Technical Bulletin 169 June 1945

Prenatal Development of the Sheep

W. W. GREEN and L. M. WINTERS
Division of Animal Husbandry

University of Minnesota
Agricultural Experiment Station

Prenatal Development of the Sheep

W. W. GREEN and L. M. WINTERS
Division of Animal Husbandry

University of Minnesota
Agricultural Experiment Station

Accepted for publication April 17, 1945

CONTENTS

3000-6-45

Prenatal Development of the Sheep

W. W. Green and L. M. Winters

Introduction and Review of Literature

THE AMOUNT AND TYPE of information available concerning the prenatal development of farm animals vary considerably. Practically all of the large volume of literature dealing with the embryology of the pig has been based upon slaughterhouse specimens, the "ages" of which have been given in terms of linear measurement (e.g., Patten, *6*, and Arey, *1*).

Very few reports have been published that have described the prenatal development of the bovine. In contrast with the pig, all of these studies have been made of specimens whose ages, in days, were either accurately known or rather closely estimated. One such report has presented pictorially the complete development of the bovine from the unfertilized ovum to the fully developed fetus (Winters, Green, and Comstock, *8*). These authors also supplied growth data concerning their various specimens and discussed the relative value of some of the commonly secured measurements.

As in the case of the bovine, most of the few papers describing the prenatal development of the sheep have been based on carefully timed and measured specimens. Some of the bodily measurements have been made on fetuses which were previously fixed in formaldehyde (Cloete, *3*, and other publications from the same laboratory). Fixation alters some of the dimensions.

All studies of prenatal development conducted in this laboratory have been based on specimens of accurately determined ages, the data from which were secured from fresh, unfixed material *(2, 4, 5, 7, 8)*. Clark *(2)* reviewed much of the early work dealing with the sheep in connection with his study of the cleavage stages of the ovum. His ova ranged in development from the unsegmented egg to the primitive streak stage (13 days postcoitus). Winters and Feuffel *(7)* recovered fetal sheep from 34 days to 140 days of age. They presented illustrations of the specimens and tabulated the dimensions of various bodily parts.

The ages from 13 days to 34 days, approximately the embryonic period of the sheep, have not been studied thus far. In addition, there is not available a complete study of the whole

prenatal period of the sheep based on fresh specimens of known ages. The desirability of and necessity for such a study have already been outlined in previous publications *(7, 8)*.

The objects of this experiment were, therefore:

1. To establish the normal prenatal development of the sheep by use of fresh, unfixed specimens of accurately known ages.
2. To secure a standard for use when studying factors responsible for variations in prenatal development.
3. To use the information for a basis for the comparison of the prenatal periods of the sheep and the bovine.

Experimental Procedures

The type of sheep and the technics used in this study were the same as or similar to those used in previous work reported from this laboratory. Estrus cycles of grade Shropshire ewes were determined by teasing the flock four times daily with a vigorous, aproned ram. The females were bred as close to the end of the heat period as possible, because the time of ovulation had been previously established as occurring approximately at that time. All ages were determined by the period from coitus to slaughter, for this was the most accurate, practical method to employ. All ages prior to 34 days will be reported in both days and hours. Fetal ages are given only in days.

Upon slaughter, the ewe's reproductive tract was taken to the laboratory and the embryo or embryos recovered. The specimens were photographed immediately while still in saline. Ova and embryos to be sectioned were then fixed in Bouin's fluid, dehydrated with alcohol, cleared in xylene or cedarwood oil, sectioned at 5μ, stained with Harris' hematoxylin, and counterstained with eosin.

The system of numbering the figures is the same as previously used (Winters, Green, and Comstock, *8*). Whole specimens are assigned whole numbers (e.g., Fig. 5, Fig. 30). Sections are given a whole number followed by a small case letter (e.g., Fig. 1a, Fig. 30d). All material is numbered in sequence. If a whole specimen for a certain age is not shown but a section of that unpresented specimen is shown, the figure assumes the same number (plus a small case letter) as it would had the whole specimen been shown in sequence with other whole specimens. For example, whole specimens are shown in figures 36 and 38. The sections accompanying figure 36 include one from the embryo shown in figure 36 (Fig. 36a) and also eight sections of an embryo which is not shown *in toto* (Figs. 37a-h).

Description and Discussion of Specimens

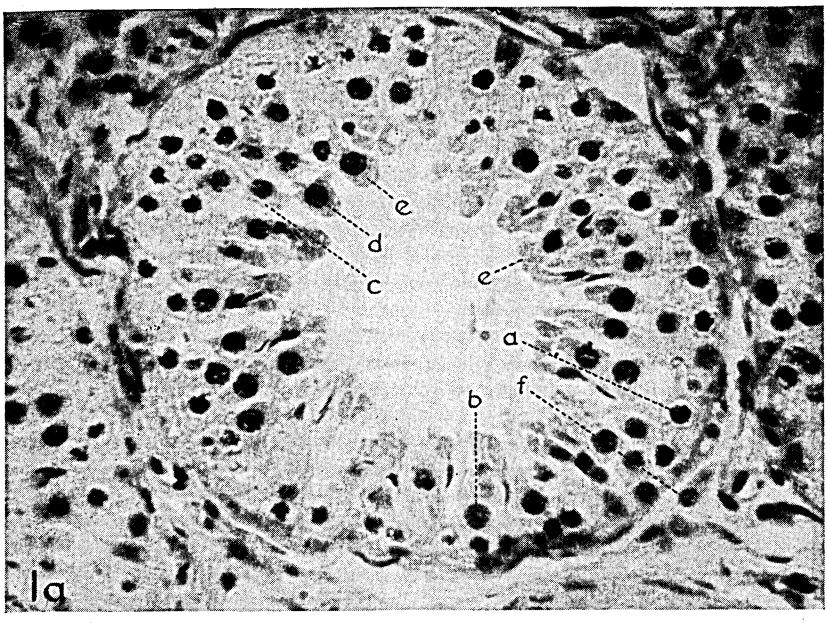
The division of the prenatal era into periods has been previously discussed *(8)* in connection with the prenatal development of the bovine. Changes in form in the sheep are similar to those in cattle, and although they may take place at ages differing from those of the bovine, the amount of qualitative development is about the same at the beginning of each new period. These similarities will be discussed at the appropriate time.

In the sheep, the period of the ovum lasts from the time of ovulation until the attachment of the blastocyst to the endometrium which occurs about the tenth day. The embryonic period, during which time the genesis of the main organs and organ systems takes place, lasts about three weeks. The fetal period begins at approximately 34 days of age; this period is devoted primarily to growth and secondarily to continuing differentiation. It lasts until the time of parturition which is about 150 days postcoitus.

GAMETOGENESIS

Ram lambs are usually capable of producing functional sperm when about seven months old. Some ewe lambs come into heat and will conceive their first fall. The usual practice, however, is to have ewes drop their first lambs when approximately two years of age. The male then produces sperm continuously and will mate at any season of the year. Ewes exhibit a definite breeding season which continues from late summer to midwinter. During that period, they will exhibit signs of estrus about every 17 days, provided they do not become pregnant, and will stay in heat approximately 12 to 48 hours at any one time. Each ewe is quite regular in the length of her cycle and heat period and this affords an opportunity to estimate rather accurately her time of ovulation.

Gametogenesis in the sheep is similar to the processes in other farm animals. The testis contains more interstitial space than does that of the bull. This is not shown in figure 1a because of the emphasis placed on the one tubule. This cross section of a seminiferous tubule contains all of the cell types usually found in spermatogenesis and illustrates especially well the transformations involved in the process of spermiogenesis ("e", Fig. 1a). Mature sperm usually observable in tubular sections do not appear in this illustration. Figure 2a shows a section of a ripe ovarian follicle. Mature extragonadal gametes are to be seen in figures 3 and 4.



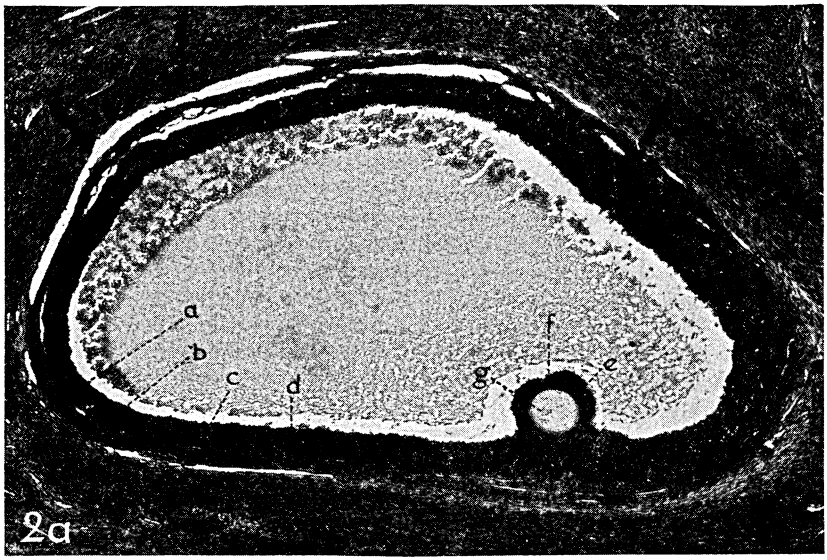
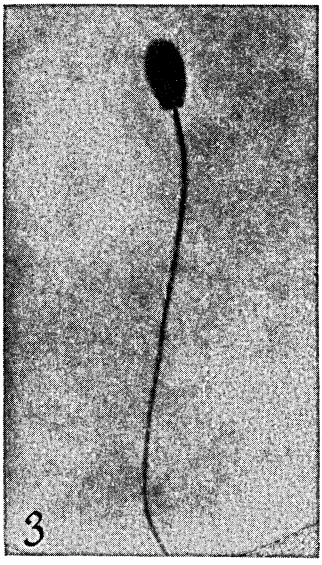


Fig. 1a. Cross section, seminiferous tubule ram testis. Bouin's fixative, Harris' hematoxylin counterstained with eosin, 5μ×413. (a) Spermatogonium, (b) Primary spermatocyte, (c) Secondary spermatocyte, (d) Spermatid, (e) Transforming spermatids, spermiogenesis, (f) Interstitial cell

Fig. 2a. Cross section ripe sheep follicle. Fixative, etc., same as Fig. 1a, ×53. (a) Tunica externa, (b) Tunica interna, (c) Membrana propria, (d) Stratum granulosum, (e) Cumulus oophorus, (f) Ovum, (g) Nucleus



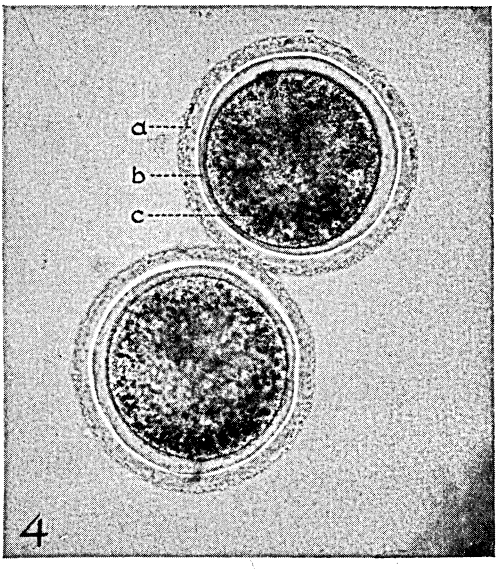


Fig. 3. Sheep sperm. Bouin's fixative, Heidenhain's iron-hematoxylin stain, ×1173

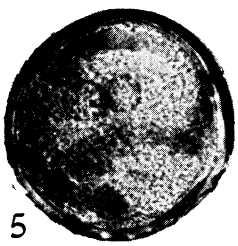
Fig. 4. Unsegmented sheep ova 37.5 hours, ×173. (a) Zona pellucida, (b) Vitelline membrane, (c) Cytoplasm

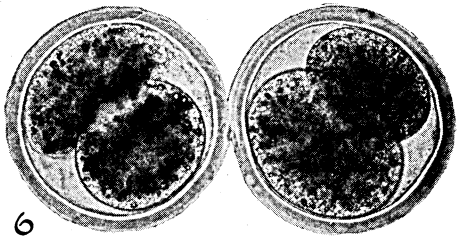
PERIOD OF THE OVUM

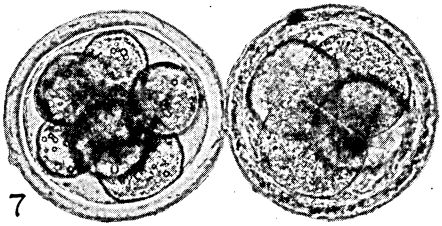
Segmentation of the sheep ovum appears to be initiated approximately a day and a half after the end of the heat period. During that interval, fertilization takes place and the two pronuclei are formed. Figure 4 shows a pair of twin ova which, although apparently fertilized, have not started to segment. They were recovered 37.5 hours postcoitus and were photographed while in saline and before fixation. Two pronuclei are to be seen in figure 5. This egg was 32.5 hours old at the time of recovery and had been fixed in Bouin's fluid and stained with Delafield's hematoxylin before photography in order to demonstrate the internal structure.

