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The Cells of the Adrenal Cortex of the Ewe During the Estrual Cycle and Pregnancy

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ABSTRACT

The adrenal glands of 49 ewes were studied. Nineteen of the sheep were killed during the estrual cycle and 30 during pregnancy. Tissues were fixed according to the methods of Champy, Helly, Bouin, and Nassonov. They were stained by the method of Kull or with aniline acid fuchsin and methyl green.

The changes observed during the estrual cycle and pregnancy were: (1) Variations in the percentages of light and dark cells in the zona fasciculata and zona reticularis, (2) variations in the amount of lipoid in cells of the zona glomerulosa and zona fasciculata, and (3) variations in the number of chondriosomes of all cortical cells. The number of dark cells and the amount of lipoid were great during estrus, early pregnancy and late pregnancy. Dark cells were less numerous from 5 to 10 days after the onset of estrus and from the 36th to the 84th day of pregnancy. The amount of lipoid was low from 3 to 12 days after the beginning of estrus, and the 24th to the 36th day of pregnancy. Chondriosomes underwent frequent changes. They were abundant 9 days after the beginning of estrus and from the 60th to the 136th days of pregnancy. Golgi bodies and spheres which stain with aniline acid fuchsin were also observed in cortical cells.

The results are believed to indicate a relationship between the cells of the adrenal cortex and the cylical changes which occur during the estrual cycle and pregnancy.

The Cells of the Adrenal Cortex of the Ewe During the Estrual Cycle and Pregnancy

LAURA J. NAHM* AND FRED F. MCKENZIE

Animal Husbandry Department, University of Missouri, and the United States Department of Agriculture Cooperating.

INTRODUCTION

Several recent investigations indicate a probable relationship between the activity of the adrenal gland and the female sexual cycle. The present study was made to determine what, if any, morphological changes in the cortical cells of the adrenal gland of the ewe accompany the physiological changes occurring during the estrual cycle and pregnancy.

This investigation is a part of a larger project in which the whole reproductive tract of the ewe is being studied, together with the pituitary, pineal and thyroid, and these in pregnant and non-pregnant individuals. Reports on these other phases follow.

MATERIALS AND METHODS

The material used in this study consisted of small pieces of adrenal glands of 49 ewes. Nineteen of the ewes were killed during the estrual cycle and 30 during pregnancy. The time after the beginning of estrus, age, breeding, weight, condition, date of slaughter, and length of previous estrual cycles of each animal are given in Tables 1 and 2.

The pieces of material were taken from comparable regions of the adrenal glands of these ewes and were fixed by the methods of Bouin, Champy, Helly, and Nassonov (Lee '28) (cf. Tables 1 and 2). Tissues were dehydrated in alcohol, cleared in xylene, and embedded in paraffin or a mixture of paraffin and bayberry wax. (Twenty-five grams of bayberry wax were added to each pound of paraffin to facilitate sectioning in warm weather).

Sections were cut 4 micra in thickness and were stained by the aniline acid fuchsin-thionin-aurantia method of Kull (Lee '28, pp. 333-34) and with aniline acid fuchsin and methyl green. For the latter method, sections were covered with a 10% solution of acid fuchsin (Grübler) in aniline water, heated to steaming, and allowed to cool for five minutes. They were rinsed in distilled water, treated 1 minute with a 1% solution of phosphomolybdic acid, and rinsed in distilled water.

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They were counterstained for 1 to 2 minutes in a 0.25% solution of methyl green in 50% alcohol, passed quickly through 70% alcohol, 95% alcohol, absolute alcohol, half alcohol-half xylene, and xylene, and were mounted in gum-damar. Material fixed by the method of Bouin was stained with Heidenhain's iron haematoxylin and eosin. Material fixed by the method of Nassonov was examined unstained after extraction with oil of turpentine for 6 to 12 days. Slides were studied within a week after staining to preclude changes due to bleaching.

EXPLANATION OF FIGURES

The details of preparations were studied with a Zeiss microscope equipped with Leitz objectives 4 and 7, Zeiss 2mm. objective, and 6X and 18X compensating oculars.

In the explanation of the individual figures, the serial number of the ewe is given first. "Estrus" refers to a non-pregnant animal. The interval from the beginning of estrus until the time of slaughter is given in days. The region from which the photomicrograph was made and the methods of fixation and staining follow. Magnifications of the photomicrograph follow. The page references are to the description of the figure in the text.

D—dark cell
L—light cell
V—vacuolated cell
ch.—granular chondriosome
ch. v.—vesicular chondriosome
cr.—chromaffin granule
fs.—fuchsin-stained sphere
g.—Golgi element
l.—lipoid sphere
n.—nucleus

