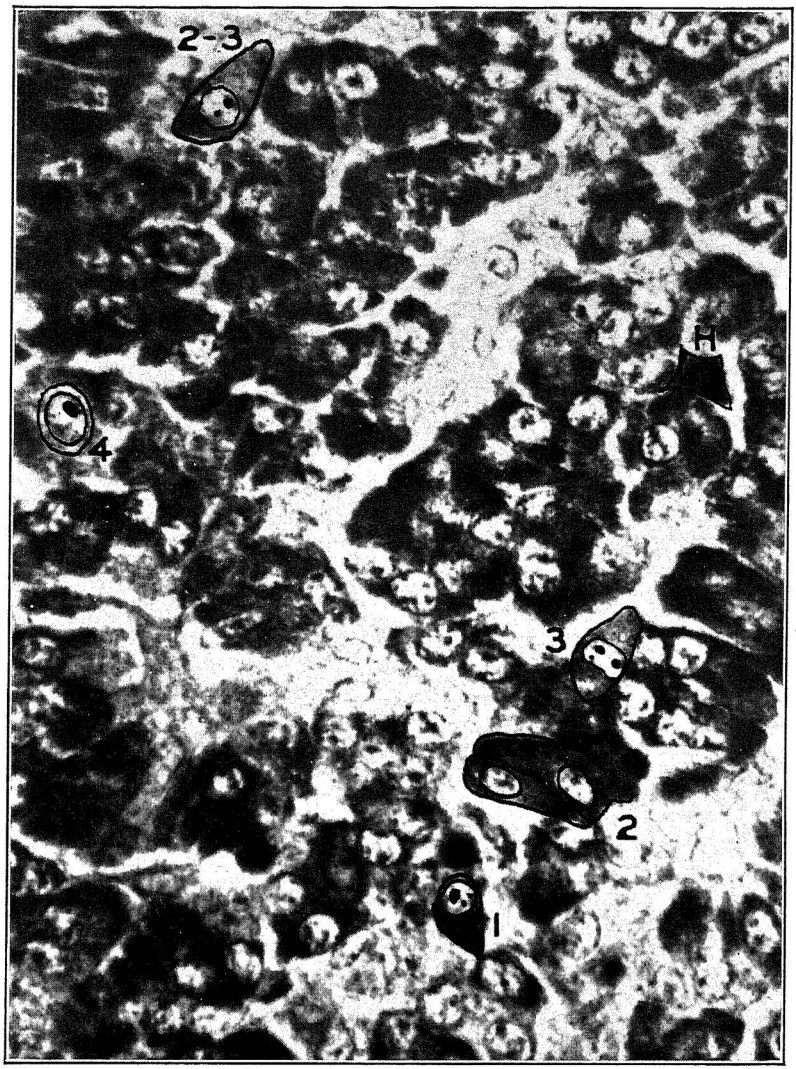

Figure 16.—The glandular lobe of the pituitary during the early-lutein phase of the cycle to show moderate granulation of cells of type 2, and small cells of type 4. Most of the dark cells are type 2 and most of the light cells are vascular elements. *H*, histiocyte; *2-3*, a cell intermediate between types 2 and 3. Ewe 22a, 1.8 days after the beginning of estrus, Bouin-Mallory (strong), green filter. X 1000.

Seriation of the pituitaries of these ewes upon the basis of age, breeding, season, and finish has shown no conspicuous variations asso-

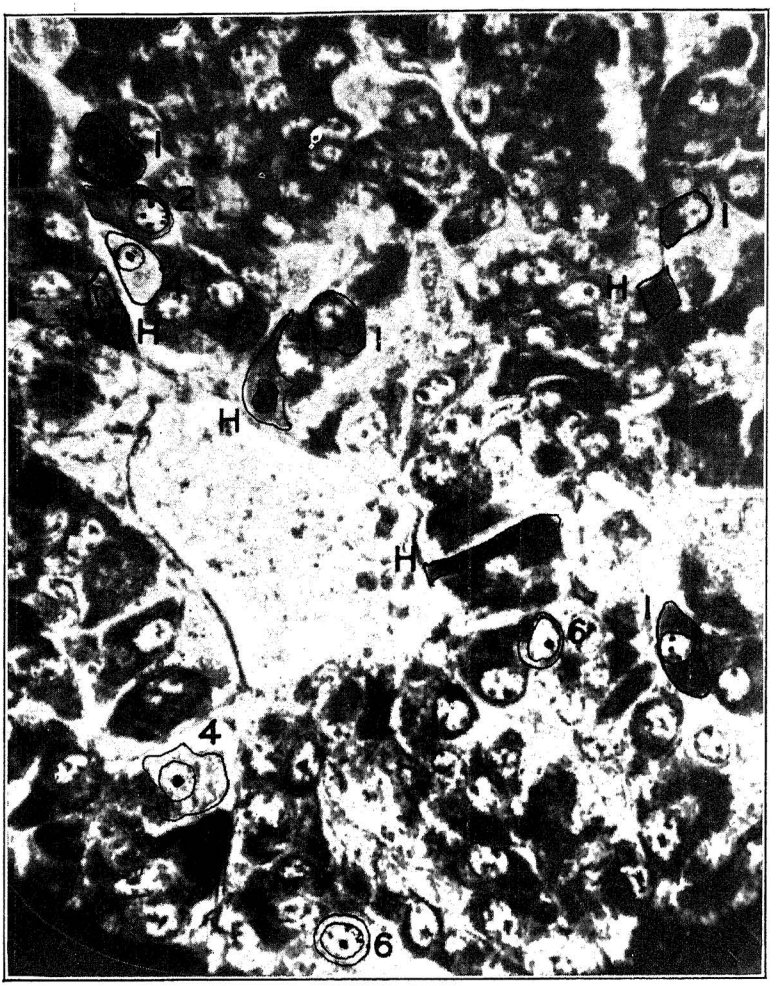

Figure 17.—The glandular lobe during the mid-lutein phase when cells of type 4 are relatively large and cells of type 2 very numerous. Cells near top of figure labeled *2*, and *4* and cell labeled *1* near bottom have maculae. The irregular histiocyte, *H*, has blue cytoplasm, the regular one has red hyaline cytoplasm and nucleus. The cell of type 6 is partly covered by a cell of type 2. Most of the dark cells are cells of type 2, but several histiocytes lie along the blood vessels. Erythrocytes are stained red, blue, or orange. Ewe 16a, 7.6 days after the beginning of estrus, Bouin-Mallory (strong), green filter. X 1000.

ciated with any of these factors. The range of variation, however, has been kept as small as was conveniently possible. Other experiments should be designed to demonstrate the effects of wide variation in these factors upon the pituitary of the ewe.

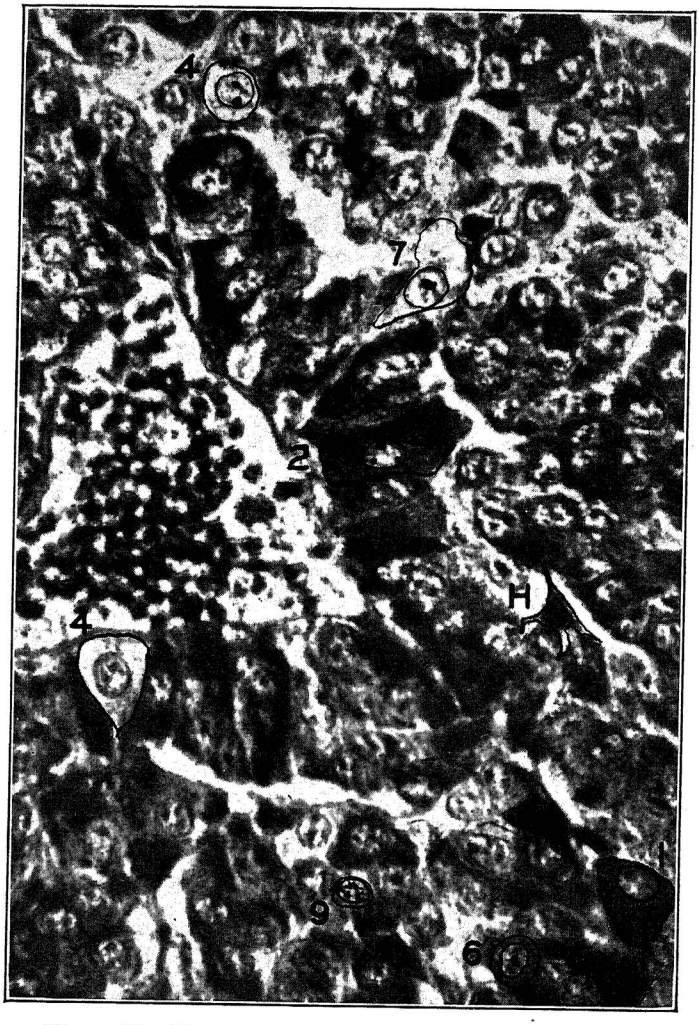

Figure 18.—The glandular lobe during early pregnancy. Cells of types 1, 2, 4, 6, 7, and 9, and histiocytes, *H*, and vascular elements are shown. The erythrocytes are stained bright red or bright blue. Almost all the dark cells are of type 2. Ewe 13a, 22 days after the beginning of the last estrus, Bouin-Mallory (strong), green filter. X 1000.