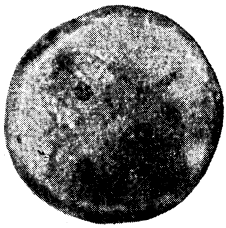
Early blastomere formation is illustrated by the two sets of twin ova shown in figures 6 and 7. The two-cell stage was found to exist 39 hours postcoitus. Four and eight blastomere ova were flushed from the oviducts 42 hours after the ewe had been bred. The egg in figure 8 was 44 hours old. It was stained with Delafield's hematoxylin but not sectioned prior to photography.

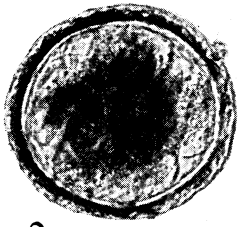
Cell division continued at a rapid rate during the second and third days. The ovum shown in figure 9 has nearly 16 blastomeres and was 2 days-17 hours old. At 3 days-5 hours, the 16-cell stage

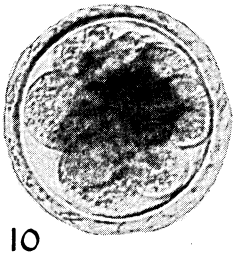

5

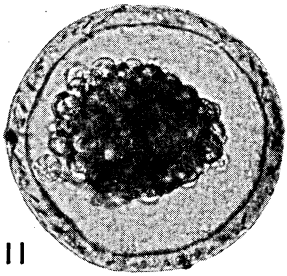

6

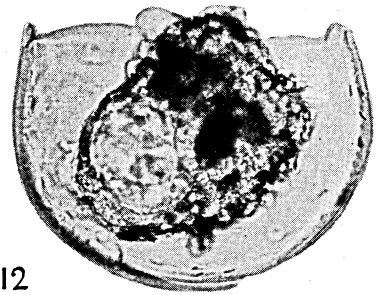

7

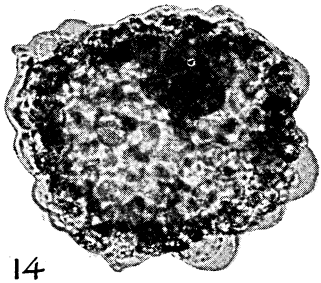

8

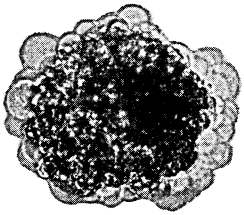

9

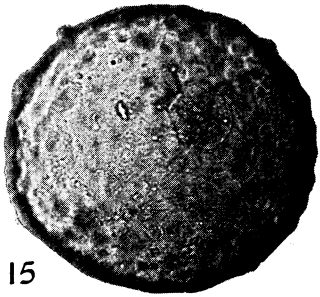

10


11


12


14


13


15

Figs. 5 and 8. Stained ova; Figs. 6, 7, 9-15. Fresh specimens; Figs. 5-14. ×168; Fig. 15. ×97
Fig. 5. 1 day-8 hours; Fig. 6. 1 day-15 hours; Fig. 7. 1 day-18 hours; Fig. 8. 1 day-20 hours; Fig. 9. 2 days-17 hours; Fig. 10. 3 days-5 hours; Fig. 11. 6 days-17 hours; Fig. 12. 8 days-2 hours; Fig. 13. 7 days-0 hours; Fig. 14. 7 days-13 hours; Fig. 15. 8 days-18 hours

was found (Fig. 10). Some of the blastomeres were of different sizes and this may be interpreted either to be the start of trophoblastic specialization or merely the natural reduction in cell size which may come at this stage of cell division. Indications of blastocoele formation appear in the specimen illustrated in figure 11. This ovum was 6 days-17 hours old. The trophoblast and inner cell mass may be noted. At this time, both in age and degree of development, the zona pellucida is about to be lost. It has changed in size and structure during the process of cell division. The thickness of the zona has progressively decreased, as indicated in table 1. In each instance the zona pellucida was measured at five points chosen at random and an average of the five readings was calculated. A concurrent change in structure was also noted and may be seen in figures 4 to 12. At first the zona pellucida seems to be made of two layers, the outer portion being composed of a matrix filled with small fibrils. The inner layer appears quite homogenous and much like the matrix of the superior layer. As the zona ages and thins, the fibrils become coarser and form a greater proportion of the whole. Accompanying this structural change is a change in the elasticity of the zona pellucida. When young, the zona is quite tough and will withstand relatively rough handling. It becomes much more fragile as time passes. This is true for both the fertilized and unfertilized egg.

The diameter of the zonal cavity is larger in the oldest specimen (Fig. 11). This change in size may be caused by a general weakening of the zona pellucida, or it may be the result of an interchange of new materials from the uterus which may have been permitted by a shift in the permeability of the zona itself.

The next specimens were arranged in accordance with their stage of development rather than by chronological age. An 8 day-2 hour blastula is shown in figure 12. It is in the process of losing the zona pellucida. The segmentation cavity is more fully developed than that of the preceding specimen and the inner cell mass shows more conspicuously. Further enlargement of the inner cell mass and the formation of the germ disc area are shown in

Table 1. Thickness of Zona Pellucida in Microns

Stage of development	Thickness of zona pellucida—average of five readings
	μ
Unsegmented	14.7
8 cell, 44 hours	13.3
16 cell, 77 hours	11.3
Blastocyst, 6 days-17 hours	8.4
Blastocyst, 8 days-2 hours	6.8

figures 13, 14, and 15. These blastules were 7 days-0 hours, 7 days-13 hours, and 8 days-18 hours old, respectively. During this time, the cells of the trophoblast change in character and form, and they become arranged into the spherical form characteristic of blastocysts at the time of attachment.

EMBRYONIC PERIOD

The blastocyst of the sheep attaches itself to the wall of the uterus by means of a surface-to-surface contact as do the blastocysts of cattle, rabbits, and swine. In the sheep, this attachment occurs about the tenth day while the blastocyst is spherical in shape.

One of these blastocysts was shown by Winters, Green, and Comstock *(8)*. It was 10 days-12 hours old and in the process of becoming attached at the time of recovery. The same situation was found for 9 day-18 hour and 10 day-22 hour old embryos shown in figures 16 and 17, respectively. Each of these gastrulas was sectioned and portions are shown in figures 16a and 17a. The trophectoderm in each was well developed; the entoderm had completely lined the inner surface of the outer layer, forming the archenteron or primitive gut. Mesoderm had not been formed. Spreading mesoderm may be seen in the 10 day-19 hour embryo of figure 18.

This embryo was recovered by dissection as were the previous blastocysts. The uterus was laid open while in a saline bath. After discovery, the blastocyst was moved gently by the use of blunt probes. It seemed to be attached to the uterus by means of an adhesive, mucin type of material. By probing the endometrium a short distance from the embryo, the specimen loosened from the uterine lining and floated into the saline. This type of attachment is very similar to that of the rabbit (Winters and Green, unpublished data).

At this time the trophectoderm is developed to the stage of rapid elongation, the spherical shape being transitory during the formation of the three germ layers. Early stages of elongation of the chorion are shown by twin embryos which are 10 days-16 hours old and are illustrated in figures 19A and B. The chorions were 5.66 mm. and 6 mm. long and the germ discs were 0.266 mm. and 0.33 mm. in longest diameter. Additional evidence of a differential growth rate between the sheep and the bovine at this early embryonic time is shown by the fact that the lengths of these twin chorions are about the same as those to be found for 13 day bovine chorions.

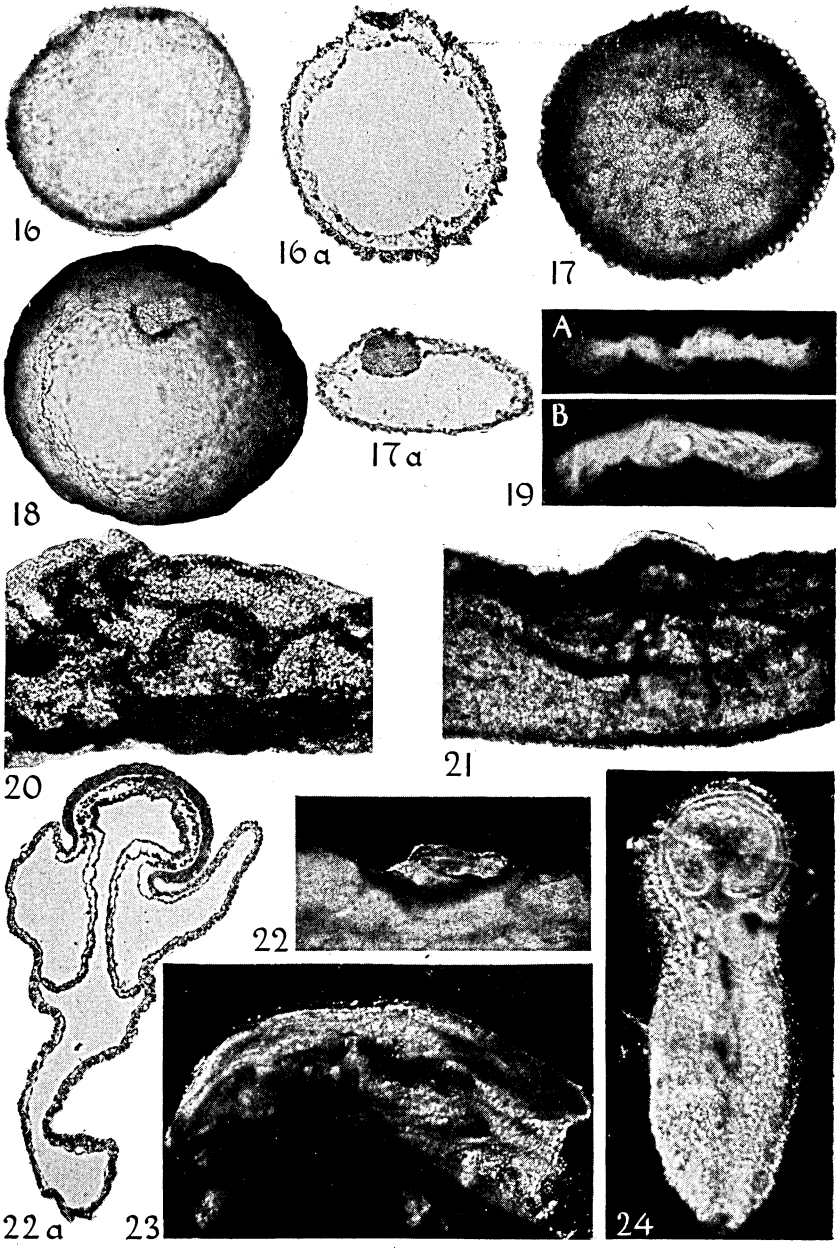


Fig. 16. 9 days-18 hours, ×40; Fig. 16a. ×72; Fig. 17. 10 days-22 hours, ×40; Fig. 17a. ×72; Fig. 18. 10 days-19 hours, ×34; Fig. 19. 10 days-16 hours, ×6; Fig. 20. 11 days-23 hours, ×36; Fig. 21. 12 days-13 hours, ×35; Fig. 22. 13 days-3 hours, ×15; Fig. 22a. ×72; Fig. 23. 14 days-17 hours, ×114; Fig. 24. 14 days-22 hours, ×162