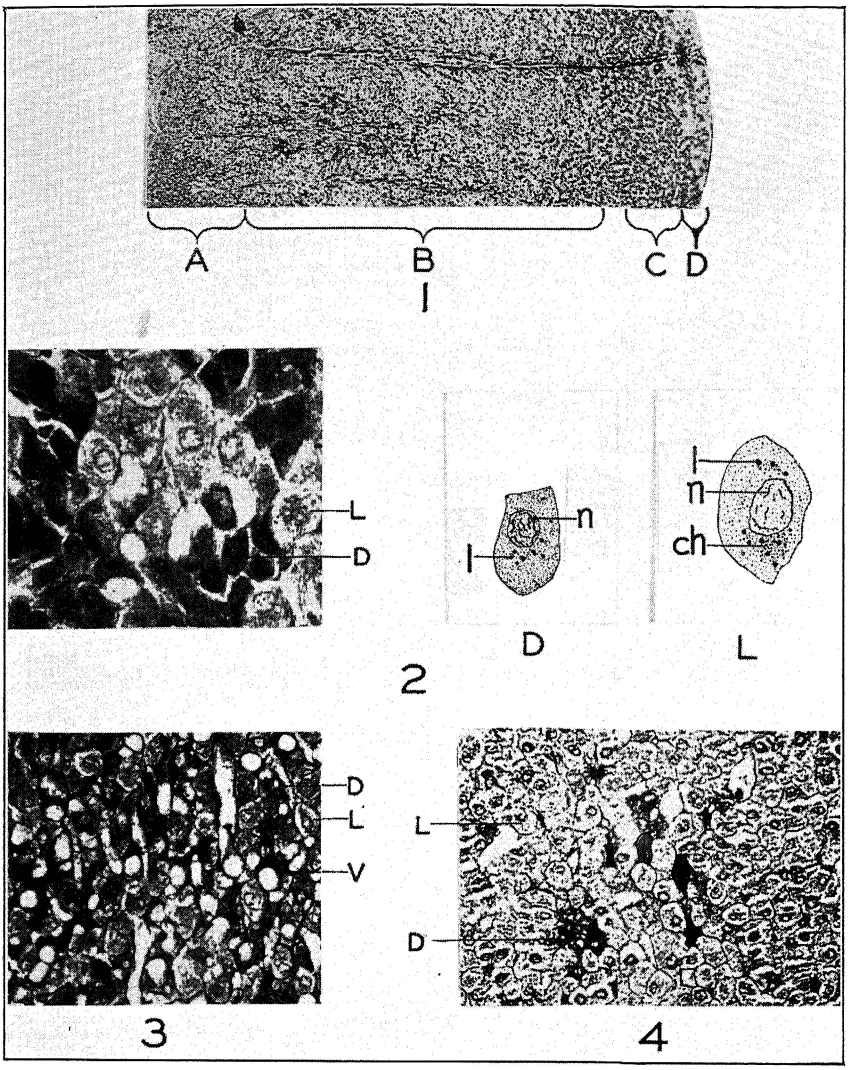

Fig. 1.—General Structure of Adrenal Gland of Pregnant Ewe 2.3 Days after the Beginning of Estrus. Champy; fuchsin-methyl green. X45. A—zona glomerulosa; B—zona fasiculata; C—zona reticularis; D—medulla. p. 12.

Fig. 2.—Ewe 2a; estrus—0.7 day; zona fasciculata; Champy; Kull. L—light cell; D—dark cell. pp. 12 and 13. Dark and light cells are present in equal numbers. X417.

Fig. 3.—Ewe 2a; Estrus—0.7 day; zona fasciculata; Champy; fuchsin—methyl green. p. 13. Same as figure 2 but showing a larger area. X270.

Fig. 4.—Ewe 14b; Estrus—7.1 days; zona fasciculata; Bouin, haematozylin-eosin; p. 13. Few dark cells are present. X270.

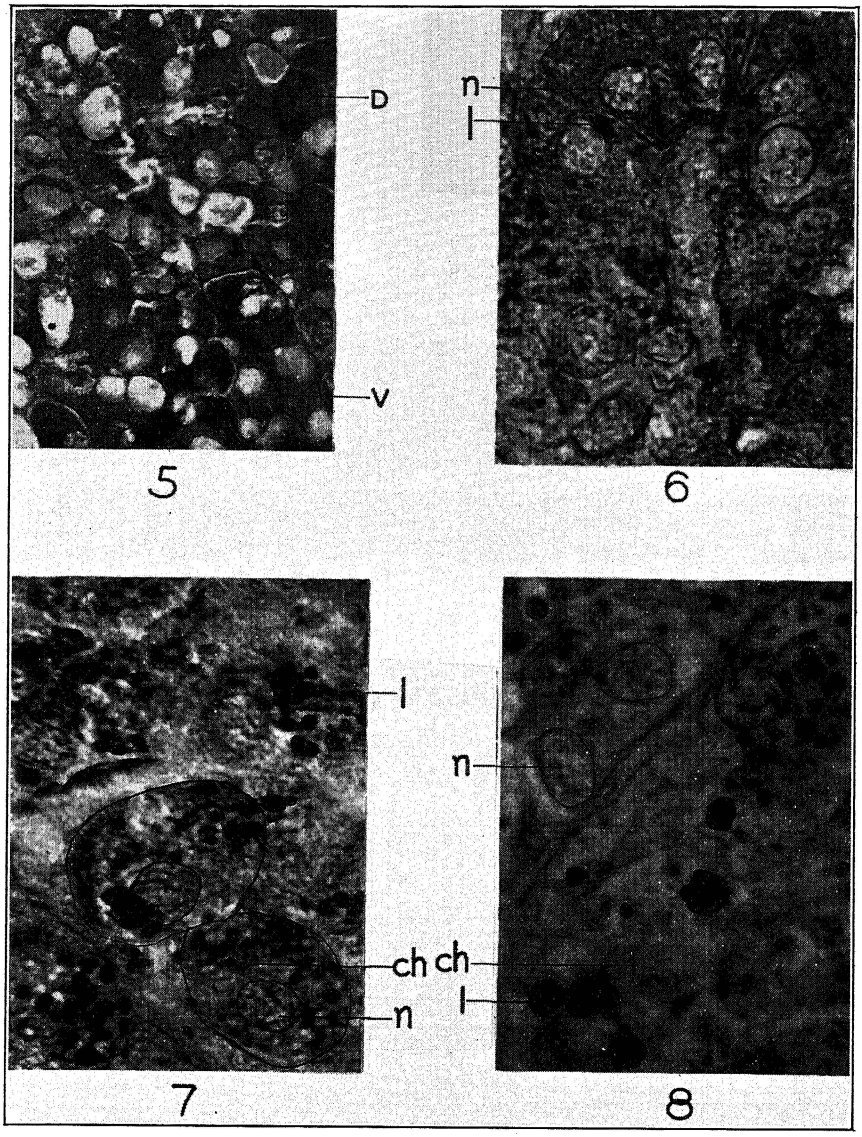

Fig. 5.—Ewe 24a; parturition—148.7 days; zona fasciculata; Champy; fuchsin-methyl green. p. 15. Large numbers of dark and vaculated cells. X417.