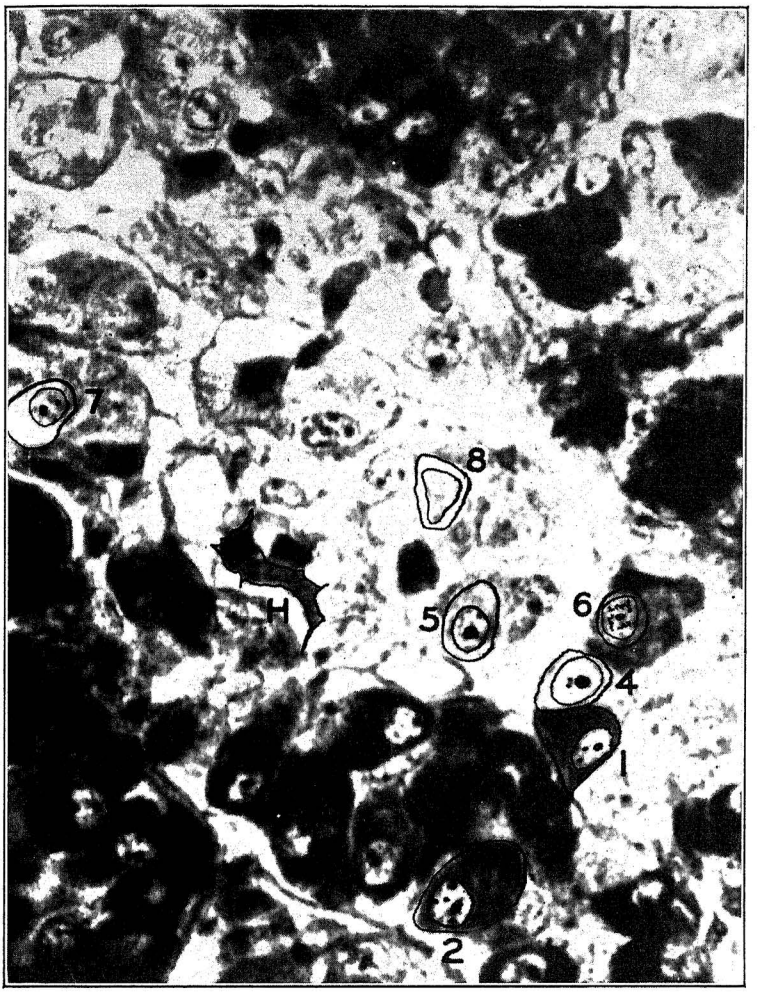

Figure 19.—The glandular lobe very near the tuberal part at mid-pregnancy. Most of the dark cells are type 2 and most of the light ones are types 5 or 7 or vascular elements. The cells of the granular types are larger in the core than at the periphery. Ewe 52a, 73 days after the beginning of the last estrus, Bouin-Mallory (strong), green filter. X 1000.

Figure 20.—The glandular lobe in late pregnancy. The gray and light cells are chiefly type 3 or cells intermediate between 2 and 3 (2-3). The dark cells are type 1 or histiocytes. Ewe 20a, 133 days after the beginning of the last estrus, Bouin-Mallory (strong), green filter. X 1000.

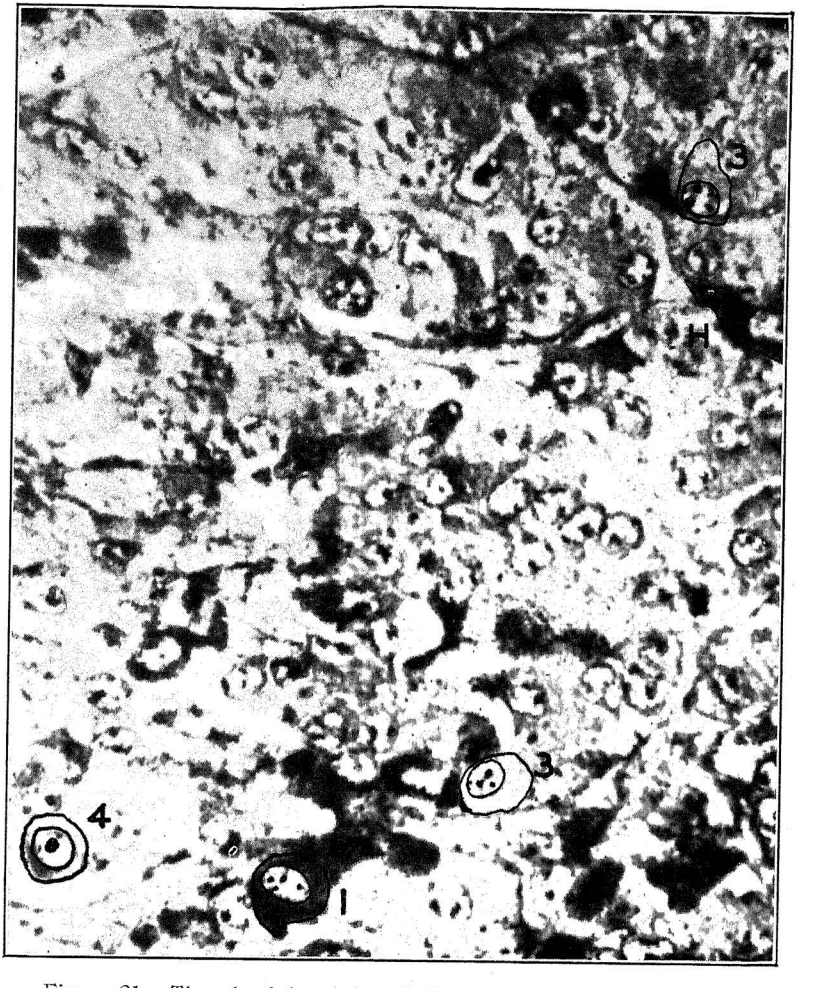

Figure 21.—The glandular lobe of the pituitary just after parturition. The dark cells are type 1. The only cell of type 4 is ringed and the cells of type 3 and elements of the vascular system constitute the rest of the picture. Ewe 24a, a few hours after parturition, Bouin-Mallory (strong), green filter. X 1000.

The Intermediate Lobe.—The intermediate lobe of the pituitary varies greatly in size, shape, and arrangement in ewes. The posterior medial portion of the intermediate lobe is separated from the glandular lobe by a conspicuous cleft which is represented in the middle of the gland by more or less cyst-like continuations. Toward the anterior end of the gland, the intermediate lobe forms a wedge between glandular-tuberal and neural lobes. In two animals, hyaline material staining

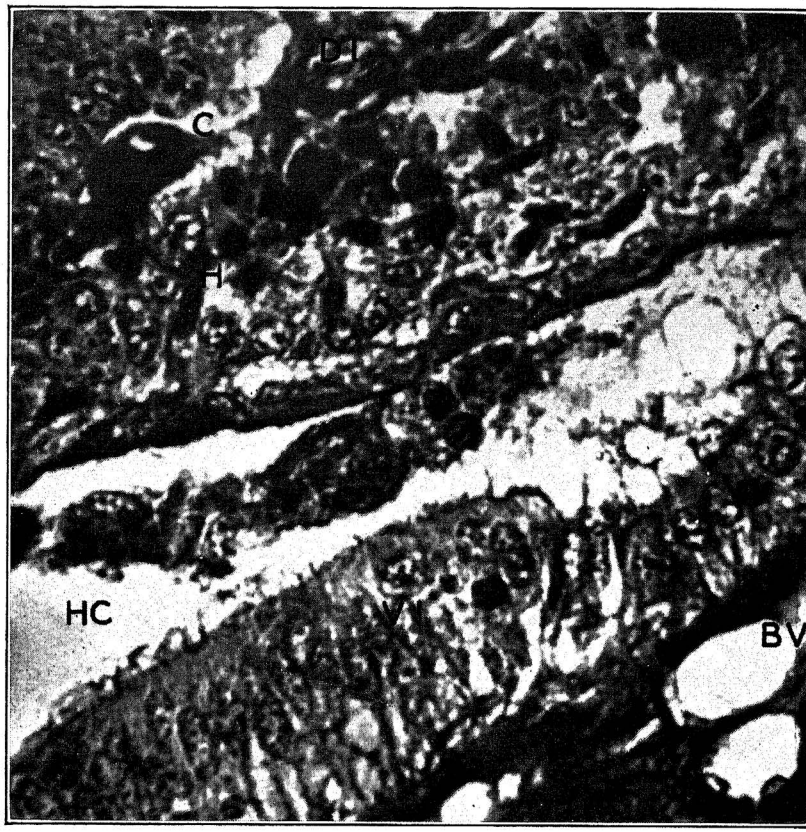

Figure 22.—The intermediate lobe of the pituitary body. Clumps of intermedial cells, histiocytes, *H*, colloid, *C*, and blood vessels constitute the dorsal part of the intermediate lobe, *DI*. A clump of cells lies in the hypophyseal cleft, *HC*. Ciliated columnar cells constitute the ventral border of the intermediate lobe, *VI*. The blood vessel, *BV*, lies in the connective tissue band between intermediate and glandular lobes. Ewe 52a, Bouin-Mallory (strong), green filter. X 1000.