Only the germ disc area and a small portion of the chorion of an 11 day-23 hour old specimen are shown in figure 20. This is somewhat similar to the 13 day-14 hour bovine specimen. A complete segment of the chorion with a side view of the germ disc area is shown in figure 21. The delicate folds near the germ area represent the first stages of the formation of the amnion in this 12 day-13 hour embryo. These folds are much more pronounced on the 13 day-3 hour specimen of figure 22. By this age the primitive streak has formed; the mesoderm has started to split and to associate itself with the ectoderm and entoderm forming embryonic and extraembryonic somatopleure and splanchnopleure; and the yolk sac has started to form. These may be seen in figure 22a which also presents a sectional view of the amnionic folds. Ectodermal specialization begins about this age and evidences of this may be seen in figures 23 and 24. The first presents a side view of a 14 day-17 hour embryo and the second figure shows a top view of a 14 day-22 hour specimen which possessed four somites. Special features of note are: the ectodermal plate, somites, neural folds, and primitive streak. Following these first differentiations, very rapid progress in the formation of new organs and organ systems ensues.

During the day following the age represented in figure 24, many changes are produced. The 15 day-20 hour embryo illustrated in figure 25 was 3.687 mm. long. The neural ridges of the

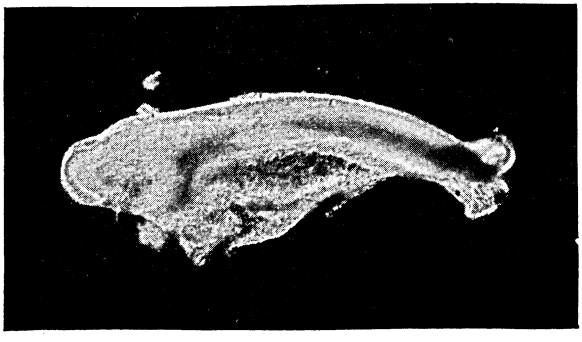


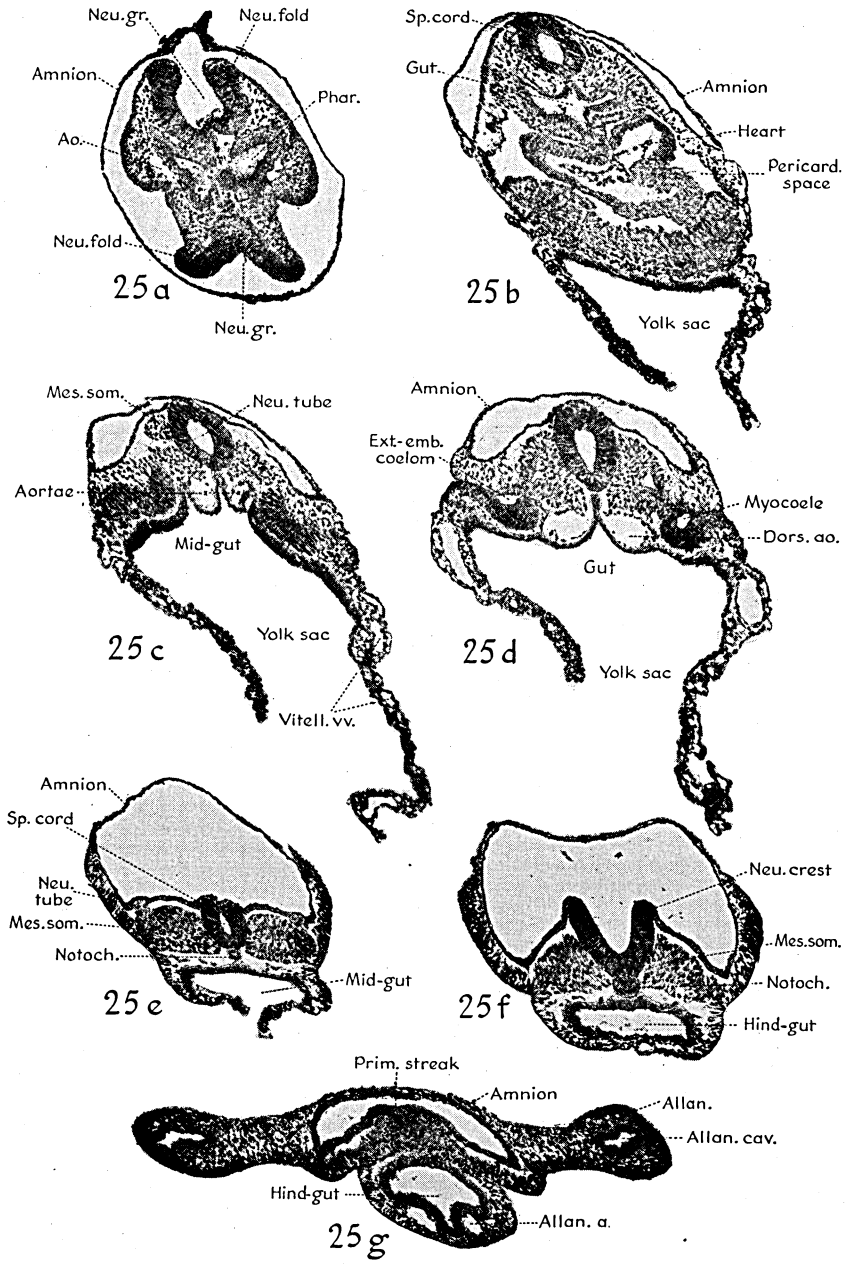
Fig. 25. 15 days-20 hours, ×16

future brain region are well formed. The neural tube has been enclosed approximately to the region of the hind-gut. Neural crests and the primitive streak were observed in the hind-gut area. In this region, the allantois had formed and had started to grow laterally. The yolk sac (cut away) had grown much larger.

A heart bulge had formed and some of the larger vessels could be seen from the exterior. This embryo was sectioned and 637 sections were secured. Representative cross-sectional areas are shown in figures 25a-g. The first picture was taken of section No. 64 (Fig. 25a) which showed the neural folds of the brain region and the neural groove immediately anterior to the neural tube. The other sections (Nos. 156, 170, 202, 401, 452, and 534), figures 25b-g respectively, illustrate the structure of the embryo at this age. The amount of vascular development associated with the allantois is indicated by the allantoic arteries shown in figure 25g and the large umbilical veins in the body wall (unlabeled) in figure 25d. The number of somites do not show clearly in figure 25. One embryo recovered at 15.5 days was 4 mm. long and 19 somites were present. The chorion of this specimen was still loosely attached.

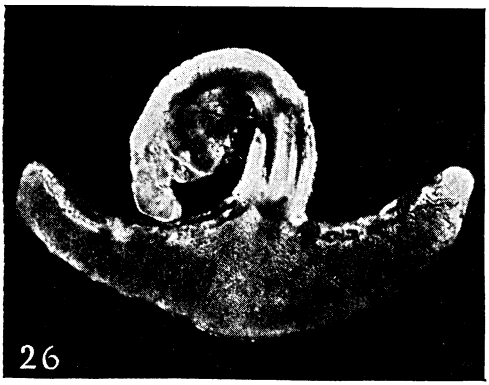
Further evidence of rapid progress during the first half of the third week of development is shown by the embryos of figures 26, 27, and 28 which were 16 days-17 hours, 16 days-19 hours, and 17 days-4 hours of age, respectively. The most striking external changes are the growth of the allantois and the flexion and torsion of the bodies. They are also passing from a straight form into the C shape so characteristic of the next phase of embryonic change. Apparently the shape of the specimen at this period may be influenced to some extent by the amnion. This is not true later in life. The embryos in figures 27 and 28 are still enclosed in their somatopleure covering while the amnion had been removed prior to photography in figure 26. Amnionic removal allowed the embryo to curve to a greater extent and also allowed the observance of more structural detail. This same phenomenon may be seen in figures 29A and B.

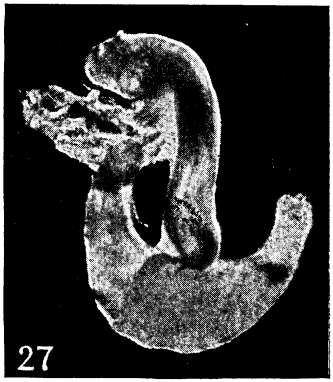
New features are to be found in this trio of embryos. The cephalic region has undergone differentiation in all its portions. Closure of the brain folds has resulted in the formation of a tubular brain and the specialization of certain areas. The lens placoid area is not as easily seen as the area of the otocyst. Lower jaw development has started. One gill cleft is formed. In the mid-region, the heart bulge has enlarged, and the heart chambers may be seen from the exterior. The mesonephros is relatively well developed and the denser tissue shows distinctly from the outside. Posteriorly, the cloaca has been formed and the sinus rhomboidalis, previously discernible, was illustrated well in figure 28. At 17 days, the chorion extends about three-fourths the length of the uterine horn which, in the sheep, is 10-12 cm. long.



Figs. 25a-25g.* ×72

* Explanation of the abbreviations appears in the appendix.