Fig. 6.—Ewe 17b; pregnant—12.1 days; zona glomerulosa; Champy; fuchsin-methyl green; pp. 12 and 15. Cells contain the average amount of lipoid. X1260.

Fig. 7.—Ewe 1a; pregnant—136.3 days: zona glomerulosa; Champy; fuchsin-methyl green. pp. 12 and 16. Cells contain abundant lipoid. X1260.

Fig. 8.—Ewe 24a; parturition—148.7 days; zona glomerulosa; Champy; fuchsin-methyl green. pp. 12 and 16. Cells contain large lipoid spheres. X1260.

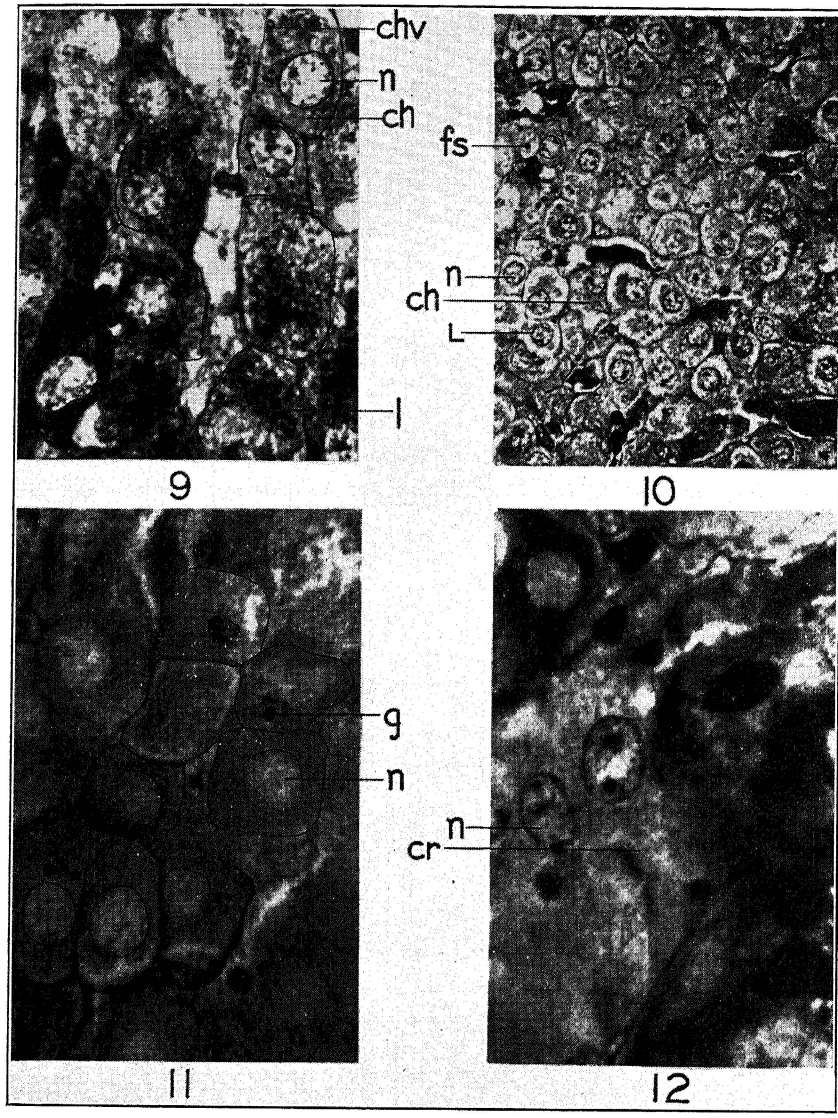

Fig. 9.—Ewe 47a; pregnant—2.3 days; zona fasciculata; Champy; fuchsin-methyl green. pp. 12, 13, and 16. Dark and light cells, granular and vesicular chondriosomes. X1260.

Fig. 10.—Ewe 15b; pregnant—20.6 days; zona fasciculata; Helly; fuchsin-methyl green. pp. 12 and 16. Few dark cells are present; fuchsin-stained spheres are seen. X417.

Fig. 11.—Ewe 6b; pregnant—7.3 days; zona fasciculata; Nassonov 10 days; turpentine 7 days. p. 12. Golgi elements are present in a few cells. X1260.

Fig. 12.—Ewe 8a; pregnant—30.8 days; medulla; Helly; fuchsin-methyl green. p. 13. Dark syncitial masses and light elongated cells; chromaffin granules and secretion spheres may be seen. X1260.

TABLE 1.—VARIATIONS IN CELLS OF ADRENAL CORTEX—ESTRUAL CYCLE.