like blood plasma rather than colloid was found in the cleft. The dorsal wall of the cleft is lined with somewhat flattened cells; the ventral wall, with flattened or columnar cells, some of which are ciliated and a few of which show drops resembling mucus (Figs. 22 and 23). Thick-walled blood vessels are relatively numerous, and capillary networks occur frequently. The epithelial cells of the intermediate lobe are arranged in clumps between blood vessls or, occasionally, in acini surrounding colloid. These cells are very small with fuchsinophilic or prominent nuclei packed with nucleoli. The cytoplasm is faintly granu-

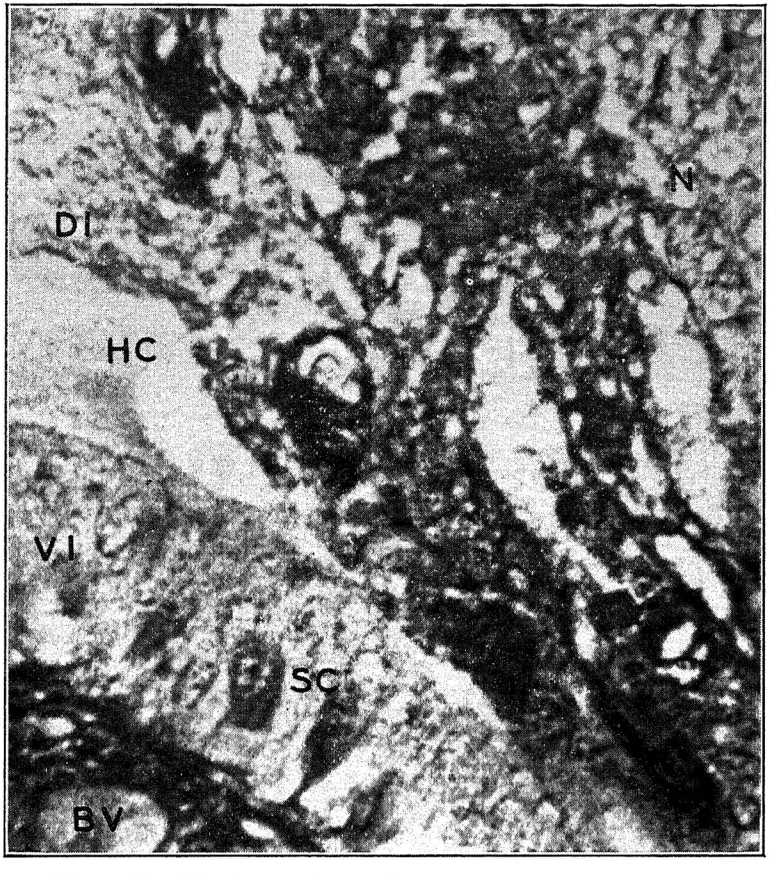

Figure 23.—The intermediate and neural lobes of the pituitary body. The dorsal intermediate lobe, *DI*, is very small and consists chiefly of connective tissue and blood vessels, dark colored areas. The ventral border, *VI*, in this region is of tall non-ciliated columnar cells with a few of a secretion type, *SC*, with cytoplasm like that of cells of type 5. The junction with the neural lobe, *N*, is shown. The hypophyseal cleft, *HC*, contains exudate. The blood vessel, *BV*, lies in the connective tissue band between the intermediate and glandular lobes. Ewe 52a, Bouin-Mallory, (strong), yellow filter. X 1100.

lar and is usually not stained intensely with any dye. Membranes cannot be observed between many pairs of nuclei; the effect is that of a syncytium. Specific granules and fat spheres were not observed in cells in any of the pituitaries from these ewes. Chondriosomes are not present in the intermediate lobe after fixation in Bouin's fluid, even when they are preserved in the cells of the glandular lobe. Histiocytes may be numerous.

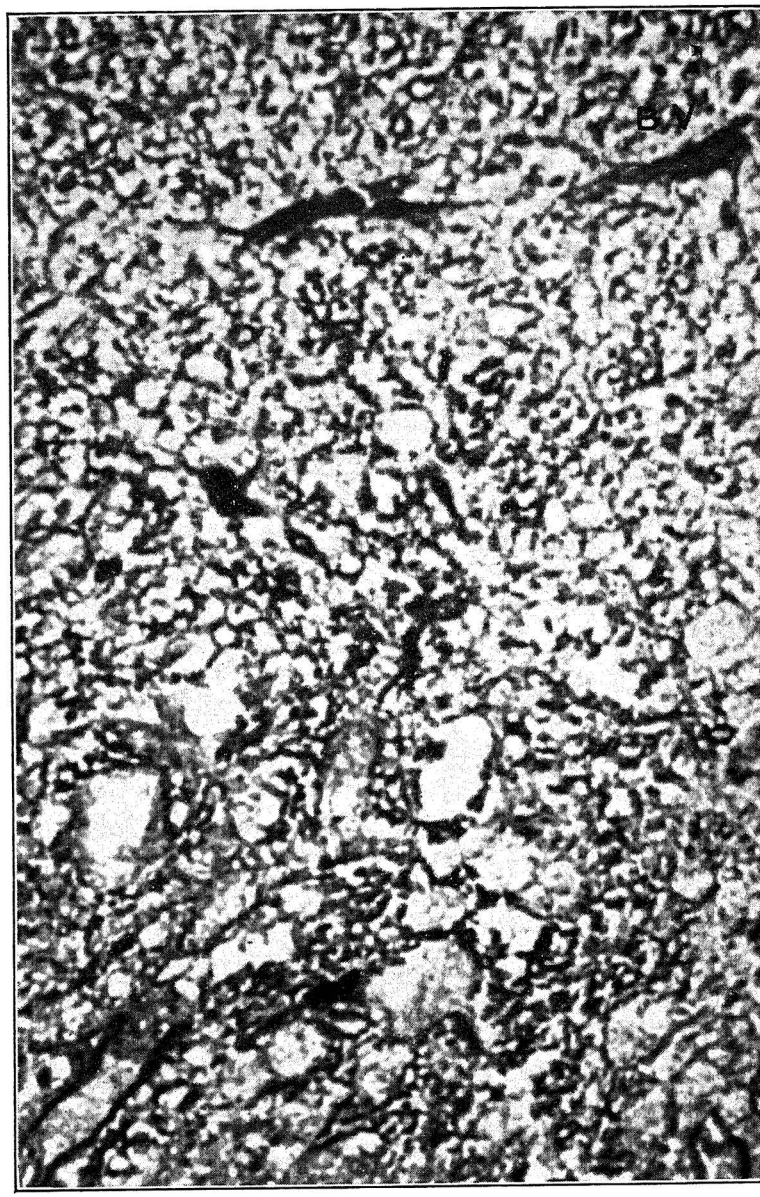

Figure 24.—The posterior lobe of the pituitary body. Blood vessels, *BV*, neuroglial fibers, and interfiber spaces are shown. Nuclei of a few cells are present in the field but are not shown with this filter. Ewe, 52a, Bouin-Mallory (strong), yellow filter. X 1000.

The Neural Lobe.—The neural lobe consists almost entirely of neuroglial fibers with several cell bodies visible. These form a loose network with many spaces among the fibrils (Figs. 23 and 24). Small cells, like those in the intermediate and tuberal lobes, sometimes in clusters, are found near the junctions with these two lobes (Fig. 25).