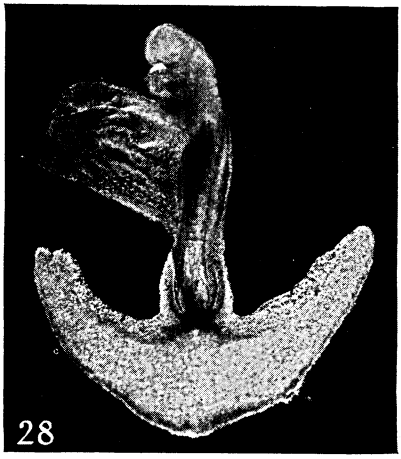
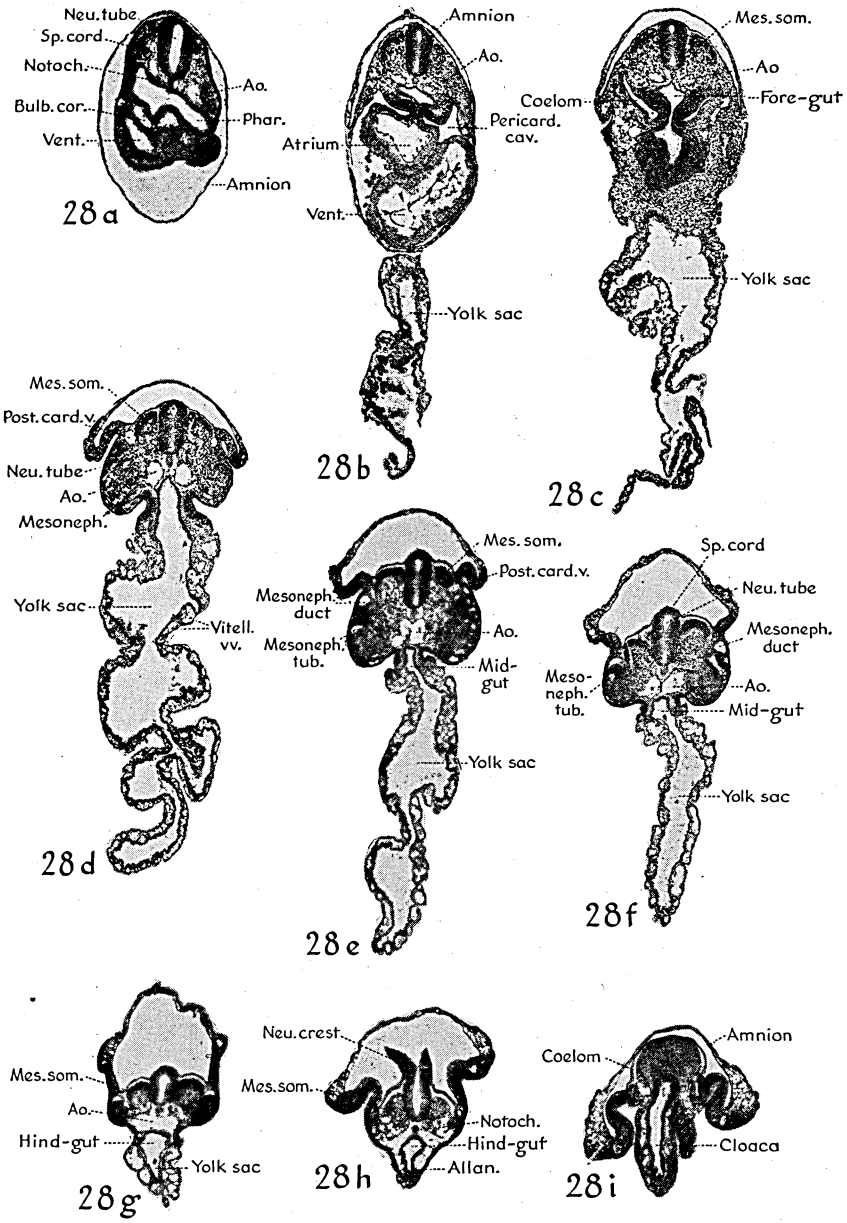


Fig. 26. 16 days-17 hours, ×8; Fig. 27. 16 days-19 hours, ×8; Fig. 28. 17 days-4 hours, ×8

The embryo and allantois shown in figure 28 were sectioned and yielded 555 units. The nine sections presented in figures 28a-i were selected to show typical cross sections at various levels. The section numbers in order of the figure letters were: 45, 88, 120, 162, 199, 227, 276, 358, and 409. Vascular structures of interest are the bulbus cordis, the further development of the heart, and the anastomosis of the aortae. Comparison with the sections of figures 25 a-g will show the rapid growth of the mesonephros.

Figures 29A and 29B are of the same 17 day-20 hour embryo enclosed in and free from the amnion. The yolk sac and allantois were removed in each instance. Differences in form again illustrate the possible effect of the amnion on bodily form at this stage. The beginning of the **C** shape of the embryo is also more



Figs. 28a-28i. ×34

pronounced but it is not fully developed until the stage shown in figure 30. Embryos at this age are rather translucent and permit external observance of many internal structures. The heart chambers of figure 29B show well because of this reason. Mesonephric regions are distinct and that tissue is progressively becoming more dense as well as larger in bulk. The optic region is to be seen here but shows more distinctly in figure 30. Two gill bars are present in the figure 29 specimen as are the upper limb buds. Lower limb buds were seen for the first time in the 18 day-22 hour specimen shown in figure 30. The third gill cleft has now made its appearance. The liver which was formed prior to this age is now becoming enlarged. Four of the 634 sections secured from this embryo are shown in figures 30a-d. They are, in order, sections No. 178, 209, 337, and 552. The amount of development of the organs illustrated should be noted. The gastrointestinal system has progressed in many respects. The stomach has started to enlarge and curve. Liver specialization has progressed and the gall bladder has formed. Intestinal growth resulted in an elongation of that structure. Cardiac development has kept pace with the rest of the body; the structure of the bulbus cordis should be noted. Figure 31a is a section of an 18 day-14 hour embryo and is used to show the mesonephros in the region antipodal to the cephalic region.

Near the end of the third week, many structures of the body cavity have been formed. For a few days henceforth there seems to be a change in developmental activity. Structures already formed continue to grow rapidly and undergo further differentiation, but few new major organs are formed during this time.

Evidence of the increase in bulk of the internal organs is shown in the next embryo and in sections of other specimens of similar ages. Figure 32 illustrates a 20 day-20 hour embryo. The accompanying sections, figures 33a-i and 34a and b, were secured from three different individuals. In the figure 33 series, portions of each of a pair of 21 day-1 hour twins are shown. One, figures 33a-e, was sectioned into 841 units and those selected were Nos. 109, 224, 309, 490, and 530. Parts of the second twin complete the series. The third specimen used was 21 days-18 hours of age. All were near enough in chronological and developmental age to allow adjacent presentation. The main features of note are the well-developed bifurcated lung bud and the general growth and progressive development of structures previously formed.

Continuing growth was still the main activity of the 21 day-19 hour and 23 day-23 hour embryos presented in figures 35 and 36.

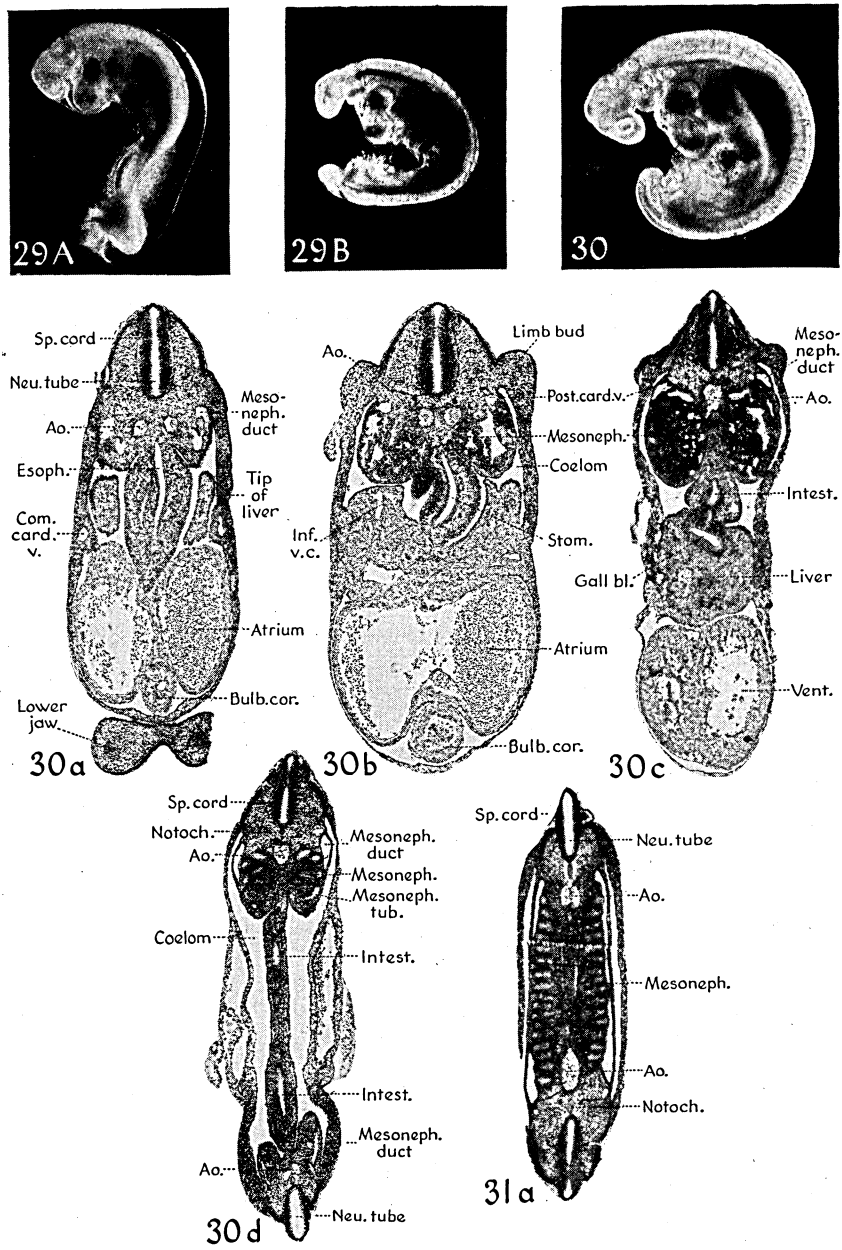
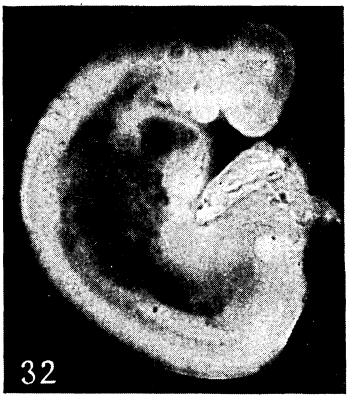
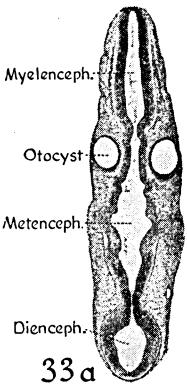
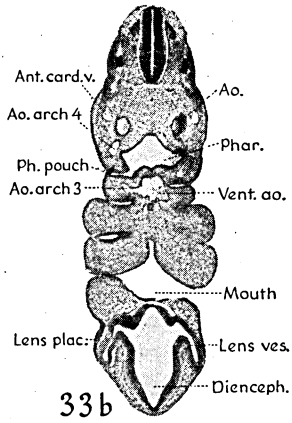
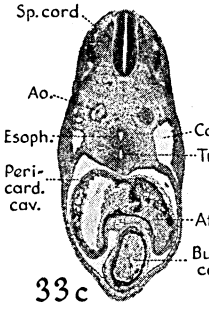


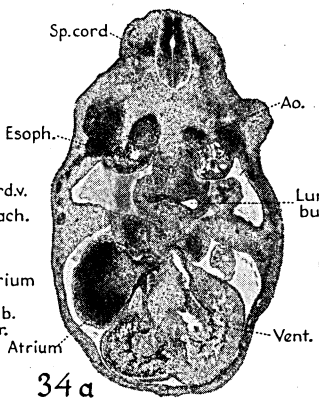
Fig. 29. 17 days-20 hours; A, Amnion intact; B, Amnion removed; Fig. 30. 18 days-22 hours, ×6.4; Figs. 30a-30d, 31a. ×28