Ewe	Days after beginning of estrus	Age	Breeding	Weight	Condition	Date of slaughter	Length of previous cycles (days)	Per cent of dark cells	Amount of lipoid	No. of chondriosomes	Regions studied	Fixatives	Remarks
1251a	0.0	3	Shrop	170	Good	12/14	17.4; 16.4; 16.7	50	†††	†††	Entire cortex	Champy; Helly	Slaughtered before ovulation
7b	0.5	8	HampX	148	Good	11/29	17.0; 17.0; 16.7	10	†††	†	Zona fasisculata; zona reticularis	Champy; Helly; Nassonov	Slaughtered before ovulation
2a	0.7	4	Shrop	---	Medium	11/17	15.4;16;32;16.2	50	††	††	Entire cortex	Champy	Slaughtered before ovulation
45a	0.9	2	X	110	Medium	11/9	16; 16; 16.0	50	††	†	Zona fasciculata; reticularis; medulla	Champy	Slaughtered just before ovulation
22a	1.8	5	HampX	116	Medium	11/28	15.4; 17.7; 17.0	20	†††	††	Entire cortex	Champy	
20b	3.3	6	HampX	150	Good	10/29	16.0; 16.7; 17.0	20	†	††	Entire cortex	Champy	
25a	3.8	5	X	164	Medium	12/1	34.4; 16.0; 16.5; 14.7; 15.8	20	†	††	Cortex and medulla	Champy; Helly	
22b	5.3	7	HampX	134	Good	11/1	16.0; 16.5; 18.2; 17.0	10	††	†	Zona fasciculata	Nassonov; Helly; Bouin; Champy	
53a	5.3	3	X	130	Medium	11/1	11.0; 10.6; 19.8; 32.5; 7.0	10	Not fixed	†	Zona glomerulosa	Helly	Cycles irregular
14b	7.1	6	HampX	120	Good	10/20	15.9; 16.6	4	Not fixed	†††	Entire cortex	Bouin; Helly; Nassonov	Possibly pregnant; no embryo recovered
23a	7.4	6	HampX	130	Medium	12/9	14.6; 14.0; 15.3; 10.7	30	†	†	Cortex and medulla	Champy	Running back to ten-day cycle.
24b	7.9	5	HampX	120	Poor	12/8	16.5; 16.0; 15.4; 16.7; 17.3	15	†	†	Zona fasciculata	Champy	Poor fixation

Ewe	Days after beginning of estrus	Age	Breeding	Weight	Condition	Date of slaughter	Length of previous cycles (days)	Per cent of dark cells	Amount of lipoid	No. of chondriosomes	Regions studied	Fixatives	Remarks
44a	7.9	6	HampX	150	Medium	12/30	16.1; 16.2; 17.9; 15.5; 17.1; 16.1; 16.3	40	†	††	Cortex and medulla	Bouin; Champy	Possibly pregnant; no embryo recovered; out of line on pituitary
28a	9.1	6	HampX	120	Medium	12/31	16.5; 17.5; 17.0; 16.2; 16.3; 17.0	16	None	††††	Cortex and medulla	Champy; Helly	End of season; twelve-day cycle
3b	9.9	7	HampX	120	Medium	10/4	17.0	10	Not fixed	†	Zona fasciculata; zona reticularis; medulla	Bouin; Nassonov	
38a	11.0	2	HampX	120	Medium	11/30	16.4; 16.1; 16.3; 15.0; 16.8	30	†	†	Cortex and medulla	Bouin; Champy; Nassonov	
21b	12.3	8	HampX	107	Medium	12/9	16.4; 15.5	10	†	††	Zona glomerulosa; zona fasciculata	Bouin; Champy	Possibly pregnant; no embryo recovered
35a	14.0	2	HampX	138	Medium	12/5	20.1; 15.7; 15.9; 51.2	25	†	†††	Entire cortex	Champy	Possibly anestrual
10b	14.3	6	HampX	111	Medium	10/31	16.0; 15.0; 15.0	25	††	†	Zona glomerulosa; zona fasciculata	Champy; Helly; Nassonov	

EXPLANATIONS

a designates animal slaughtered in 1931-32.
b designates animal slaughtered in 1932-33.
Age is given in years.
Breeding Shrop = Shropshire; Hamp = Hampshire; South = Southdown; X = Grade.
Weight is given in pounds at time of slaughter.
Condition refers to fatness of carcass.
†Indicates presence of chondriosomes or lipoids in the cells. Increase in numbers or amount is indicated by ††, †††, and ††††.
Percentages of dark cells are based on zona fasciculata.
Amount of lipoid is based chiefly on the zona glomerulosa.

TABLE 2.—VARIATIONS IN CELLS OF ADRENAL CORTEX—PREGNANCY.

Ewe	Days after beginning of estrus	Age	Breeding	Weight	Condition	Date of slaughter	Length of previous cycles (days)	Per cent of dark cells	Amount of lipoid	No. of chondriosomes	Regions studied	Fixatives	Remarks
50a	1.8	-	X	106	Medium	12/1	16.3	33	††	†	Zona glomerulosa; zona fasciculata	Champy	Zona glomerulosa absent from Champy
47a	2.3	7	X	126	Medium	11/10	16.2; 17.6; 16.3; 16.0	33	††	†††	Cortex and medulla	Champy; Helly	
6a	4.8	5	South	138	Medium	11/16	17.1; 15.9; 16.5	25	††	†	Zona glomerulosa; zona fasciculata	Champy	
6b	7.3	6	HampX	114	Medium	11/8	16.0; 16.4; 16.6	20	†††	†	Cortex and medulla	Helly; Nassonov	
5b	9.3	5	HampX	112	Medium	11/4	30.6; 21.0	25	---	†	Cortex and medulla	Champy; Helly	Zona glomerulosa absent from Champy
43a	11.3	6	HampX	112	Medium	11/17	17; 16.1; 16.7; 17.2; 16.6	25	---	½†	Cortex	Champy; Helly	Zona glomerulosa absent from Champy
17b	12.1	5	HampX	140	Good	12/31	16.3; 17.6; 17.5; 16.6; 17.0	25	†††	††	Cortex and medulla	Champy; Helly; Nassonov	
16b	14.0	4	HampX	141	Good	10/7	None observed	12	††	††	Cortex	Champy; Nassonov	
23b	18.9	6	HampX	113	Medium	10/11	None observed	10	††	†	Cortex	Helly	
21a	20.0	2	HampX	110	Medium	12/9	Not observed	15	0	†	Cortex and medulla	Champy; Helly	
15b	20.6	6	HampX	117	Poor	10/7	16.4	10	††	†	Cortex and medulla	Champy; Helly; Nassonov	
51a	24.9	-	Shrop	70	Poor	12/2	51.4; 17.1	25	†††	½†	Cortex and medulla	Champy; Helly	
9b	27.3	6	HampX	130	Good	12/13	17.0; 16.3; 18.6; 17.0	20	††	†	Cortex and medulla	Champy; Helly	
2b	29.9	4	HampX	124	Medium	11/10	16.0	10	½†	0	Cortex and medulla	Champy; Helly; Nassonov	
8a	30.8	5	Shrop	122	Medium	12/7	23; 17; 33.0; 17	33	Not fixed	---	Cortex	Helly	Lipoid not fixed
19b	32.3	8	HampX	105	Medium	11/1	16.0	15	†	††	Zona glomerulosa; zona fasciculata	Nassonov; Champy; Helly	