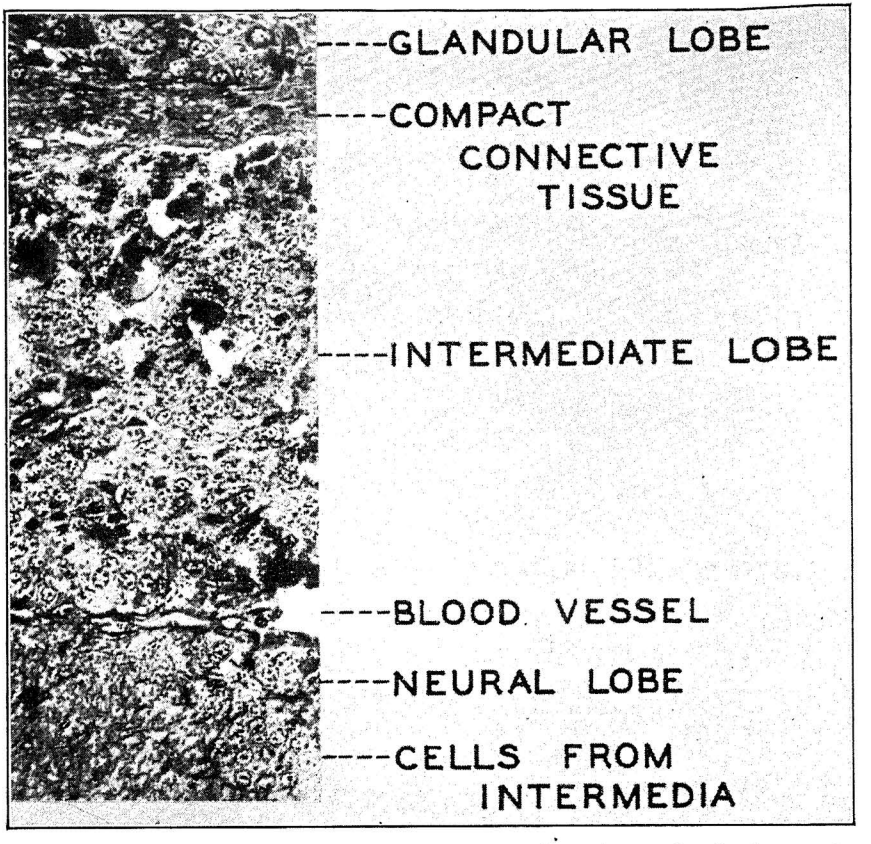

Figure 25.—The three lobes of the pituitary. The clump of cells from the intermediate lobe almost surrounded by the neuroglial cells and fibers represents the only epithelial type of cell in the neural lobe. The blood vessels of the intermediate lobe are rather thin walled. Histiocytes (cells with dark nuclei) are numerous. Ewe 24a, Bouin-Mallory (strong), green filter. X 320.

A little colloid with staining properties somewhat like that of the other lobes is preserved in the posterior lobe of one animal. Only thick-walled blood vessels have been observed in the neural lobe (Fig. 24).

The Tuberal Part.—The tuberal extension of the pituitary body is difficult to delimit. It merges almost completely with the intermediate lobe. The core of the glandular lobe is very similar to the

tuberal region. The cells are chiefly of the types 4, 5, 7, and 8 but all the granular types of cells found in the glandular lobe are present occasionally. Colloid follicles are more numerous in the tuberal region than they are in the glandular or intermediate lobe. These follicles are lined with cells of type 8 which are not ciliated. The tuberal part almost surrounds the stalk of the neural lobe at the anterior end, and the tuberal cells invade it much more than do those of the intermediate lobe in some animals.

DISCUSSION

Pituitary bodies of sheep are similar in general structure to those of other species of mammals. The anterior lobe in the ewe is relatively larger in median section than those shown in the diagrams of Cowdry (1928 and 1934) and Maximow (1930), and it occupies a ventral rather than an anterior position. The differentiation of the glandular lobe into a central core and a peripheral zone has been mentioned in very few other species. The proportions of cells in these two regions differ decidedly and the core resembles the tuberal part more than it does the peripheral zone. The intermediate lobe and the remnants of the hypophyseal cleft are as variable in the ewe as in other species. The cleft separates the intermediate from the glandular lobe in many regions, but no sharp boundary occurs between intermediate, posterior, and tuberal lobes. The tuberal lobe resembles the central core of the glandular lobe and contains several granular cells. Cells with secretion granules like those Maurer and Lewis (1922) described in the pars intermedia of swine have not been observed in the intermediate lobe of the pituitary of the ewe. The posterior lobe is relatively small and contains very few of the colloid spheres usually described in this region. Epitheloid cells are lacking, except at points of invasion from the intermediate and tuberal regions.

The tuberal part of the pituitary must be regarded as chiefly accessory to the glandular lobe until someone finds a function for pituitary colloid.

The evidence for active secretion from the intermediate lobe of the ewe is scant. The absence of specific granulations and the small amount of cytoplasmic material suggest that if secretion has ever occurred, it is not continuing in ewes during their period of breeding activity. Melanophores, or pigment cells of some type, are numerous in certain breeds of sheep; but no one has suggested that intermedin may be related to the control of pigmentation in mammals. Intermedin is more like the hormones of the posterior than those of the anterior lobe and may have a source similar to the neural hormones. Maurer and Lewis (1922) believed that secretion granules were usually

absent from the intermediate region because they were lost during the time elapsing between slaughter and fixation. If that is the explanation in these pituitaries, others collected more recently and fixed within ten to twelve minutes of the time the animals were slaughtered should contain granules. The presence of the vesicular and nucleolated, or distinctly fuchsinophilic, nuclei discredits the idea that the cells of the intermediate lobe are truly embryonic.

The neural lobe of the ewe contains no epitheloid cells except those invading it from other lobes, no cells of the type ordinarily considered secretory. However, since nerve fibers, at least those of the sympathetic and parasympathetic systems, seem to achieve their effects through the release of chemical compounds, it is perhaps not fantastic but only logical to assume that cephalic fibers produce similar substances in the neural lobe of the pituitary. The resemblances of the physiological effects of pitocin and pitressin from the pituitary and the stimulation of the sympathetic and parasympathetic nervous systems are worth mentioning. Many substances that would not be preserved for staining by ordinary histological techniques could be stored in the interstices of the fibers and the neuroglia.

The cells of the glandular lobe of the pituitary body have usually been described as of three types, acidophilic, basophilic, and chromophobic. However, Trautman (1909) in sheep and other domestic animals and Howes (1930) in the ox have listed five types, subdividing the two chromophilic types into weak and strong. Cleveland and Wolfe (1932) described four principal types of cells with several variations in their size, granulation, and staining capacity but did not stress the relation to acidophils and basophils. Sawyer (1936) described five morphologically distinct types of cells in the bat pituitary but named them from her interpretation of their function. It has been difficult to decide how the nine types of cells recognized in the pituitaries of these ewes would be classified by other investigators upon the basis of their favorite techniques, but an attempt to do this has been made in Table 5. The conspicuous differences in the classification may be due to our misinterpretations of the various authors' criteria for distinguishing cell types, but they suggest that the custom of separating pituitary cells into acidophils, basophils, and chromophobes may have been difficult for previous investigators to follow. The problem becomes doubly difficult when at least three-fourths of the staining combinations do not contain basic dyes, or when basic dyes stain the supposedly acidophilic cells, or when the same dye stains a different group of cells after a change in the method of fixation. A numeral or lettering method of designating cells is not altogether satisfactory but seems more desirable than the use of inappropriate descriptive terms.

TABLE 5.—CLASSIFICATION OF CELLS INTO CONVENTIONAL TYPES UPON THE BASIS OF THE STAINING REACTIONS DESCRIBED BY VARIOUS AUTHORS.

Author or Method	Chromophil		Chromophobe
	Acidophil	Basophil	
Nelson[1]	1, 2, 3	4, 5, 6	7, 8, 9
Severinghaus	1	2, 3, 4, 5, 6	7, 8, 9
Cleveland & Wolfe	2, 3	1, 4, 5, 6, 7	8, 9
Martins	1, 2, 3, 4, 5, 6		7, 8, 9
Kindell	1, 2, 3	4, 5, 6	7, 8, 9
Howes	1 strong	3 weak, 2 strong	4, 5, 6, 7, 8, 9
Maurer & Lewis[2]	2, 3	1, 4, 5, 6	7, 8, 9
Wright stain	1, 2, 3, 4, 6		5, 7, 8, 9
Kull stain	1, 2, 4, 5, 6		3, 7, 8, 9
Heidenhain's[3] hematoxylin-eosin	4, 5, 6 1, 2, 3, 4, 5, 6	1, 2, 3	7, 8, 9 7, 8, 9

1 Personal communication after observation on some of the sections of the pituitaries of these ewes.
2 Maurer and Lewis describe the acidophilic cells as showing an affinity for the basic dye safranin in the acid violet-safranin neutral dye solution.
3 The first row treats hematoxylin as a basic dye; the second as an acid dye.