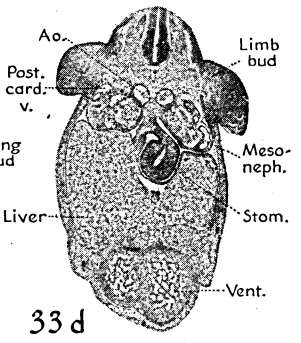


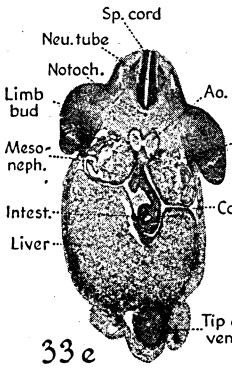


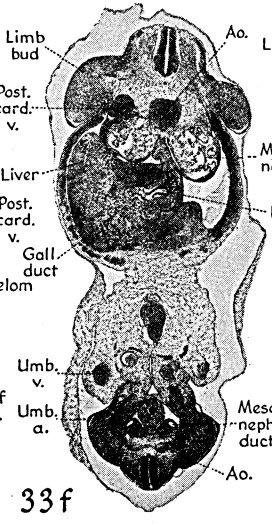












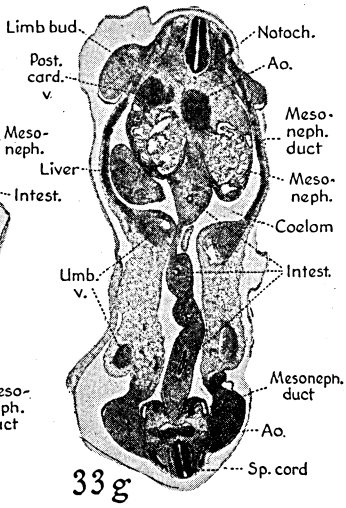
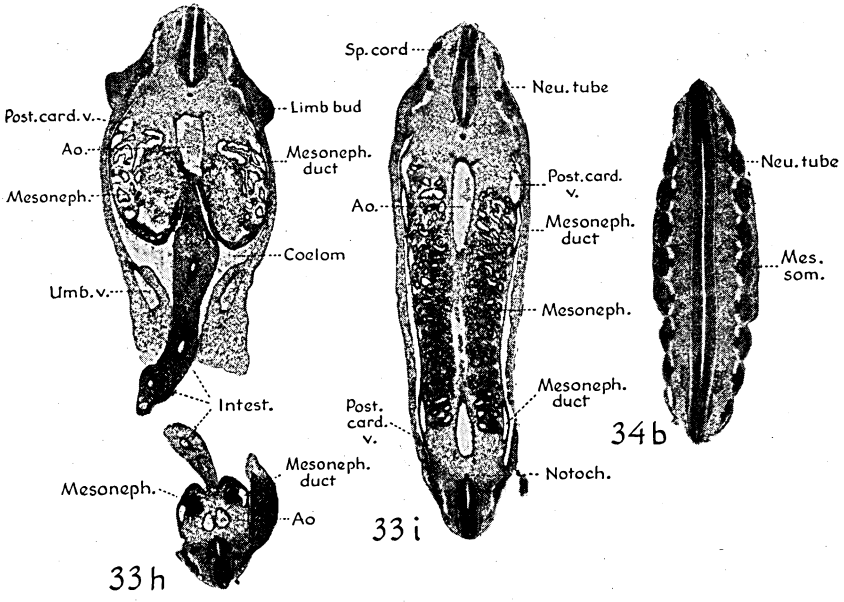
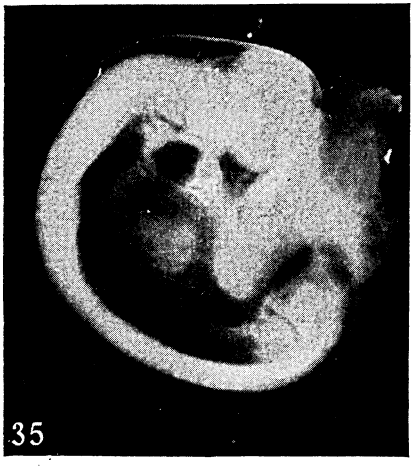
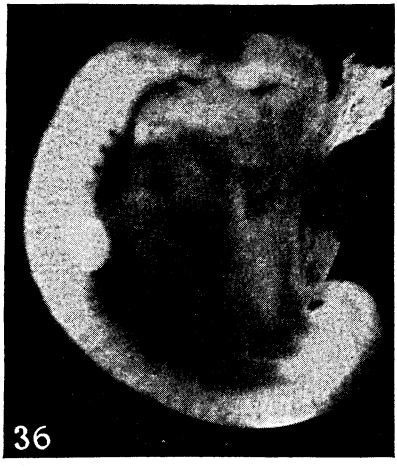


Fig. 32. 20 days-20 hours, ×6.4; Figs. 33a-33e. ×16; Figs. 33f-33g. ×12.8; Fig. 34a ×16







Figs. 33h-33i. ×20; Fig. 34b. ×20

Fig. 35. 21 days-19 hours; Fig. 36. 23 days-23 hours

The body at these ages still is of a C shape. Internal features of a 23 day-14 hour embryo are illustrated in figures 37a-h. When cut, 1,215 sections were secured. Those used in order of their lettering were: 213, 384, 480, 589, 789, 857, 1053, and 1121. The section in figure 36a was secured from the embryo shown in figure 36 and was used because it better illustrated the structures of the stomach region than did similar sections of the embryo used for the figure 37 series. Special note should be made of the bulbus cordis in figure 37c.

Between 25 and 29 days a change in bodily form from the C shape to that more nearly resembling the fetus takes place. It is during this time that the gonad and metanephros begin to form. The specimens shown in figures 38 and 40 were 25 days-19 hours and 28 days-23 hours of age, respectively, while the one used for sectioning, figures 39a-g, was exactly 27 days old. Throughout the head region to the level of the liver, the sections were cut at 5μ as usual. From then on, the thickness was changed to 8μ, i.e., through the liver and posterior regions. The change took place at section No. 1205 and this accounts for the relatively fewer sections, 2,057, for an embryo of that size. Progressive changes in growth may be seen in figures 39a-c (section Nos. 120, 720, and 1091) if reference is made to the preceding sections. Starting of the urogenital system may be seen in the next pictures. The gonadal ridge is labeled in figures 39d and e which represent section Nos. 1226 and 1367. Both mesonephric and metanephric ducts are to be seen in figure 39f (section No. 1570). In all of these figures, there is a greater condensation of mesoderm in the areas of future bone formation than was found in previous sections.

This outlining of future cartilaginous tissue is more distinct in the extremities of the 30 day-22 hour embryo of figure 41. When sectioned, this embryo yielded 2,079 sections and Nos. 723, 894, 1069, 1269, and 1496 are shown in figures 41a-e. The main new feature of this specimen is the metanephros. The distal ends of the limb buds appeared furrowed, foreshadowing digital formation. The brain cavities of this embryo may be seen in relief and give external evidence of internal structure and development.

The next two specimens demonstrate the situation in the developing sheep at the transitional stage between the embryonic and fetal periods. As previously stated, the setting of an exact age for the ending of the embryonic period is purely an arbitrary, artificial, but nevertheless useful expedient not without rationalization. Winters and Feuffel *(7)* used 34 days as the beginning of the fetal period and the results of this study justify that choice.

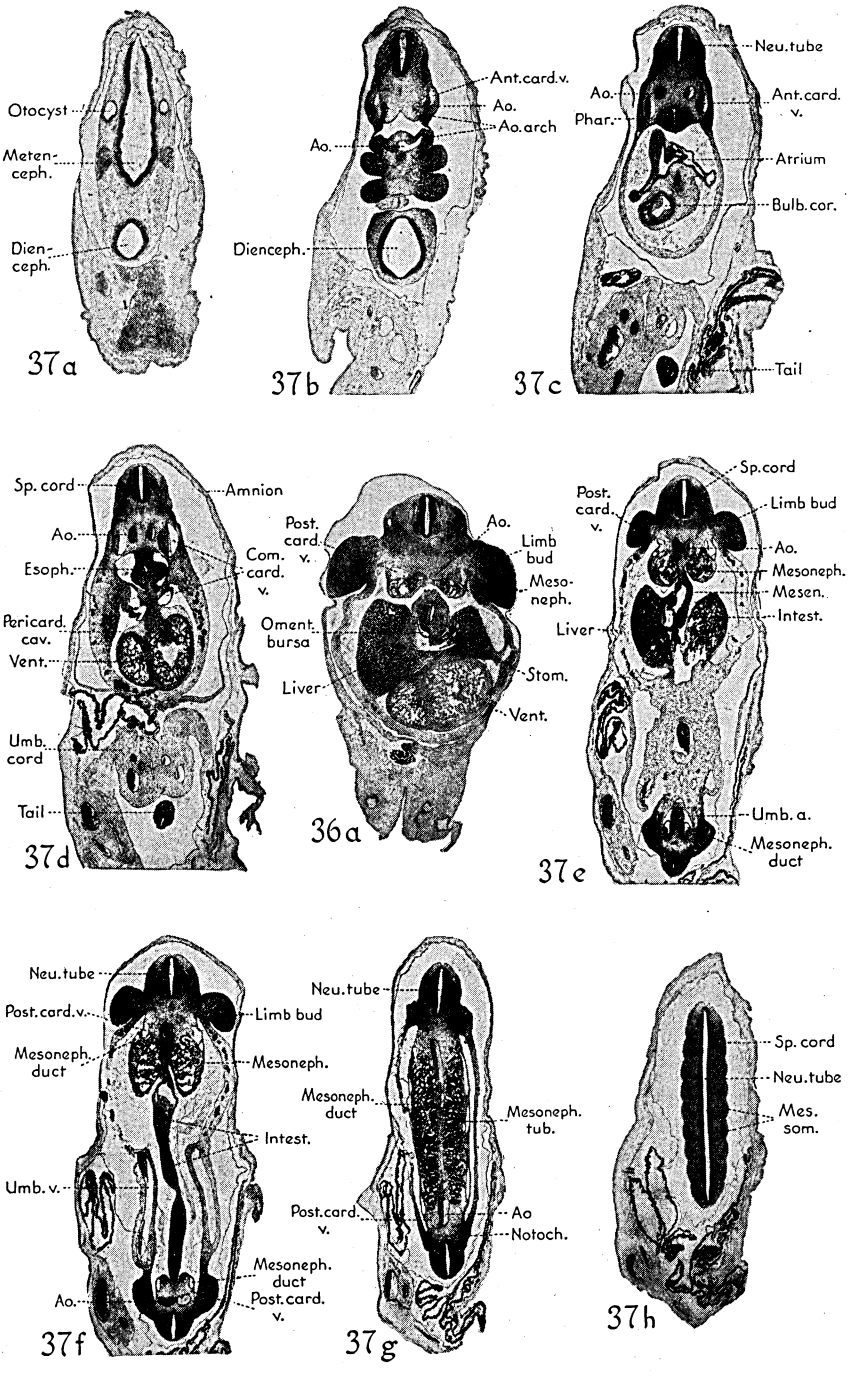
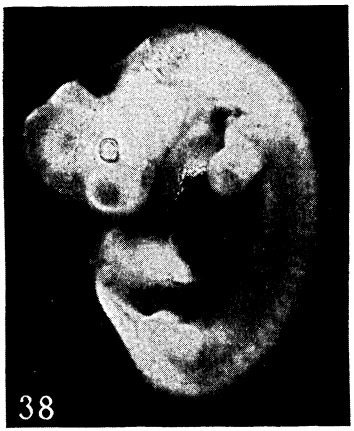
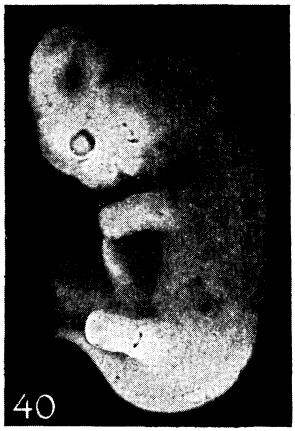