Ewe	Days after beginning of estrus	Age	Breeding	Weight	Condition	Date of slaughter	Length of previous cycles (days)	Per cent of dark cells	Amount of lipoid	No. of chondriosomes	Regions studied	Fixatives	Remarks
4b	35.0	5	HampX	120	Good	12/17	15.6; 32.0; 5; 16.0; 16.1	4	††	†	Cortex and medulla	Champy; Helly	Champy sections poorly fixed
48a	36.3	5	ShropX	138	Medium	12/2	21.5; 30.0	20	---	†	Cortex and medulla	Champy; Helly	No zona glomerulosa on Champy sections
26b	45.7	5	HampX	116	Good	1/2	17.6; 48.4	4	††	†	Cortex and medulla	Champy; Helly	
30a	48.2	6	HampX	130	Medium	12/16	16.5; 35.6	25	†††	†	Cortex and medulla	Champy; Helly	
19a	60.0	5	HampX	214	Good	1/11	36.5; 16.0	25	---	††	Cortex and medulla	Champy; Helly	No zona glomerulosa on Champy sections
52a	72.9	3	HampX	128	Medium	1/9	17.0; 15.0; 30.6	4	††	††	Cortex and medulla	Champy; Helly	
41a	85.1	5	HampX	138	Good	12/5	18; 16.0	33	††	†††	Cortex and medulla	Champy; Helly	
37a	97.1	5	X	118	Poor	12/17	None observed	33	††	††	Cortex and medulla	Helly; Champy	
9a	108.3	5	South	104	Medium	12/18	None observed	25	Not fixed	†	Cortex and medulla	Helly	Lipoid not fixed
1161a	120.5	4	Hamp	161	Medium	3/11	68.8	30	---	††	Cortex and medulla	Helly; Champy	No zona glomerulosa on Champy sections
20a	133.1	5	X	110	Medium	1/8	None observed	30	Not fixed	††	Cortex and medulla	Helly	Lipoid not fixed
1a	136.3	5	Shrop	142	Medium	1/15	None observed	33	††††	†††	Cortex and medulla	Champy	
33a	141.0	2	HampX	170	Medium	1/18	None observed	33	†††	†	Cortex and medulla	Champy; Helly	
24a	148.7	-	HampX	150	Poor	1/26	None observed	50	††	††	Cortex and medulla	Champy; Helly	

EXPLANATIONS

a designates animal slaughtered in 1931-32.
b designates animal slaughtered in 1932-33.
Age is given in years.
Breeding: Shrop=Shropshire; Hamp=Hampshire; South=Southdown; X=Grade.
Weight is given in pounds at time of slaughter.
Condition refers to fatness of carcass.
†Indicates presence of chondriosomes or lipoids in the cells. Increase in numbers or amount is indicated by ††, †††, and ††††.
Percentages of dark cells are based on zona fasciculata.
Amount of lipoid is based chiefly on the zona glomerulosa.

OBSERVATIONS

Typical Glandular Structure

The adrenal gland of the ewe is covered by a rather heavy capsule of connective tissue. The cortex is divided into the three usual regions; (1), a narrow zona glomerulosa just beneath the capsule; (2), a much wider zona fasciculata; and (3), a zona reticularis. (Fig. 1). If the point at which differentiation into "light" and "dark" cells begins is considered as the boundary between the zona fasciculata and zona reticularis as Hoerr ('31) suggests, the width of both zones is quite variable. If the usual criterion of cell arrangement is used, the zona fasciculata is considerably wider than the zona reticularis.

The cells of the zona glomerulosa and the "light" cells of the zona fasciculata and zona reticularis are quite similar in general morphology. (Compare Fig. 2 *L* and Fig. 6). They are rather large, rounded in outline, and contain large oval or round nuclei. Each nucleus contains granular clumps of deeply stained chromatin and one or several conspicuous nucleoli. The cytoplasm of the cell stains lightly. Distributed through it are chondriosomes, usually lipoid spheres, and often other structures.

When present, the lipoid spheres vary in size from those just large enough to be visible at a magnification of 1360 diameters to those which are almost as large as the nucleus. They vary in number from 2 to 25 per cell. (Fig. 2 *L*, 6, 7, and 8). Lipoid is most abundant in cells of the zona glomerulosa and least so in cells of the inner half of the zona fasciculata.

The chondriosomes are chiefly small and of the granular type. After fixation by Champy's method, the larger chondriosomes are frequently vesicular with dark red rims and clear centers. (Fig. 9, 2 *L*, and 7).

Golgi bodies are visible in some of the cells after incubation with osmic acid at 35° C. for 9 to 14 days. They consist of masses of fine blackened granules or slender filaments which form a net-like structure adjacent to and slightly smaller than the nucleus. (Fig. 11). Golgi bodies occur in a limited region several cell-layers beneath the periphery. Bleaching with turpentine does not remove them.

After fixation in Helly's fluid, the cells of the zona glomerulosa frequently contain spherical or irregular bodies which stain bright red with fuchsin. These structures are approximately the size of the nucleoli and differ from chondriosomes only in size. (Fig. 10). These spheres also occur in smaller numbers in the zona fasciculata and zona reticularis.