Just how great the differences in the cells of the pituitaries of the sheep and other animals may be is hard to say. A few observations on pituitaries of rats, mice, and rabbits indicate that some of the types of cells found in sheep are not present in these species. The descriptions of the various cells included within one of the three usual groups, acidophils, basophils, and chromophobes, however, often suggest that some of the types found in the ewe have been lumped for the purpose of making cell counts. The cell counts, which would have been necessary to establish the degree of variability in the ewe, were not made because sufficiently thin sections, of entire or half glands, to satisfy the requirements of Rasmussen (1924) have not been available.[1] The regions of the pituitary are so conspicuously different that counts made at one level would not be valid for another or for the whole gland.

Although at least nine morphologically different types of cells are present in the pituitary, they do not necessarily represent distinct functional types. There is some evidence of interrelations between certain of the groups.

Some cells of type 1 are much like cells of type 9 in size and nuclear character but contain a few granules like those of the larger and more highly differentiated cells of type 1. This is interpreted as meaning

1 A preliminary study involving cell counts was demonstrated by Warbritton and McKenzie (1935). The 'a' type acidophils correspond to the cells of type 1, the 'b' type acidophils to types 2 and 3, and the basophils to types 4, 5, and 6.

that cells of type 1 have arisen from cells like type 9, and the possibility exists that they return to this form by the loss of granules.

Cells of types 2 and 3 differ from each other chiefly by the presence or absence of granules. The macula which appears more distinctly in the granular cells is not lacking in the non-granular forms. These cells occur in the same regions of the pituitary, and the sum of the two types remains approximately constant in spite of variation in the relative proportions of the two types. The non-granular cytoplasm of type 3 is smooth like that of types 5, 6, and 7 but its affinity for stains is somewhat different. A few small cells of type 2 approximate type 9 very closely and are considered as evidence of transition from this type.

Among the cells classified as type 4 are cells no larger than those of type 9, but they have small nucleoli and contain granular masses of protoplasm with the staining properties of type 4. These cells are considered transitional stages from type 9 to type 4. There are many indications that granules are lost or gained and that cells of type 4 may decrease or increase in size. No nuclear changes occur as long as any granules remain, and no non-granular cells of type 9 with large nucleoli have been observed. This argues against a return of the cells to type 9. Vacuolation of large cells of type 4 may cause them to look somewhat like cells of type 7, but cells of type 7 are rarely found in the regions where cells of type 4 are most abundant.

The cells of types 5, 6, and 7 have a characteristically smooth cytoplasm with an affinity for acid dyes like acid violet, aniline blue, and eosin. Cells intermediate in appearance between these types have been observed in most pituitaries. The size and chromatin arrangement of the cells of type 6 suggest that they may have been derived from type 9. By a growth process during which a dilution of the cytoplasm occurred type 6 could develop into type 5. If the vacuolation present in most cells of types 5 increased, cells of type 7 would result. Since there is no indication that cells of type 7 degenerate, it may be assumed that the material stored in the vacuoles is eliminated and that the cells return to a form resembling type 6.

The origin, previous history, and fate of cells of type 8 are unknown. Their position in the center of an alveolus surrounding the colloid suggests that they have been concerned with its production; but the cells contain no elements resembling colloid; both nucleus and cytoplasm seem completely passive; and no indication of the transformation of the cells of types 1, 2, or 3, which surround the cells of type 8, into such a cell as that of type 8 is seen. After fixation according to the method of Champy, the cells of type 1 resemble the colloid in staining capacity and might be considered to transform directly into colloid, or to secrete it and leave behind the cells of type 8. This would solve the problem; but the observations on material

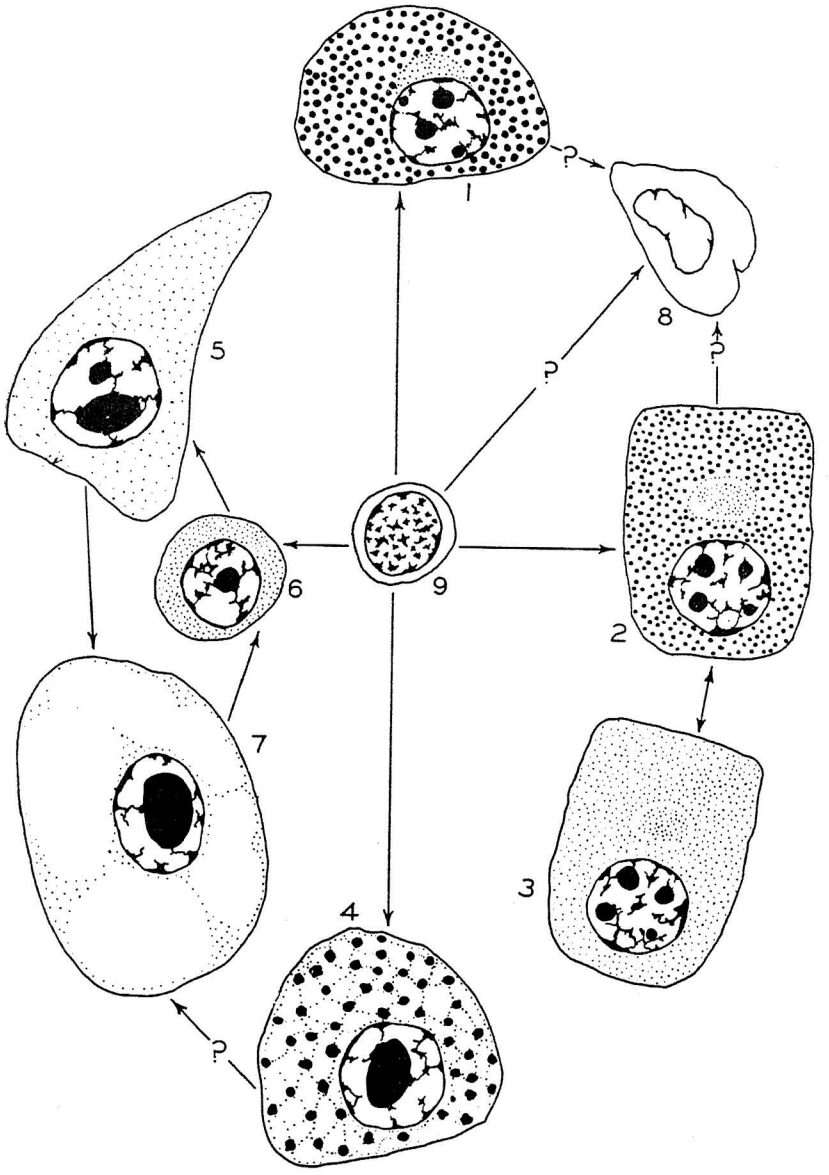

Figure 26.—A diagram of the relations of cell types found in the pituitary body of the ewe. The arrows indicate the directions in which differentiation is believed to take place.

fixed by other techniques lends no support to the view. With no other fixative does the colloid take the same stains as the cells of type 1. Cells of types 2, or 3, often form a ring about the cells of type 8. Cells of type 1 occasionally occur in these rings. It is, at least, equally probable that a different type of cells may have arisen from type 9, produced the colloid, and assumed the exhausted appearance of type 8.

Cells of type 9 are regarded as undifferentiated cells like those from which all other pituitary cells may have been derived and capable of giving rise to any of the other types.

A summary of the interrelations of the types of cells found in the pituitary of ewes is shown in Fig. 26. The nine morphologically different cells seem to belong to six functionally different groups as follows: (1) type 1, (2) types 2 and 3, (3) type 4, (4) types 5, 6, and 7, (5) type 8, and (6) type 9. This grouping could be explained more or less plausibly by assuming that growth and differentiation along four or perhaps five lines from a single type of embryonic cell occurred before the stage of sexual maturity. A few of these embryonic cells persist as type 9 but differentiate infrequently in the mature ewe. The cells of type 8 become inactive after they have produced the colloid but do not degenerate. The cells of type 2 degranulate to form cells of type 3, and the cells of type 3 secrete material and store it as granules to form cells of type 2. The cells of type 1 and type 4 alternately release secretions and store them in the form of granules. The cells of types 6, 5, and 7 pass through a cycle in which a secretion of some type is stored in vesicles or vacuoles and discharged from them. Cellular degeneration is rare in the pituitary of the ewe and mitosis has not been observed; hence the supply of cells must remain relatively constant during the period of sexual maturity. Pregnancy and the anestrual season affect only the rate at which cells store and release substances. "Pregnancy" cells do not occur. Castration seems to disturb the type of storage in cells of types 2 and 3, and to result in the formation of cells resembling signet ring "castration" cells.