Fig. 36a, Figs. 37a-37h. ×9.3





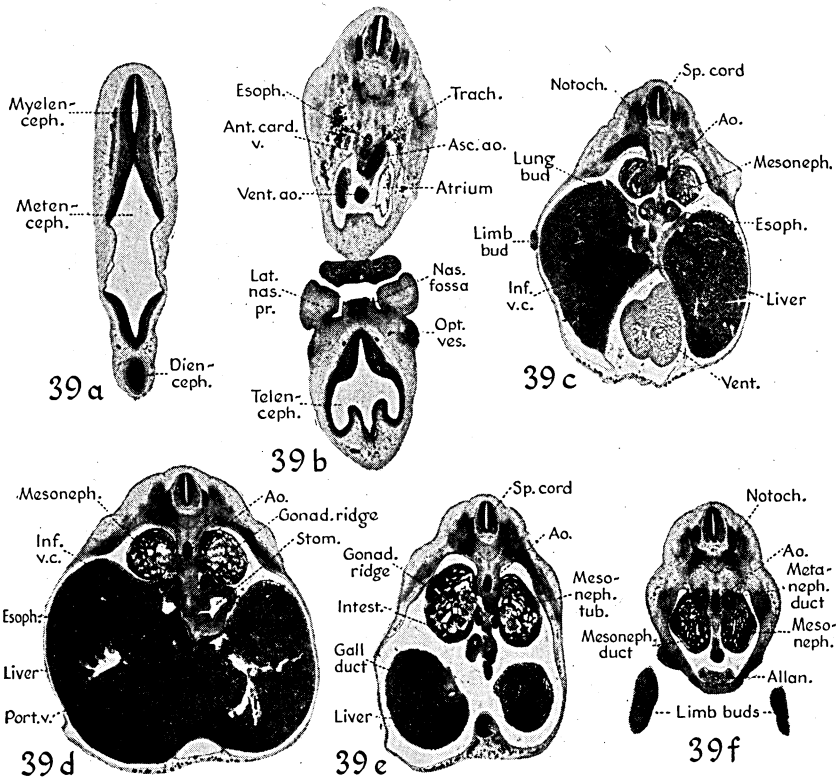


Fig. 38. 25 days-19 hours; Figs. 39a-39f. ×9; Fig. 40. 28 days-23 hours

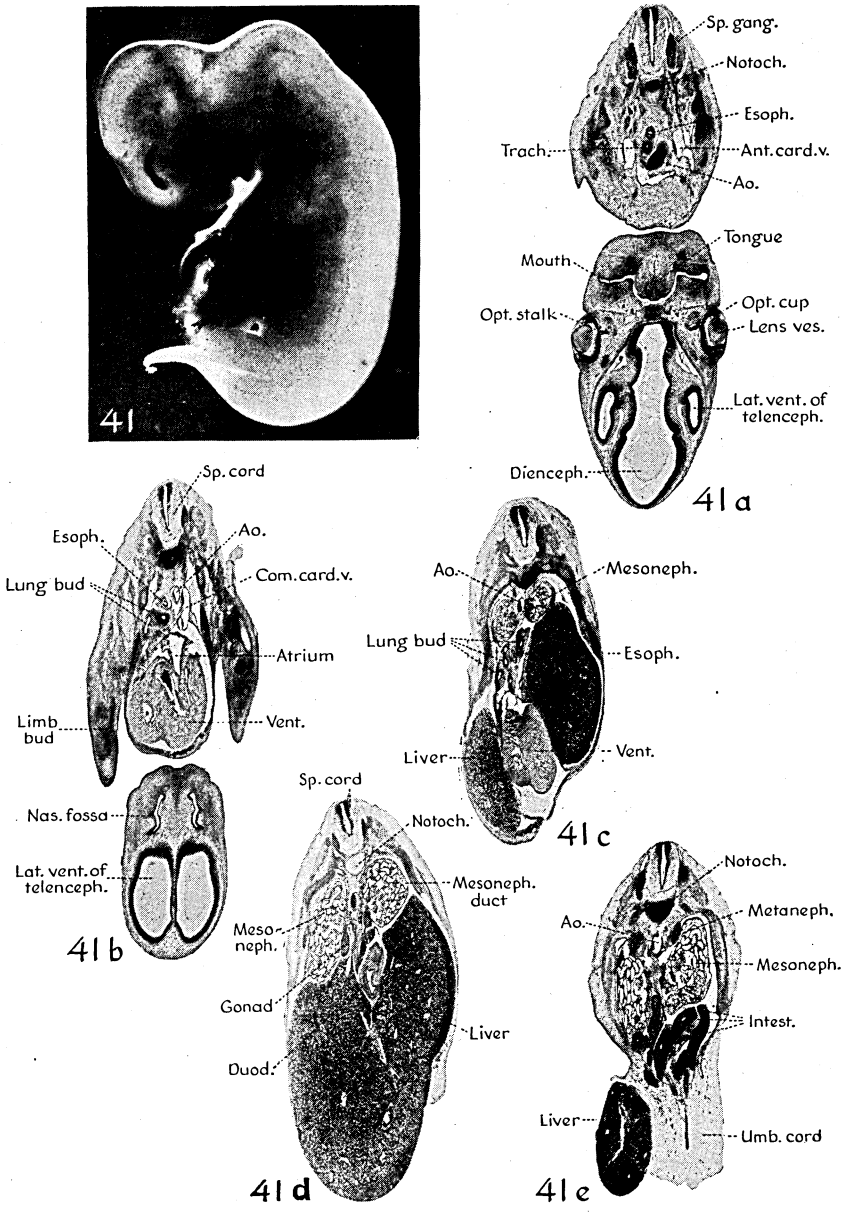
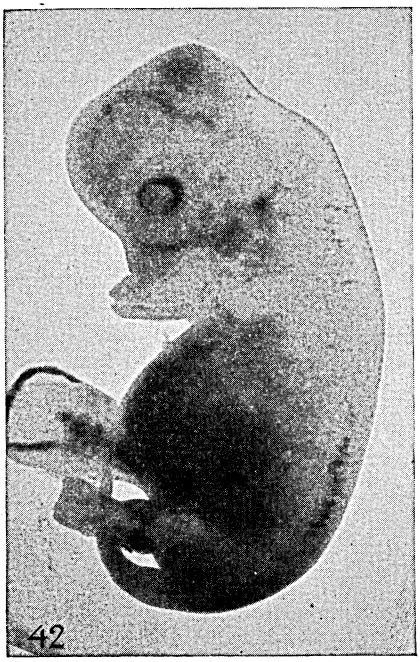


Fig. 41. 30 days-22 hours, ×4.8; Figs. 41a-41e. ×9

Prior to that age (see figure 42) the activity of organogenesis was prominent. These changes were observable both in section and in external appearance. The embryo passed through a series of shapes which may be used to characterize stages of the embryonic period. During the middle of the fifth week it undergoes another shift in appearance from that of the embryo to that of the fetus. It is true, of course, that future bodily outline will be different from that of the 34 day point but those contour shifts will result mainly from variation in actual and relative sizes of organs and will be due mainly to changes in bulk and density of structures and less because of continued differentiation of new formations. Although the 34 and 36 day specimens of figures 42 and 43 could rightfully appear with the fetuses of the following section, they are still near enough to the late embryonic stage to be grouped with the embryos if one so desires. Because they show the structure at this transitional period and because of the manner in which the material is pictorially presented, the two will be used to close the discussion of the embryonic period.

Some external changes exhibited by these specimens are of importance. The external ear which had previously started to



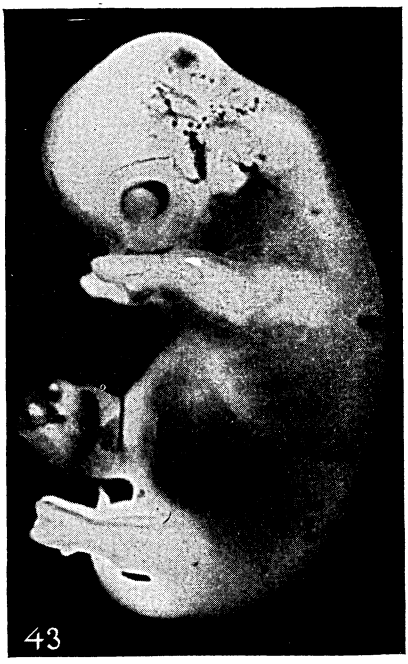


Fig. 42. 34 days, ×2.8; Fig. 43. 36 days, ×2.8

form is becoming prominent on both embryos. Regional specialization of the appendages is evident in the 34 day fetus and is much more prominent in figure 43. Outlines of future osseous areas seen only in sections at earlier ages are now visible from the exterior. Sections of the 36 day fetus are presented in figures 43a-l. The structures are sufficiently well developed and are near enough to adult positions to eliminate the necessity of giving sectional numbers.

FETAL PERIOD

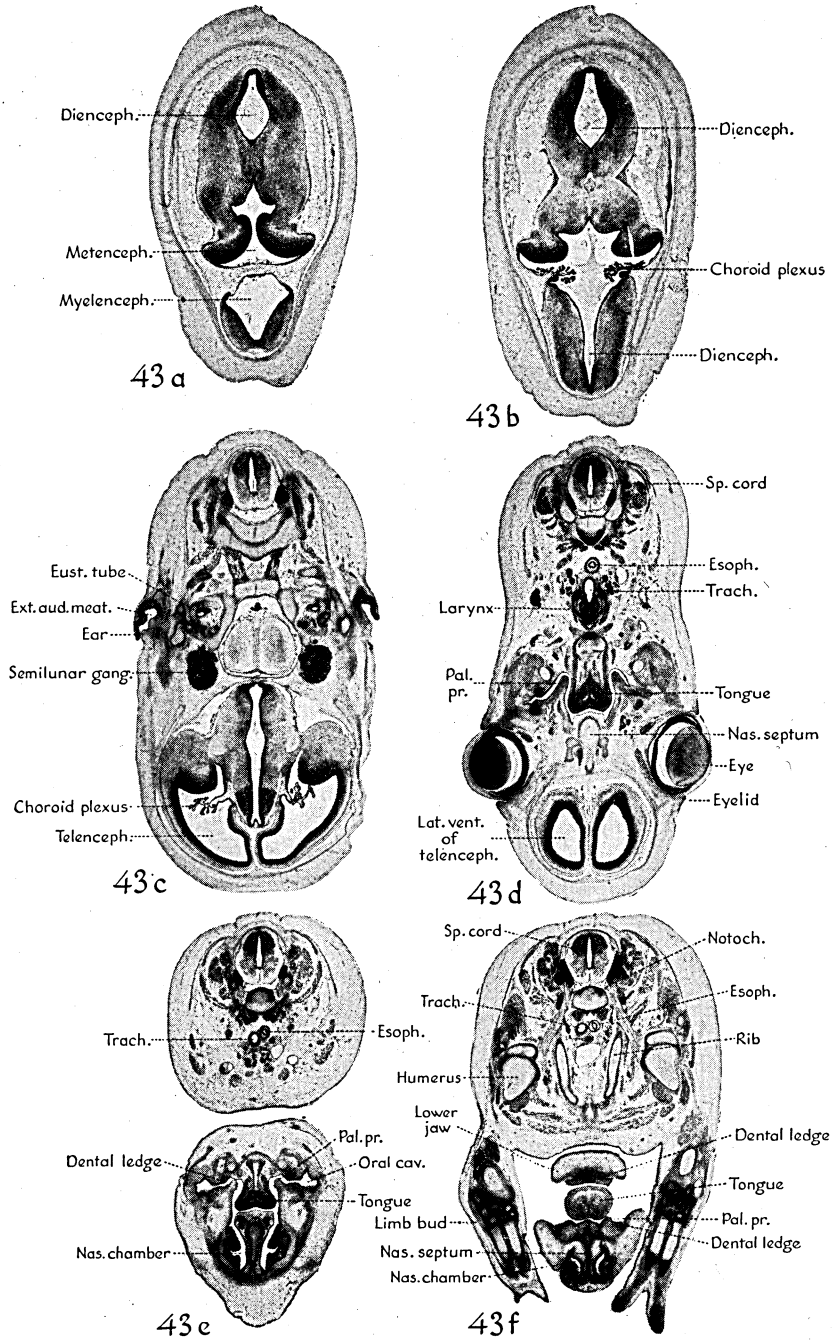
At the beginning of the fetal period, the sheep and bovine are in similar stages of development and are also alike in appearance. There is, however, an apparent discrepancy in the percentage of the gestation period which is consumed by the fetal period. In the sheep it accounts for about 77 per cent of the total gestation time and about 84 per cent of the total in the bovine.