The "dark" cells of the zona fasciculata and zona reticularis are slightly smaller than other cells of the adrenal cortex. They are not rounded but often have concave margins and appear shrunken. Their cytoplasm stains deeply, chondriosomes can sometimes be distinguished, and rather irregular lipoid spheres are common. (Fig. 2 *D* and 9). Vesicular chondriosomes entirely fill some cells.

The medulla is composed of groups of slender elongated cells held together by thin filaments of connective tissue. The cells appear to form tubules. Membranes between adjacent cells may be absent or so obscure that the cells seem to form a syncitium. The nuclei are large and contain deeply stained nucleoli and clumps of chromatin. The cytoplasm is usually stained green with methyl green and contains a few small chondriosomes and lipoid spheres. Chromaffin granules and fuchsin-stained spheres are sometimes seen after fixation in Helly's fluid. (Fig. 12).

Staining reactions are not entirely specific. The cells of the zona glomerulosa and the dark cells of the zona fasciculata are usually stained red by fuchsin after fixation in Champy's fluid. However, they may fail to stain with fuchsin and become blue, purple, green, or orange instead. The light cells of the zona fasciculata and zona reticularis vary in color from light red to deep blue, violet, or green after staining with fuchsin and methyl green. The cells nearest the periphery of the piece of fixed material usually absorb the fuchsin more readily than others and are frequently somewhat shrunken. Cells near the center of large pieces of fixed material are usually stained by methyl green, and chondriosomes are not fixed.

Changes During the Estrual Cycle

The principal differences observable in cells of the adrenal cortex during the estrual cycle are variations in (1), the percentage of dark cells; (2), the lipoid content of the cells; and (3), the number of chondriosomes.

In early estrus, 50% of the cells are of the dark type. (Fig. 2 and 3). This percentage continues for 0.9 day, but is reduced to 20% after 1.8 days. After 3.3 and 3.8 days the dark cells still constitute 20% of the total but at 5.3 days a further reduction to 10% occurs. The number remains at a low level until the seventh or ninth day (Fig. 4) and then begins to rise, reaching 25% to 30% in 12 to 14 days. (Table 1 and Fig. 13 *A*)*.

The amount of lipoid is also greatest during early estrus. While no actual quantitative measurement of the amount present could be made, estimates indicate a definite trend. Lipoid is present in largest quantity 1 hour and 0.5 day after the onset of estrus. A slight reduction

*Stages described are based on animals with cycles of typical length (15 to 18 days).

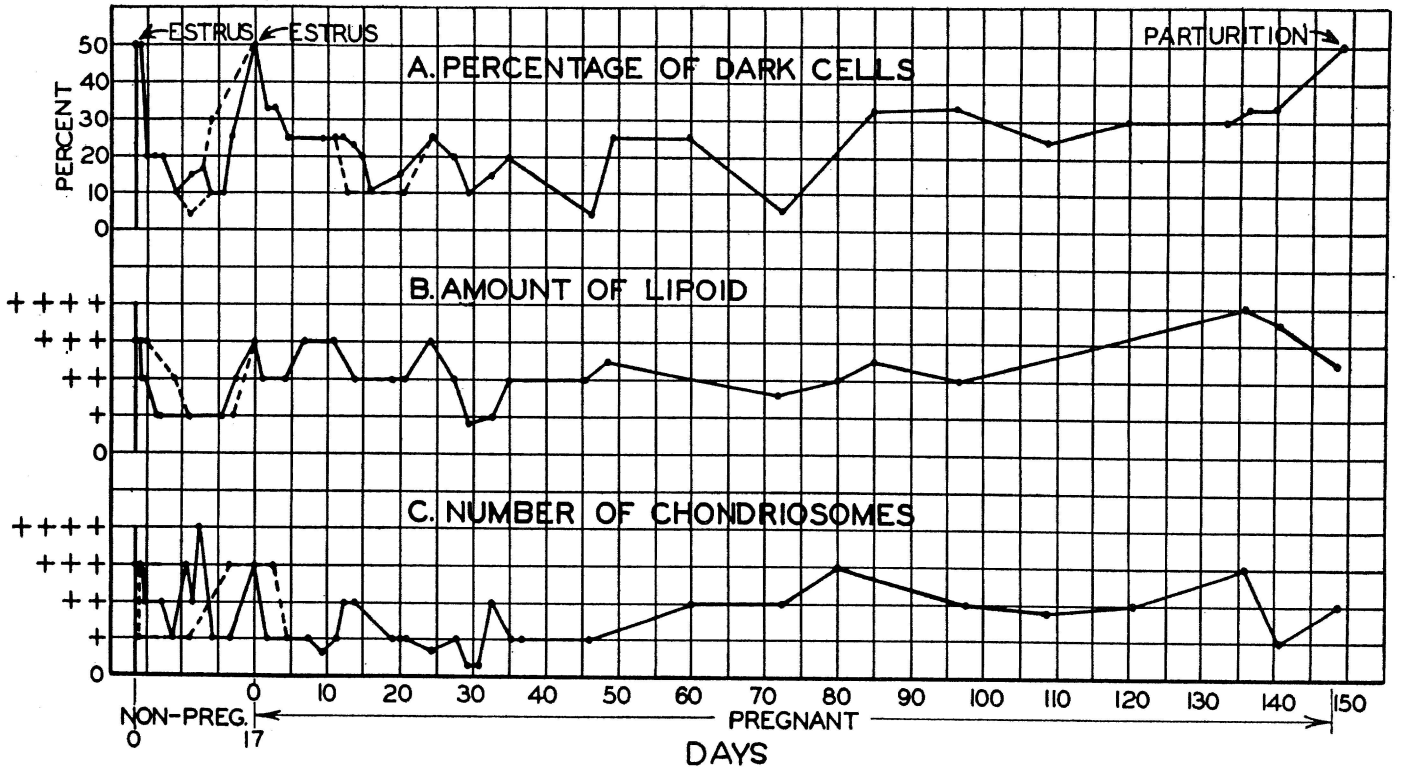

Fig. 13.—Changes in the cells of the adrenal cortex during estrus and pregnancy. Solid lines are based on animals with cycles of 15-18 days; broken lines are based on animals with cycles suspected of being abnormal. (Cf. Tables 1 and 2).

in amount at 0.9 day is followed by a rise to the estrus level at 1.8 days. The amount is greatly reduced 3.3 days after the beginning of estrus and remains low for 11 days. An increase appearing at 14.3 days indicates the beginning of the rise to the estrus level. (Table 1 and Fig. 13 *B*).