The presence of six fundamentally different cell types in the anterior pituitary of the ewe, in contrast to the one, two, three, four, or five types described in other species, opens rather than solves the secretion problems attributed to the gland. The hormones of the anterior lobe fall into two general classes, those concerned with metabolism and those concerned with reproduction. The metabolic factors suggested include the growth hormone, thyrotropic, parathyrotropic, ketogenic, pancreatotropic, and adrenotropic principles, and perhaps a factor regulating water balance. The proposed reproduction hormones include follicle-stimulating, luteinizing, and lactogenic principles. Metabolic conditions in these ewes were not altered intentionally, and cells producing hormones affecting metabolism should not vary marked-

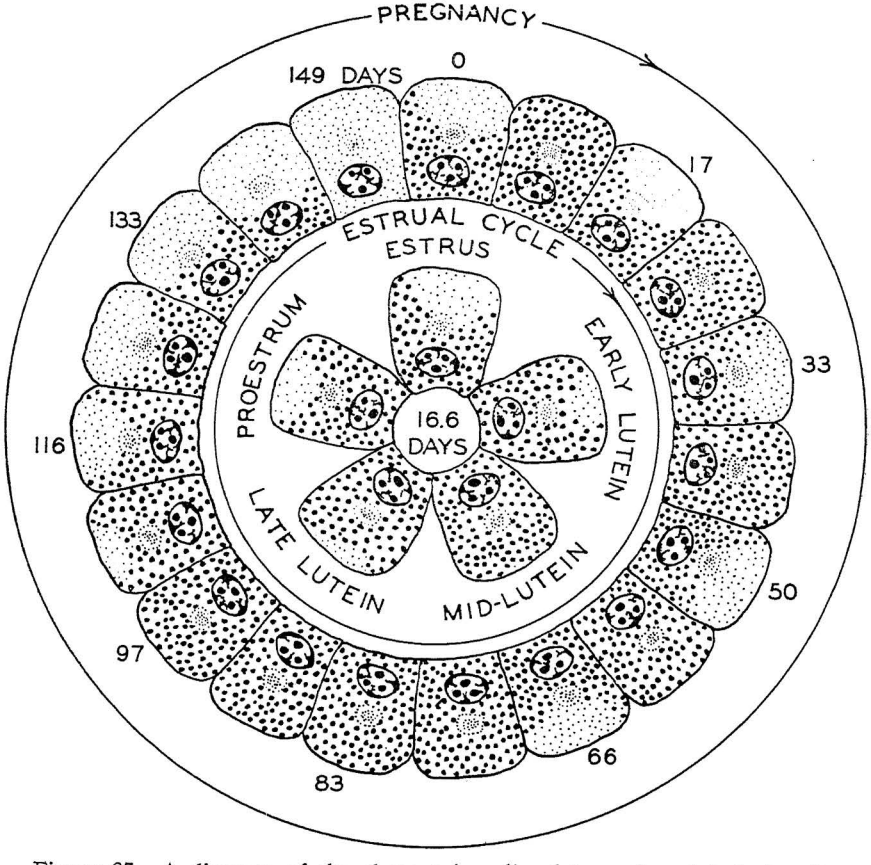

Figure 27.—A diagram of the changes in cells of types 2 and 3 during the estrual cycle, and pregnancy. The cells are drawn to represent both the number of granulated cells and the degree of granulation of cells rather than the appearance of the majority of cells at the given period. The inner circle shows the changes during an estrual cycle averaging 16.6 days in duration; the outer circle shows the changes during pregnancy.

ly unless there was some correlation with the process of reproduction. Reproductive phases were deliberately varied.

In the interpretation of the activity of cells from fixed material, there are two schools of thought. One assumes that a cell is producing and excreting large quantities of a product when the cell itself is filled with specific granules; the cell and the circulating medium contain maximum quantities of the product at the same time. The other assumes that the actively excreting cell quickly depletes its stored granules and either excretes its secretion as rapidly as it is produced or ceases excretion and begins storage; if the cell is filled with the product, the circulating medium will be low; and if the circulating medium contains a high concentration of the substance, the cell will contain little unless the rate of production is high enough to provide

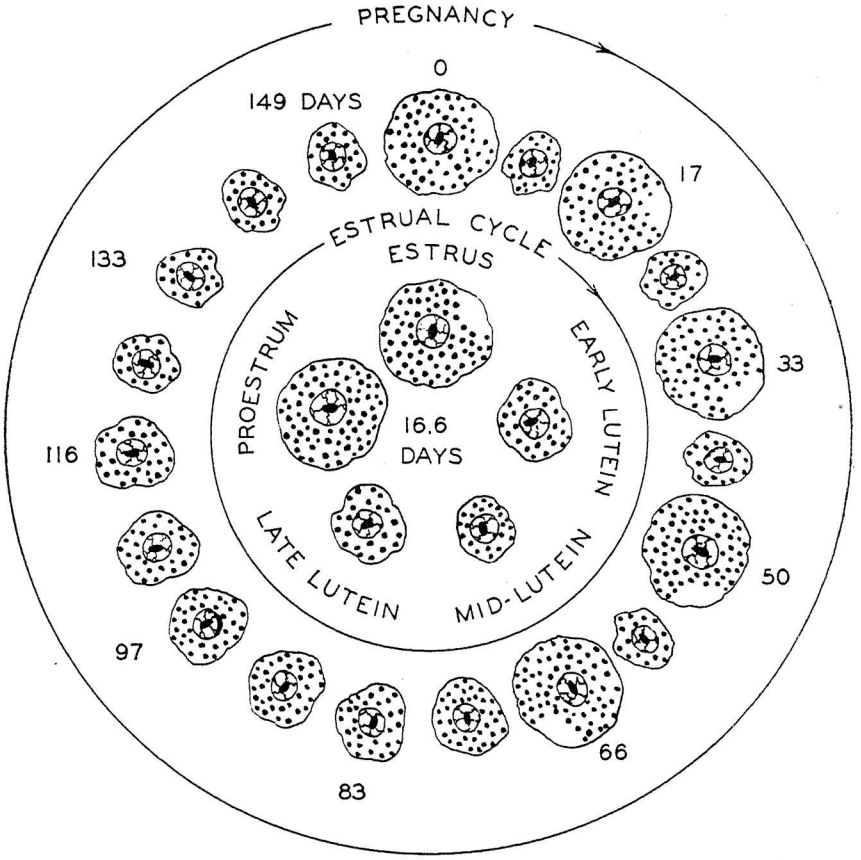

Figure 28.—A diagram of the changes in cells of type 4 during the estrual cycle and pregnancy. The cells are drawn to represent the average size and degree of vesiculation at a particular period. The inner circle represents the changes during the estrual cycle; the outer circle, the changes during pregnancy.

for both storage in and elimination from the cell. The second concept of cellular activity seems more in keeping with modern chemical and physical theories.

If the observations on the cellular variations in the pituitaries of the ewe are interpreted according to this concept, the change from type 2 to type 3 is associated with elimination of a stored product from the cell; decrease in the size of cells and preponderance of type 3 indicate a low storage and secretion level; the increase in the size of the cells with vacuolation and preponderance of type 3 may mean as increased storage of a somewhat different product. The reduction in size and in number of granules should indicate elimination of material from the cells of type 4. Reference to Table 4 shows how the cells of animals in different reproductive phases vary in these respects. The tendency seems to be for excretion to occur from cells of type 2-3

during the second half of the estrual cycle and throughout estrus, and to repeat the same cyclic changes at intervals of fifteen to eighteen days during the first half of pregnancy. (Fig. 27). Stored secretions are eliminated slowly during the latter third of pregnancy and rapidly at parturition. Storage takes place in cells of types 2 and 3 during the first half of the estrual cycle, the corresponding periods in the first half of pregnancy, from the sixtieth or seventieth day of pregnancy until the one hundred twentieth day, and after castration. During the anestrual season these cells appear inactive. The cells of type 4 have a storage phase during the second half of the estrual cycle, begin secretion during estrus, and continue to secrete during the early part of the cycle (Fig. 28). Their secretion continues cyclically through the first half of pregnancy. There is some indication of secretion during the latter half of pregnancy, particularly at parturition and during anestrum, but not after castration.

The period of excretory activity in the cells of types 2 and 3 coincides fairly well with changes in the ovary associated with the presence of follicle-stimulating hormones. The anestrual changes seem most easily explained by the absence of such secretion. The elimination of stored products from the cells of type 4 begins a few hours before the changes associated with the presence of the luteinizing hormone occur in the ovary. The low storage level in the cells of type 4 during the second half of pregnancy, and particularly the latter fourth, suggests that they may produce a mammotropic hormone, concerned with the development of the mammary gland. The complete degranulation of cells of type 2 at parturition makes them the more plausible source for the lactogenic hormone, which initiates milk secretion.

If the cells of type 2 store the follicle-stimulating hormone or its antecedent, the pituitary of the sheep is a relatively rich source of this substance. The glands from animals slaughtered in the mid-estrual interval and in mid-pregnancy should be most valuable. The luteinizing factor, if it is produced by cells of type 4, would be much less abundant than the follicle-stimulation factor in sheep; but compared with other species, the cells of type 4 are probably more numerous than the granular basophils which are often said to produce the gonadotropic substances. The time to slaughter for a high pituitary content of luteinizing factor would be just before, or in early, estrus.