Early changes in form are parallel in the two species. Anteriorly, they consist of: reduction in size of the cephalic prominence, modification of the cervical flexure, formation of facial features, remodeling of head structure, growth of the eyelids, elongation of the neck, and rotation of the axis of the head. The liver prominence becomes reduced and the body wall thickens. All external features become more sharply defined. This is especially true of the appendages. Most of these changes have occurred by the time the sheep is 46 days of age, figure 46, or at the end of approximately 30 per cent of the gestation period. Cattle fetuses are at the end of the first one-fourth of the pregnancy period at the comparable stage.

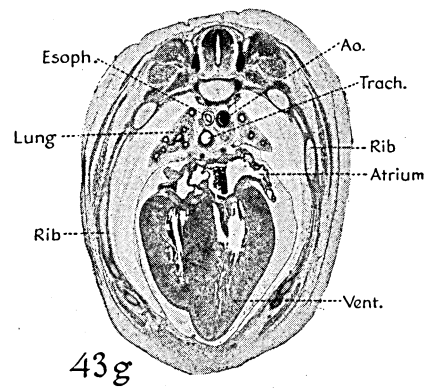
Study of figures 42 to 56 will show that the last 90 per cent of the sheep's fetal period is concerned chiefly with growth and continuing differentiation. External qualitative changes during these weeks include the appearance of a few hairs about the muzzle and eyes at about 90 days and a complete hair covering at approximately 116 days. Pigment was first observed on the 104 day specimen shown in figure 53. A newly born lamb less than 12 hours "old" is shown in figure 57.

Discussion

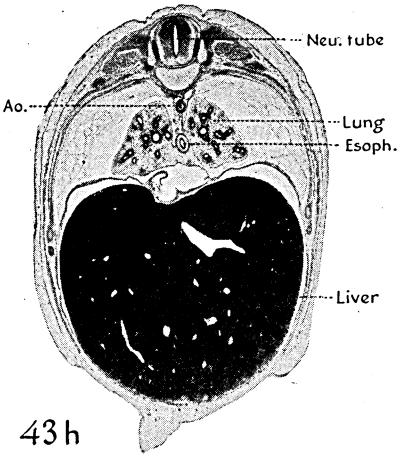
The prenatal development of the sheep is very similar to that of the bovine; however, the two species do differ in their rates of development. This, of course, is obvious as the bovine has 1.87 times as long a gestation period as the sheep and the young are



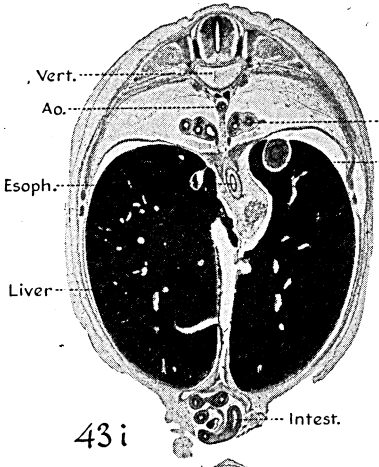
Figs. 43a-43f. ×6.4



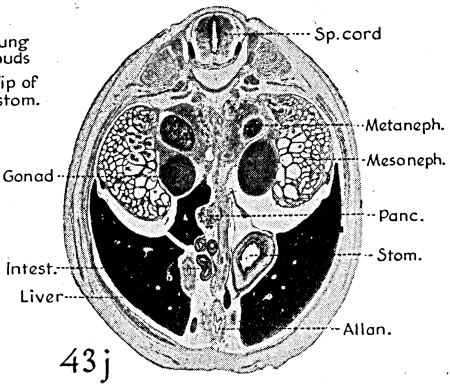
43g



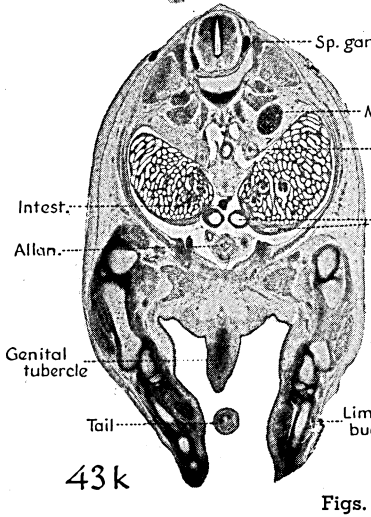
43h



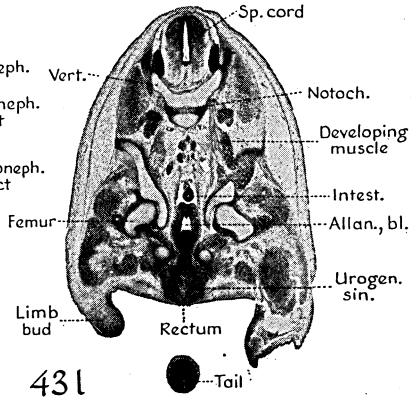
43i



43j



43k



43l

Figs. 43g-43l. ×6.4

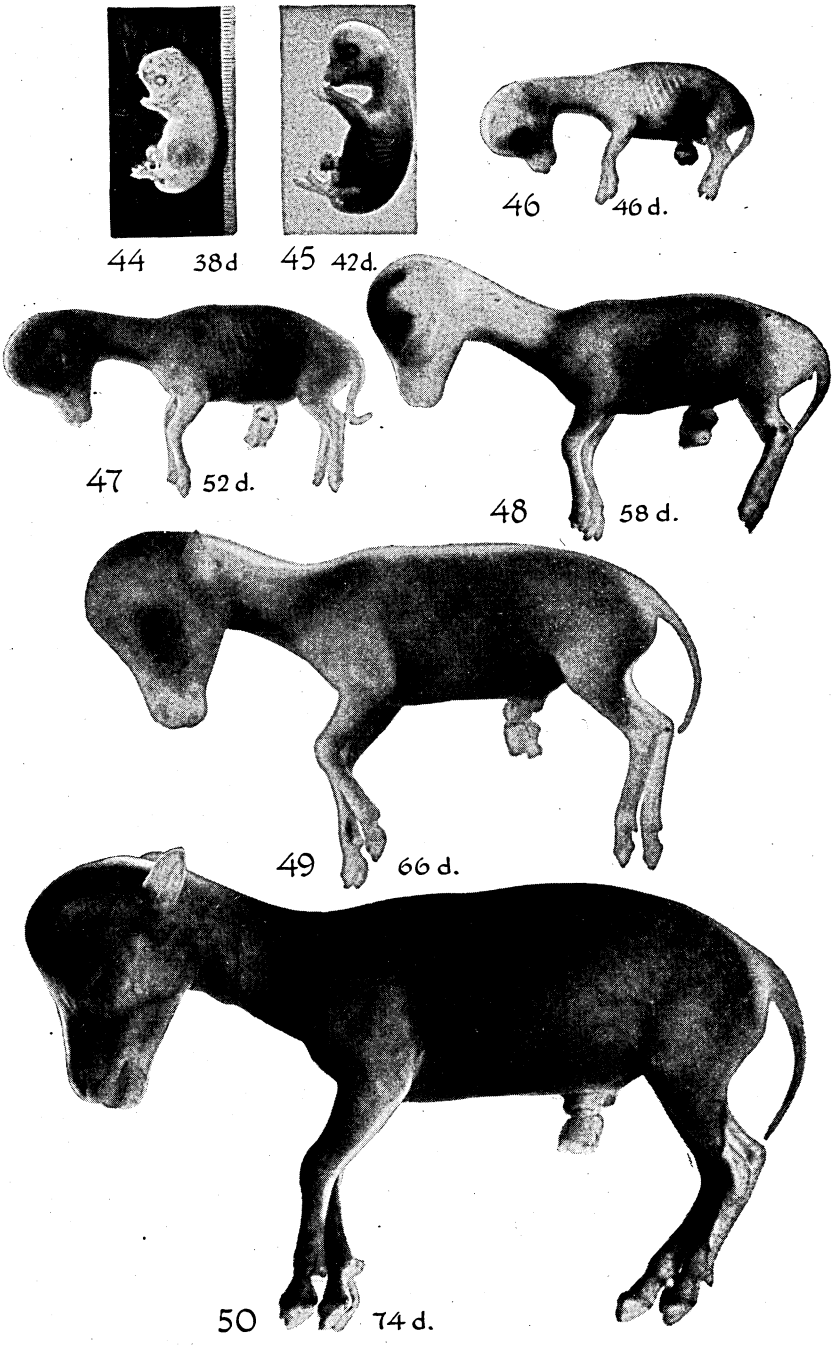
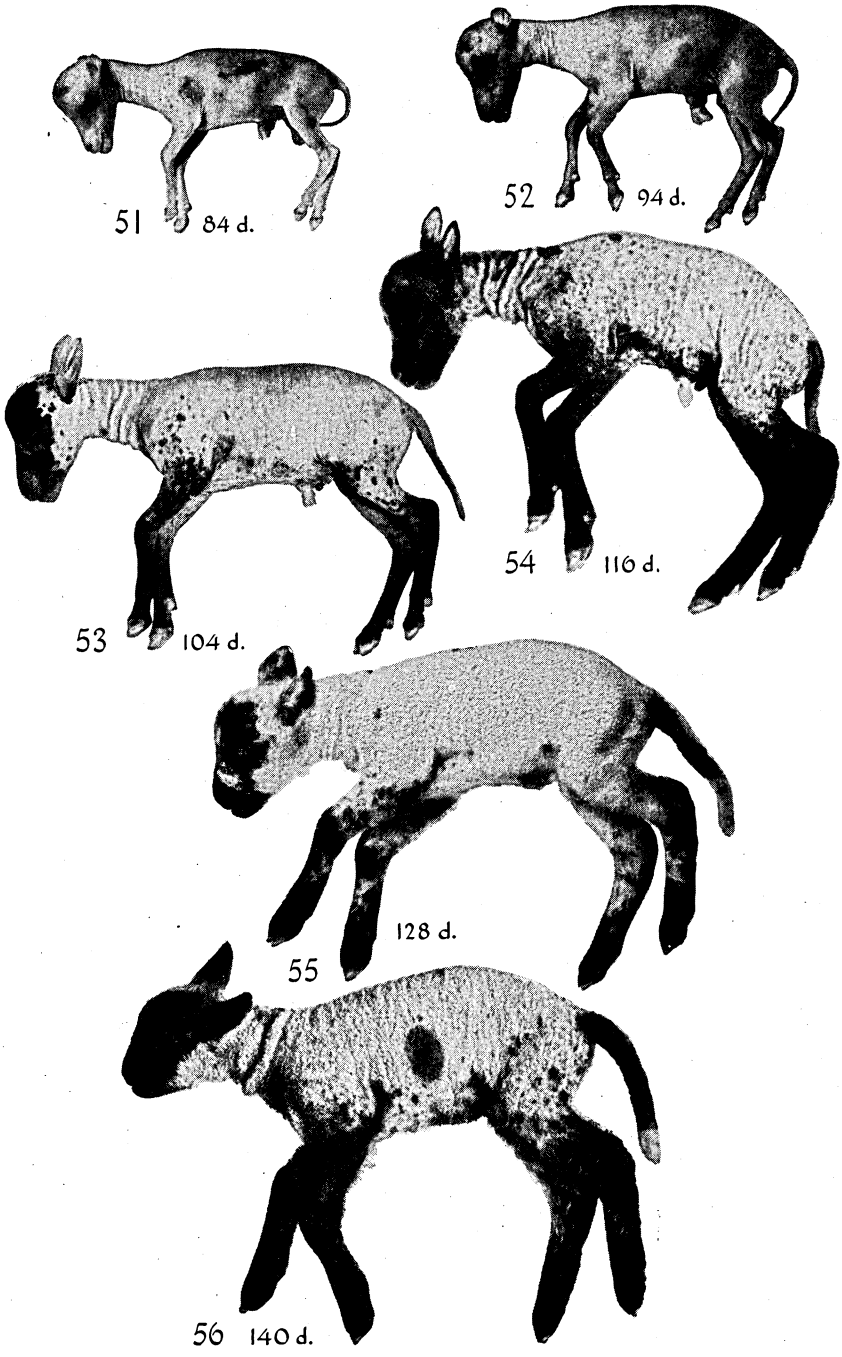


Fig. 44. ×0.46; Figs. 45-50. ×0.5.