The number of chondriosomes fluctuates frequently during the estrual cycle. The number is greatest 9.1 days after the beginning of estrus. The number is slightly lower just before and during estrus. The smallest numbers are to be found in the cells at 0.5, 0.9, 5.3, 7.4, 7.9, 9.9, 11, and 14.3 days after the onset of estrus. (Table 1 and Fig. 13 *C*).

Slight variations in the number of fuchsin-stained spheres which appear after fixation in Helly's fluid also occur. The number is greatest 7.1 days after the beginning of estrus, and but few are visible 1 hour and 12 hours after its onset.

Changes Associated with Pregnancy

The types of cellular changes in the adrenal gland during pregnancy are similar to those which occur during the estrual cycle.

In pregnant ewes 1.8 and 2.3 days after the beginning of estrus, approximately one-third of the cells are of the dark type. Many of these are vacuolated. A decrease to 25% occurs at 4.8 days and persists until 12.1 days after the beginning of estrus. From 14 days to 72.9 days the proportion shifts upward and downward between 4% and 33%. A rhythm is apparent with low points at 15-day intervals. The dark cells are also much less frequently vacuolated than in earlier stages. From 85.1 to 141 days the number of dark cells is equal to one-fourth or one-third of the total number in the zona fasciculata and zona reticularis. A great variety of cell types is seen at these stages. Some of the dark cells are stained the typical dark red with fuchsin, some are orange or yellow, and a few are purple after staining with fuchsin and methyl green. Immediately following parturition 50% of the cells are of the dark type. A large number of these are vacuolated. (Fig. 5). In general, the number of dark cells is higher during the estrual cycle than during pregnancy. (Compare Tables 1 and 2 and Fig. 13 *A*).

Variations in the amount of lipoid in the cells occur throughout pregnancy, but the most conspicuous variations are to be seen near the end of the gestation period. The amount of lipoid drops rapidly in early pregnancy (1.8, 2.3, and 4.8 days) as it did in early estrus. A slight increase is seen at 7.1 days. Similar amounts are present 12.1, 24.9, and 48.2 days after the beginning of estrus (Fig. 6), but at periods between these points the amount is considerably smaller. Curve B (Fig. 13), accordingly, shows a number of low peaks. The largest

amount of lipoid present at any stage is in the cells of a ewe killed 136 days after the beginning of estrus. (Fig. 7). Immediately following parturition, lipoid spheres, which are larger than those found at any preceding stage, occur in cells of the zona glomerulosa. However, the total amount of lipoid is somewhat smaller than in the preceding stages. (Fig. 8). In general, lipoid is more conspicuous and more abundant during pregnancy than during the estrual cycle. (Compare Tables 1 and 2 and Fig. 13 *B*).

The number of chondriosomes present in cortical cells fluctuates throughout the gestation period. A rather large number of both granular and vesicular types is found 2.3 days after the beginning of estrus. (Fig. 9). From 4.8 to 11.3 days the number falls off rapidly. A slight increase in number occurs 12 and 14 days after the onset of estrus. This is followed by a decided reduction. A slight increase again occurs at 32.3 days and is again succeeded by smaller numbers in stages immediately following. From 60 days to the end of the gestation period, the number of chondriosomes is greater than in earlier stages. The largest numbers occur 85.1 and 136.3 days after the beginning of estrus. (Fig. 13 *C* and Table 2).

The fuchsin-stained spheres of tissues fixed in Helly's fluid are first evident 7.3 days after the beginning of estrus. They occur chiefly in cells of the zona glomerulosa, and, in smaller numbers, in cells of the zona fasciculata and zona reticularis. The number of spheres fluctuates throughout the gestation period. Figure 10 shows the condition 20.6 days after the beginning of estrus when the number of spheres is rather great.

SUMMARY OF OBSERVATIONS

1. The adrenal gland of the ewe is composed of a narrow zona glomerulosa, a wide zona fasciculata, and a somewhat narrower zona reticularis.

2. Both light and dark cells occur in the zona fasciculata and zona reticularis.

3. Lipoid spheres, chondriosomes, fuchsin-stained spheres, and Golgi elements have been indentified in the cytoplasm of cortical cells.

4. Variations in types of cells and in their constituents are seen during the estrual cycle and pregnancy.

5. The number of dark cells and the amount of lipoid are great during early estrus, early pregnancy, and late pregnancy.

6. The amount of lipoid is reduced from the 136th day of pregnancy to the time of parturition, but the number of dark cells increases during the same period.

7. Chondriosomes undergo frequent changes in number. Their number is high 9.1 days after the beginning of estrus, and also from the 60th to the 136th day of pregnancy.