The relatively high gonadotropic content of the pituitary of the ewe suggests that the ovarian response may be difficult to induce in this species if the amount of hormone in the blood is proportional to the pituitary content. The ratio of follicle-stimulating to luteinizing hormone in the blood must be very large, though decreasing, at the time of ovulation. Failures to induce estrus during the anestrual period by means of gonadotropic substance, as reported by Cole and

Miller (1933) and in agreement with observations in this laboratory, may be due to an inadequate dose of the follicle-stimulating hormone to produce a full estrogenic response before ovulation. The pituitary of the anestrual animal is not deficient in cells of type 4 and is probably capable of supplying the required luteinizing hormone whenever the ovary can respond to it. Most commercial preparations contain the complete gonadotropic fraction. The preparations from menopausal urine, relatively high in the follicle-stimulating hormones, should be more valuable in inducing estrus and ovulation in anestrual ewes than those from other sources.

The mechanisms of pituitary and gonadal interrelations have a fascination for most investigators. The assumption that the accumulation of the product of a cell in the blood reverses the reaction by which these substances are produced can account for many of the observations on ewes. The application of this mechanism has been limited to the phenomena in ewes showing an anestrual period because observations on ovaries and pituitaries of the same animals are available. The interpretation is as follows: The pituitary gland, probably in the cells of type 2, synthesizes and stores a follicle-stimulating hormone (FSH) as a precipitate in the form of granules; the small amount of hormone in solution in the cell is in equilibrium with the hormone in the blood:

FSH (granules) $\longleftrightarrow$ FSH (in solution) $\longleftrightarrow$ FSH (blood).

During the anestrual season the precipitated form in most cells is lacking; and the concentration in the blood is very low, for synthesis has stopped. Toward the end of the non-breeding period the cells begin to synthesize FSH, the amount in solution in the cell and in the blood increases, and granules are formed. Some factor in the germinal epithelium reacts with this hormone in the blood reducing its concentration in the blood until the granules are partially dissolved and forming small follicles:

germinal epithelium + FSH (blood) $\longrightarrow$ small follicles

FSH (granules) $\longrightarrow$ FSH (in solution) $\longrightarrow$ FSH (blood).

When the substance in the germinal epithelium fails to react further with the FSH, it accumulates in the blood until the reaction is reversed:

FSH (blood) $\longrightarrow$ FSH (in solution) $\longrightarrow$ FSH (granules).

The cells which produce luteinizing hormone (LH), probably those of type 4, also contain the hormone stored in the granular form in equilibrium with the soluble form in the cells and in the blood:

LH (granules) $\longleftrightarrow$ LH (in solution) $\longleftrightarrow$ LH (blood).

The hormone in the blood reacts with substances in the small follicle resulting in its growth to a large immature one and reducing the LH (blood) until more is released from the cell:

small follicle + LH (blood) ⟶ large immature follicle

LH (granules) ⟶ LH (in solution) ⟶ LH (blood).

As soon as the reaction is complete, the LH accumulates in the blood until storage begins in cells of type 4.

LH (blood) ⟶ LH (in solution) ⟶ LH (granules).

The FSH of the blood can react with something in the immature follicle causing it to mature and to form estrogenic cells as soon as sufficient FSH has been drawn from the granules:

large immature follicle + FSH (blood) ⟶ mature follicle

FSH (granules) ⟶ FSH (in solution) ⟶ FSH (blood).

The estrogenic cells produce estrogens which react with factors in the uterus, vagina, and mammary glands and, possibly, on the autonomic nervous system:

uterus + estrogen ⟶ estrual uterus

mammary gland + estrogen ⟶ growth of duct system (?)

vagina + estrogen ⟶ cornification of epithelium

autonomic nervous system + estrogen ⟶ estrual behavior (?).

If the FSH reserve is not sufficient to maintain the blood FSH, the follicles may mature without producing many estrogenic cells. The amount of estrogens may be reduced until silent heat, ovulation without estrual behavior, should result. This phenomenon occurs frequently in ewes before, and near the end of, the breeding season and may continue throughout the anestrual period. As soon as the reactions cease, the estrogens accumulate in the blood until they exceed the concentration in the estrogenic cells; and no more can be released. The cholesterol-like material which collects in the estrogenic cells leads to their destruction. Much of the estrogen is excreted:

estrogen + estrogenic cell ⟶ destruction.

The FSH has no material with which to react; and it accumulates in the blood until storage commences in cells of types 2 and 3, soon after the end of estrus:

FSH (blood) ⟶ FSH (in solution) ⟶ FSH (granules).

The mature follicle contains substances that can react with the LH in achieving ovulation and the formation of a corpus luteum:

mature follicle + LH (blood) ⟶ ovulation + corpus luteum (lutein cells);

LH (granules) ⟶ LH (in solution) ⟶ LH (blood).

The lutein cells produce progesterone which reacts with substances in the sensitized uterus until these substances will no longer react; if fertilization does not occur, this would be but a few days; if pregnancy supervenes, the progesterone may be used for several months:

progesterone + estrual uterus ⟶ progravid uterus

progesterone + progravid uterus + embryo ⟶ pregnancy.

As soon as the progesterone fails to react with other substances, it accumulates in the blood until the lutein cells can release no more of it. The fatty substances accumulate in the cells until the corpus luteum undergoes retrogression:

progesterone + lutein cell ⟶ fatty destruction.

The LH plays a minor role, if any, in the maintenance of the corpus luteum. Soon after the corpus luteum forms, the factor which reacts with LH is depleted; and LH accumulates in the blood until storage is resumed in cells of type 4:

LH (blood) ⟶ LH (in solution) ⟶ LH (granules).

During early pregnancy in the ewe the germinal epithelium and the small follicles still are able to react with FSH and LH, respectively, forming small and immature follicles; and the cyclic storage and release of the hormones continues. For the failure of the immature follicle to mature during pregnancy, two explanations can be advanced: (1) the factors with which the FSH reacts in the immature follicle may be reduced by competition with the placenta, or some other organ, for one of its components in the blood until the follicle fails to mature; or (2) the concentration of estrogens in the blood may be so great that the estrogens of the cell are never released and cause generation before the follicle matures. The number of luteinized follicles during pregnancy suggests that the second explanation is more plausible, for luteinization usually follows the FSH reaction with the immature follicle. The small follicles found at parturition and in the true anestrual ovary may have been left from the last stimulation of the germinal epithelium before the reserve of FSH was depleted. No observation is available for ewes before the onset of their first breeding season. Some other species show a period before puberty when no amount of hormone will induce an ovarian response, probably because of the general immaturity of the tissue, that is, an absence of the factor in the germinal epithelium which can react with FSH. The ewe lamb must pass through such a state, possibly somewhat complicated by the coincident anestrual period. Most of the reproductive phenomena of ewes, as they have been observed in flocks showing anestrual periods, can be explained by these assumptions. The value of this scheme in explaining other variants of the reproductive process and other types of hormone behavior is yet to be tested. It does provide a mechanism for the retrogression of the corpus luteum which has been lacking in most of the previous schemes.

Although the metabolic functions of the cells in the pituitary of the ewe have not been considered in this study, a few observations suggest some interesting interrelations.

The growth hormone has been attributed to acidophiles because of the increase in relative numbers of such cells in acromegaly and the absence of acidophils in dwarf mice. If the cells of type 1 only correspond to the acidophils of other investigators, the sheep approximates the pituitary condition of the dwarf mice more closely than it does that of other species for which cell counts are available. Sheep pituitaries are rarely preserved for commercial preparations of growth hormones and possibly have but little value.

The evidence for a thyrotropic hormone in the pituitary gland seems quite convincing. Its relation to the follicle-stimulating hormone does not seem to have been discussed. In the summer anestrual period the cells of types 2-3, believed to be the ones responsible for the follicle-stimulating hormone, decrease in size and apparent activity a short time before colloid accumulation occurs in the thyroid of sheep. Leonard (1936) found that thyroidectomy increased the effectiveness of a follicle-stimulating hormone, which could be freed from traces of the thyrotropic hormone. These observations might be explained by assuming that the same cell produced the two hormones and incorporated them in the same molecule. The molecules released in the blood stream, were received by the thyroid and ovary in proportion to their respective blood supplies. The thyroid reacted with most of the molecules reaching it and reduced by that number the molecules reaching the ovary. The ovary, of course, prevented its share of the molecules from reaching the thyroid, but this would be more difficult to demonstrate. In the absence of the thyroid gland, the ovary received the entire product of the pituitary cells and gave a proportionately greater response. Apparently the thyroid gland reacts with the thyrotropic hormone only when it is synthesizing thyroxin. The feeding of thyroxin seems to prevent its release from the thyroid gland, probably because of the relative concentrations in blood and cell. By feeding thyroxin to prevent the thyroid from synthesizing it, the follicle-stimulating hormone of the animal might be conserved sufficiently to overcome hypogonadism and sterility. The treatment is successful in some cases of human sterility and amenorrhea.