Figs. 51-54. ×0.165; Fig. 55. ×0.134; Fig. 56. ×0.120

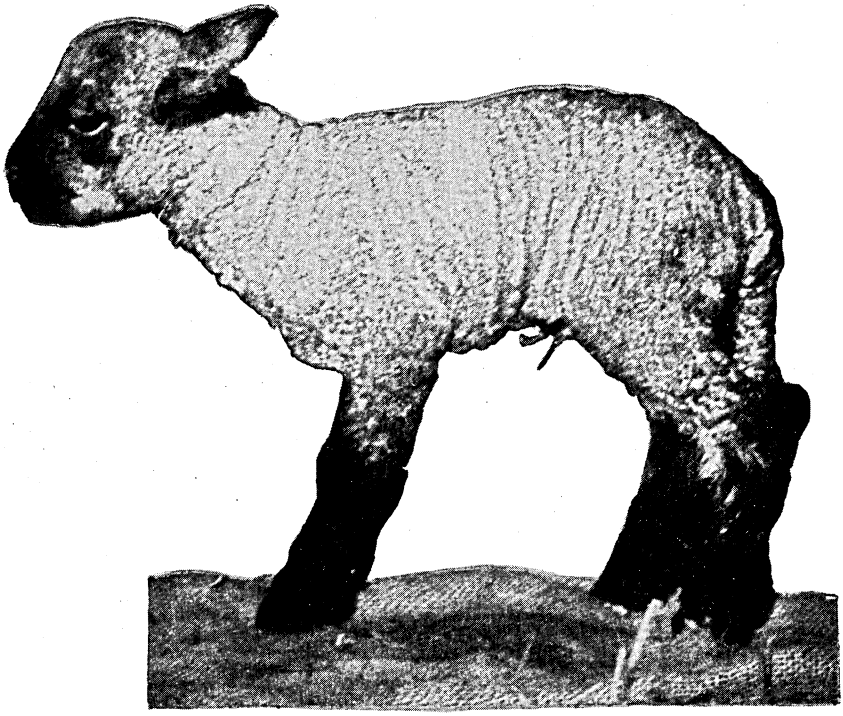


Fig. 57. Newborn lamb

in approximately similar stages of development at the natal period. Various periods or the occurrence of similar events do not, however, occupy equal portions of the gestation period.

During the first week, the development of both species progresses at the same rate. This similarity is quite noticeable when the ages of the specimens are based on postovulatory time rather than on the time postcoitus. The ewe ovulates approximately at the end of estrus and the cow liberates the ovum during the first day postestrum. Subtraction of one day from the ages of those given for the bovine would approximate the postovulatory age of the sheep. The data of table 2 are based upon this method of evaluating age and disclose the parallelism in developmental rates up to the time of formation of the spherical blastocoele.

Descent of the ovum into the uterus likewise occurs at similar times in the two species: during the fourth day in the sheep and during the fourth postovulatory day in cattle, as the first uterine egg was found 4 days-14 hours postcoitus in the latter species. In each instance the ova were in 16- to 32-cell stages.

Table 2. Rate of Postovulatory Development of the Sheep and Bovine

Stage of development	Postovulatory age at time of recovery	
	Sheep	Bovine
2-cell	39 hours	26, 36 hours
4-cell	42 hours	.....
6-cell	.....	38 hours
8-cell	42, 44 hours	40 hours
16-cell	3 days-7 hours	3 days-14 hours
32-cell	4 days-17 hours	4 days-14 hours
Blastula formation	6 days-17 hours	6 days-14 hours
Zona pellucida lost	End of 6th day—start of 7th day	End of 6th day—start of 7th day
Spherical blastocoele	8 days-18 hours	10 days-20 hours

Attachment of the embryo to the uterine mucosa occurs about the tenth day in the sheep and approximately one day later in the bovine. In both cases the gastrocoele is spherical in shape, the entoderm is present, the germ disc may be seen, and the trophoblast is ready to elongate; they are essentially equal in development but not in age. At the time of attachment the percentage of the gestation period which has elapsed is 6.66 for the sheep and 3.93 for cattle. A rapid divergence in developmental rates was noted during the embryonic period. Both species were similar in development at both the initiation and end of the period; yet the sheep had 10 to 11 fewer days in which to form its main organ systems. Formation and differentiation proceeded at similar, relative rates, as the embryos of the two species were practically identical at the same stages of embryonic development even though their chronological ages differed. In the bovine 12.5 per cent of the gestation period was consumed during embryonic development, while 16 per cent was used by the sheep. Prefetal times were 34 days or 23 per cent of the gestation period for sheep and 46 days or 16 per cent for the bovine. A study of growth data previously published for both species indicates that the relative growth rates of some bodily parts are similar during the fetal period.

Changes in the thickness and structure of the zona pellucida so evident in the sheep were not found to any similar degree in the bovine ova. Neither was the change in the size of the zonal cavity. Perhaps a real species difference exists in the structure and aging processes of the zona. Further study would have to be completed to verify the observation.

X-ray and some other features of the bovine study were not conducted for the sheep, as the sheep data were collected prior to those for the bovine, and certain additions to the general prob-

lem were suggested to the cattle study from this study of the sheep. With the exception of the 36-day sheep specimen, all of the sheep embryos were stored in 70 per cent alcohol for 10 to 12 years before they were sectioned and stained. Surprisingly little difficulty was encountered in either procedure. One specimen was a little difficult to stain and the liver of another gave some trouble when sectioned. Otherwise the embryos reacted almost as well as specimens which were stored for a matter of a few weeks or months.

In contrast with the relatively large number of abnormal ova found in the cattle study, very few were recovered from the sheep. This may have been due to the fact that all of the ewes were mature.

The conclusions reached in the discussion of the bovine data were substantiated by this study. Length measurements are apt to be unreliable unless the embryos are treated in an identical manner. The presence of the amnion, at least for the sheep, so alters the shape and thereby the length measurements that treatment of the specimens must be fully described if measurements are to be of value for comparison of the results of different workers. This study also verifies the thought previously suggested that certain growth measures such as weight, volume, etc., while giving a very general picture of the gross changes really are not reliable for accurate studies of growth. The relative size, density, and other features of various tissues and organs change so rapidly and so much in absolute terms that the assignment of growth eras for the specimen as a whole loses much of its significance.

Summary and Conclusions

1. The prenatal development of the sheep was studied by use of specimens of known ages.

2. Photographs of specimens made prior to fixation and photomicrographs of sections were presented and discussed.

3. Pronuclei formation and the segmentation of the ovum into two blastomeres as well as the four- and eight-cell stages were found during the second day of development.

4. Ova reach the uterus during the fourth day and in about the 16- to 32-cell stage.

5. Blastocoele formation was first noticed in a 6 day-17 hour old specimen.

6. The zona pellucida was lost near the beginning of the seventh day. Up to this time sheep and bovine ova develop at the same rate.

7. The zona pellucida and the zonal cavity of the sheep ovum undergo profound changes in size and structure not seen in the bovine.

8. The sheep blastocyst attaches itself to the wall of the uterus about the tenth day. The chorion then elongates rapidly.

9. The archenteron was fully formed at the end of the ninth day.

10. Mesoderm was first observed in a 10 day-19 hour specimen.

11. Four somites were found at 14 days-22 hours.

12. Allantois formation was evident on a 15 day-20 hour embryo.

13. Very rapid development takes place in the sheep embryo during its third week of development. The basic structures for most of the organs and organ systems are laid down at that time.

14. The fetal period begins at approximately 34 days. A specimen showing the structure of the sheep at this stage was presented and sections illustrating the internal structure were also pictured.

15. The relative rates of development of the prenatal sheep and bovine were discussed.

Acknowledgments

Most of the specimens used in this study were gathered by Dr. R. T. Clark and Mr. George Feuffel while graduate students of the junior author. The authors wish to express their appreciation for the help given by Louise Combs Weber in sectioning and staining the embryos. The photographs and photomicrographs were made by Mr. V. P. Hollis, Director of the Photographic Laboratory of the University of Minnesota. The photographs were mounted and labeled by Miss Jean Hirsch, Director, Medical Art Shop, University of Minnesota.

LITERATURE CITED

1. AREY, LESLIE B. Developmental Anatomy. Ed. 3, 593 pp. W. B. Saunders Company, Philadelphia. 1934.

2. CLARK, RICHARD T. Studies on the physiology of reproduction in the sheep. II. The cleavage stages of the ovum. Anat. Rec. **60**:135-159. 1934.

3. CLOETE, J. H. L. Prenatal growth in the Merino sheep. Onderstepoort Jour. Vet. Sci. and Anim. Indus. **13**:417-558. 1939.

4. GREEN, W. W., BARRETT, CATHERINE, and WINTERS, L. M. A technic for the sectioning of mammalian ova and blastocysts. Stain Technol. **12**:43-47. 1937.

5. ———— and WINTERS, L. M. Studies on the physiology of reproduction in the sheep. III. The time of ovulation and rate of sperm travel. Anat. Rec. **61**:457-469. 1935.

6. PATTEN, BRADLEY M. The embryology of the pig. Ed. 2, 327 pp. P. Blakiston's Son and Co., Philadelphia. 1931.

7. WINTERS, L. M., and FEUFFEL, GEORGE. Studies on the physiology of reproduction in the sheep. IV. Fetal development. Minn. Agr. Expt. Sta. Tech. Bul. 118. 1936.

8. ———— GREEN, W. W., and COMSTOCK, R. E. Prenatal development of the bovine. Minn. Agr. Expt. Sta. Tech. Bul. 151. 1942.

APPENDIX

Explanation of Abbreviations

A.—artery
Allan.—allantois, allantoic
Ant.—anterior
Ao.—aorta, aortic
Asc.—ascending
Bl.—bladder
Bulb. cor.—bulbus cordis
Card.—cardinal
Cav.—cavity
Com.—common
Dienceph.—diencephalon
Dors.—dorsal
Duod.—duodenum
Esoph.—esophagus
Eust.—eustachian
Ext. aud. meat.—external auditory meatus
Ext.-emb.—extraembryonic
Gang.—ganglion
Gonad.—gonadal
Gr.—groove
Inf.—inferior
Intest.—intestine
Lat.—lateral
Mes.—mesodermal
Mesen.—mesentery
Mesoneph.—mesonephric, mesonephros
Metaneph.—metanephric, metanephros
Metenceph.—metencephalon
Myelenceph.—myelencephalon
Nas.—nasal
Neu.—neural
Notoch.—notochord
Oment.—omental
Opt.—optic
Pal.—palatine
Panc.—pancreas
Pericard. cav.—pericardial cavity
Ph.—pharyngeal
Phar.—pharynx
Plac.—placode
Port.—portal
Post.—posterior
Pr.—process
Prim.—primitive
Sin.—sinus
Som.—somite
Sp.—spinal
Stom.—stomach
Telenceph.—telencephalon
Trach.—trachea
Tub.—tubule
Umb.—umbilical
Urogen.—urogenital
V.—vein
V. c.—vena cava
Vent.—ventral
Vent.—ventricle
Vert.—vertebra
Ves.—vesicle
Vitell.—vitelline
Vv.—veins