DISCUSSION

The adrenal gland has long been believed to be closely related to reproductive processes. A procedure frequently used in investigating this relationship has been to note the effects of bilateral adrenalectomy on the estrual cycle and pregnancy. The white rat was the principal experimental animal, but varying results were obtained. Lewis ('23) found that 60% to 80% of the rats survived bilateral adrenalectomy and that reproductive functions and pregnancy appeared normal. When Wyman ('28) performed similar experiments, 70% to 82% of the animals died, and in those which survived, the estrual cycle was inhibited or prolonged. Wyman found accessory adrenal tissue in all but one of the animals which survived for longer than one month. Schiffer and Nice ('30) on the other hand, observed only a slight and insignificant lengthening of the estrual cycle following removal of the adrenal glands. They observed adrenal "rests" (vestiges or accessory adrenal tissue) in all animals which survived for more than three weeks. Martin ('31) confirmed Wyman's results, for 80.1% of the female rats he used died after removal of both adrenal glands, and in those which survived, estrus was completely inhibited. Normal estrual behavior was restored by transplanting a piece of adrenal cortex into an ovary, but if that ovary was removed later, the animal died.

Recently the adrenal gland itself has been studied. Anderson and Kennedy ('32 and '33) found that the cortex of the adrenal gland of the rat increased in size during estrus. The cells of the zona fasciculata and the cell nests near the medulla were enlarged and contained an increased amount of lipoid. During pregnancy there were no striking changes from the non-pregnant diestrual animal. Foster ('34), in his analysis of the reproductive cycle of the female ground squirrel, noted a marked change in the number of cells, vascularity, and gross size of the gland during the estrual cycle and pregnancy. Fat globules were present in the cells during periods of inactivity but disappeared during periods of high reproductive activity. Zalesky ('34) likewise observed a significant increase in size and weight of the adrenal gland of the ground squirrel during the breeding season. This hypertrophy occurred largely within the zona reticularis and was due to the differentiation within it of a highly developed outer sub-zone termed "reticularis A."

The results presented in this paper also indicate a functional relationship between the cells of the adrenal cortex and the estrual cycle and pregnancy. The number of dark cells is high during early estrus, early pregnancy, and late pregnancy, all of which are periods of great activity. Zwemer ('36) described the changes which occurred in cells of

the mammalian adrenal cortex during periods of activity. He found that as a cell gave off its secretion it was reduced in size and stained more deeply. This description corresponds to the dark cells of the adrenal cortex of the ewe. If the theory of Hoerr ('31), namely, that the differentiation into light and dark cells is the first step in their degeneration, is accepted, then the increased number of dark cells found during and immediately following periods of activity is to be expected. If cortical cells are very active in the production of secretion, many of them will become exhausted and degenerate. As activity in the formation of secretion is reduced, the dark cells will tend to disappear from the gland because they will be removed more rapidly than they are produced. In pregnant animals the number of dark cells remains large for a longer period after the beginning of estrus than it does in non-pregnant animals. This is also in line with expected results since in pregnant animals the period of greater activity continues for a longer time after the beginning of the estrus in which the ewe was bred than it does in non-pregnant ones.

The significance of large amounts of lipoid in cells of the adrenal cortex has been interpreted in a variety of ways. Zwemer ('36) found that the lipoid storage type of gland might be produced as a result of mild stimulation. He also noted, however, that lipoid might be retained in large drops in the presence of an acute demand for cortico-adrenal secretion. Deansley ('31) found that one type of adrenal enlargement, stimulated by injections of killed *B. Gaertner* or of thyroxin consisted of an increase of the amount of fat and lipoid in the cells. Zwemer ('36) interpreted the lipoid as either the raw substance from which the hormone was made or the vehicle in which it was stored.

If lipoid is utilized in the production of secretion, the gradual increase in the amount of lipoid which takes place up to the time of early estrus and its rapid diminution immediately thereafter is to be expected. (cf. Fig. 13 A). One of the changes which indicates the advent of another estrus consists of the accumulation of lipoids in the cells. The fact that the amount of lipoid is diminished less rapidly immediately after the onset of estrus in ewes which become pregnant may be due to a more continuous demand for increased secretion during pregnancy and a corresponding increase of activity in the cortex. The tremendous increase in amount of lipoid in late stages of pregnancy and its reduction after parturition indicate again the utilization of lipoid for the production of secretion about the time of parturition. The fact that the average amount of lipoid present in cortical cells is greater during pregnancy than during the estrual cycle indicates an increased activity of the gland throughout the gestation period.

The more frequent fluctuations in the number of chondriosomes which occur during the estrual cycle and pregnancy of the ewe may indicate that they are more transitory substances which accumulate in the cells when excesses of reserve materials are present and are then rapidly converted into other substances. The vesicular chondriosomes found in materials fixed in Champy's fluid are apparently due to the swelling of the granular ones.

The fuchsin-stained spheres frequently seen in cortical cells after fixation in Helly's fluid probably represent some lipoidal constituent of the cell which is blackened by osmic acid after fixation in Champy's fluid, but is not neutral fat. The presence of large numbers of these in middle stages of pregnancy when lipoids are not abundant indicates that they are probably intermediate substances destined to be transformed into fat.

The results described here are based on those portions of the adrenal glands which seemed properly fixed. Hoerr ('31) emphasized the importance of proper fixation. He found that the deeper regions of whole glands were fixed by the most rapid penetrant only, and it was often difficult to tell the difference between poorly fixed cells and those which had been degenerating before the death of the animal. Results noted in this study point in the same direction. The outer cells of a piece of fixed material are often shrunken and distorted, and the inner cells do not contain chondriosomes. It is difficult under such circumstances to determine which cells are normal. Since those several cell-layers beneath the periphery appear least injured, the observations described are based on these. In spite of this difficulty and the fact that only one complete series of glands was available, the uniform trends which occur in the cells of the adrenal cortex during the estrual cycle and pregnancy justify at least tentative conclusions.

CONCLUSIONS

1. The increased number of dark cells in the adrenal cortex of the ewe during early estrus, early pregnancy, and late pregnancy indicate increased secretory activity of the cells at these stages.

2. The large amounts of lipoid present in the cells during these same periods appear to be due to the accumulation of materials to be used in the production of secretion.

3. The transitory nature of the chondriosomes indicates that they are used in the production of secretion or of other reserve material.

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