The interdependence of the adrenal cortex and the granular basophilic cells of the pituitary is indicated in the study of Hawking (1936) and others. Since hypophysectomy leads to a cortical degeneration which can be alleviated by injections of adrenotropic hormone, it seems probable that the pituitary is responsible for the maintenance of the cortex rather than the reverse. The cells of type 4 in the sheep pituitary, which seem to correspond most closely to the granular basophiles of other investigators, may produce an adrenotropic as well as a luteinizing and a mammotropic principle. No experiments can be cited to

show that the three hormones are linked in the same molecule; but it seems rather probable that, at least, two of them might be.

One of the fundamental cell types, 6, 5, 7, which seems to be functioning in the mature ewe has been assigned no hormones but several metabolic hormones have not been attributed to any cell.

SUMMARY

1. Pituitary bodies from sixty ewes slaughtered in different phases of the estrual cycle have been studied after more than fifty combinations of methods of fixation and staining. Slight modifications of Mallory's triple stain for connective tissue following fixation in relatively large pieces in Bouin's, Helly's, or Regaud's fluids have differentiated the various cells.

2. The pituitary of the sheep is divided into four parts; the glandular is largest and is ventrally located; the neural is much smaller and is dorso-medial in position; the intermediate is variable in size and lies between the glandular and neural lobes; the tuberal is also variable in size and occupies an antero-dorsal region about the stalk of the pituitary. The hypophyseal cleft extends between the intermediate and glandular lobes in the mid-line. The structure of the gland is quite similar to that described in other mammals.

3. The vascularization of the pituitary is abundant. The glandular and tuberal lobes are supplied with sinusoids and capillaries. The only arteries and veins in this region run in a connective tissue layer below the hypophyseal cleft. Capillaries are numerous in the intermediate lobe, but only thick-walled vessels occur in the neural lobe.

4. The absence of glandular cells from the intermediate and neural lobes, in view of persistent, well-confirmed reports of the extraction of chemical coordinators from these regions, suggests that the substances must be released by nerve fibers in a fashion and, perhaps, of a kind similar to the nerve fibers of the sympathetic and parasympathetic systems.

5. On the basis of ten diagnostic characteristics, the cells of the glandular lobe have been grouped into nine different types which were numbered to facilitate description. Three of these types, 1, 2, and 4, are granular; three, 3, 5, and 6, have non-granular chromophilic cytoplasm; and three, 7, 8, and 9 rarely show much cytoplasm after fixation. It is difficult to classify these cells, according to the descriptions given by other investigators, into the conventional acidophils, basophils, and chromophobes. The presence of cells with characteristics

intermediate between some of the nine types suggests there are but six fundamental types. If so, one of these is probably embryonic, and another appears to be permanently inactive in the mature ewe. Of the four groups which are potentially active, two types of cells showed changes, suggestive of secretion, which could be correlated with the phase of reproduction during which the ewe was slaughtered. No type of cell could be designated as a "pregnancy" cell, but vacuolated cells of types 2 and 3 appeared after castration suggesting the "signet ring" or "castration" cell. Cell division has not been observed in the pituitary of the ewe and cellular degeneration was very rarely seen. Cell counts have not been reported because sufficiently thin and complete sections of the pituitaries have not been available.

6 Cells designated as types 2 and 3 may be instrumental in the production of a follicle-stimulating substance and possibly of a lactogenic one that would initiate lactation after parturition.

7. Cells designated as type 4 may produce a luteinizing hormone and possibly a mammotropic substance necessary for the growth of the mammary gland during the latter half of the gestation period.

8. The pituitary of the ewe should be a relatively rich source of gonadotropic hormones, particularly the follicle-stimulating, if the cells of types 2, 3, and 4 produce them. Cytological observations indicate that a large ratio of follicle-stimulating to luteinizing hormone is necessary for estrus and ovulation. Since the luteinizing hormone is available from the pituitary during the anestrual season, follicle-stimulating hormone alone might induce the complete reaction if the commercial preparations containing relatively large quantities of luteinizing factor induce vulation without estrus.

9. A scheme based on a physical and chemical equilibrium between the cell and its products has been used to account for most of the observed phenomena of reproduction and the pituitary changes accompanying them.

10. The thyrotropic and follicle-stimulating hormones may be synthesized in the same cell and, possibly, carried in the same molecule. The adrenotropic and luteinizing hormones may have a similar association.

LITERATURE CITED

Baker, J. R. 1933. *Cytological Technique.* Methuen & Co., Ltd., London.

Baley, J. H. 1937. *Staining methods for the islets of Langerhans.* Jour. Path. and Bacteriol., *44,* 272-276.

Casida, L. E. and F. F. McKenzie. 1932. *The oestrous cycle of the ewe; histology of the genital tract.* Mo. Agr. Exp. Sta., Res. Bul. 170.

Cole, H. H. and R. F. Miller. 1933. *Artificial induction of ovulation and oestrum in the ewe during anoestrum.* Am. Jour. Physiol. v. 104, 165-171.

Conn, H. J. 1929. *Biological Stains.* Geneva, N. Y.

Cleveland, R. and J. M. Wolfe. 1932. *A differential stain for the anterior lobe of the hypophysis.* Anat. Rec., v. 51, 409-413.

Cowdry, E. V. 1928. *Special Cytology.* Paul B. Hoeber, New York. Chapter XV by Percival Bailey.

1934. *Textbook of Histology.* Lee and Febiger, Philadelphia.

Ephraim, F. 1934. *A Text-book of Inorganic Chemistry.* Translated and enlarged by P. C. L. Thorne. 2nd Eng. ed. Gurney and Jackson, London.

Galigher, A. E. 1934. *The Essentials of Practical Microtechnique in Animal Biology.* A. E. Galigher, Inc., Berkeley, Calif.

Hawking, F. 1936. *Differential cell counts of the pituitary gland in hypotension and endocrine disturbances.* Jour. Path. and Bact., v. 42, 689-702.

Howes, N. H. 1930. *A histological study of the ox pituitary gland after freezing and exposure.* Jour. Exper. Biol., v. 7, 255-259.

Kindell, F. B. 1933. *Two useful staining methods for the human hypophysis.* Johns Hopkins Hosp. Bull., v. 53, 56-59.

Lee, A. B. 1928. *Microtomist's Vade-Mecum* 9th ed., Blakiston, Philadelphia.

Leonard, S. L. 1936. *Hypophysis-thyroid-gonad relationship.* Proc. Soc. Exper. Biol. and Med., v. 34, 599-600.

Martins, T. 1933. *Sur les methodes de coloration histologique de l'hypophyse anterieure.* Compt. rend. Soc. de Biol., v. 113, 1275-76.

Maurer, S. and D. Lewis. 1922. *The structure and differentiation of the specific cellular elements of the pars intermedia of the hypophysis of the domestic pig.* Jour. Exper. Med., v. 36, 141-156.

Maximow, A. A. 1930. *A Textbook of Histology.* Ed. by W. Bloom. Saunders, Philadelphia.

McKenzie, F. F., E. Allen, M. J. Guthrie, V. Warbritton, C. E. Terrill, L. E. Casida, L. J. Nahm, and J. W. Kennedy. 1933. *Reproduction in the ewe.* Proc. of Amer. Soc. Animal Production, 1933.

Nahm, L. J. and F. F. McKenzie. 1937. *The cells of the adrenal cortex of the ewe during the estrual cycle and pregnancy.* Mo. Agr. Exp. Sta., Res. Bul. 251.

Rasmussen, A. T. 1924. *A quantitative study of the human hypophysis cerebri or pituitary body.* Endocrinology, v. 8, p. 509.

Sawyer, E. L. 1936. *The cytology of the hypophysis cerebri of the bat.* Jour. Morphol. v. 60, 127-157.

Severinghaus, A. E. 1932. *A cytological technique for the study of the anterior lobe of the hypophysis.* Anat. Rec., v. 53, 1-5.

Terrill, C. E. 1935. *Estrus and ovulation in the ewe, a preliminary report.* Proc. of Amer. Soc. Animal Production, 1935.

Trautmann, A. 1909. *Anatomie und Histologie der Hypophysis cerebri einiger Säuger.* Arch. f. mikr. Anat., v. 74, 311-367.

Warbritton, V. 1934. *The cytology of the corpora lutea of the ewe.* Jour. Morphol., v. 56, 181-202.

Warbritton, V., and F. F. McKenzie. 1935. *The anterior lobe of the pituitary body of the ewe during the estrous cycle and pregnancy.* Anat. Rec. Suppl. v. 61, p. 63.