Prenatal Growth in the Merino Sheep.*

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CONTENTS.

CHAPTER 1.—INTRODUCTION.

OBJECT OF WORK.

THAT growth during the foetal period does not differ qualitatively from that subsequent to birth is the view generally accepted. It is maintained that, in spite of its greatly varying rates and its change of direction from progressive during the developmental period to regressive during senescence, growth is one continuous process starting with fertilization of the ovum and ceasing with death of the individual. The progressive part of the cycle is " divided by the incident of birth into pre- and postnatal periods " (Arey, 1931). When it is considered how readily the latter period lends itself to investigation, it is not surprising to find that much work has been carried out in this connection. Reference to Brody *et al* (1926) or to Hammond (1932) will give an idea of the extent of the available literature.

In direct contrast to this wealth of material is the paucity of information regarding the prenatal phase of development. What knowledge does exist has been gained in studies mostly on small laboratory animals, such as the rat (Stotsenberg, 1915), the mouse (MacDowell *et al*, 1927), and the guinea-pig (Draper, 1920). In general the results of this work have confirmed the thesis of continuity of the growth curve. However, MacDowell and his associates believe that on account of qualitative differences in growth during the earliest stages, age for the purpose of growth curves should be calculated from the time of formation of the " embryo proper ".

For man and the domestic mammals there is a lack of extensive and reliable data. That such work on the human being presents well nigh insurmountable difficulties is only too readily understood. A brief indication of the complexity of the problem is given by Mall (1910). Not being bound by the same ethical laws as his medical colleague, the worker in the veterinary field encounters but one major obstacle—the brake of economic considerations. To expand on the seriousness of this problem would merely be labouring the point. Therefore, it is apparent that any work which aims at making available additional material advances greatly the facilities for the investigation of prenatal growth.

Scattered throughout the veterinary literature are isolated references to weights and dimensions of small numbers of foetuses of known or assumed ages. Due to the diversity of conditions under which the observations were made and the almost general lack of accurate definition of procedure, these figures cannot be employed in the compilation of one comprehensive table.

In this field most workers have avoided committing themselves as to the age of their material. Invariably they have relied upon either length or weight as an indication of age-sequence. Consideration of breed and individual variability will show that such criteria are apt to give misleading results. Especially would this be the case where the material is obtained from such an

uncontrolled source as a public abattoir. A further disadvantage of this procedure, exposed by Lowrey (1911), is that in the absence of data regarding age, absolute growth-rates cannot be given. The worker has to content himself with expressing growth of systems and organs relative to body weight.

The existence of adequate ageing standards would make available for studies on prenatal growth the abundance of material always obtainable at large abattoirs. As a result of the work to be reported in this paper, there is now available at Onderstepoort a complete series of accurately aged ovine foetuses. By direct comparison with this standard unknown foetuses are aged with a fair degree of accuracy.

The very nature of this standard, excellent as it is, militates against the extension of its sphere of usefulness beyond local limits. The primary object of this paper is to place this standard at the disposal of workers farther afield. It is hoped that this aim will be achieved by the publication of a partly descriptive and partly statistical study of those features of the collection which appear to be of importance in age determination.

As the development of the foetus is intimately connected with changes in the maternal genitalia, organs of lactation and endocrine glands, all these aspects of the reproductive process have received attention and will be considered in this paper. In some instances, it is felt, the numbers of observations are too limited to allow of definite conclusions. Nevertheless, the figures are given in the hope that, with appropriate additions by other investigators, eventually a large mass of data will be accumulated.

In connection with the literature it must be pointed out that every effort has been made to avoid what may be termed " second-hand " quotations. However, where it has been impossible to obtain the original article in this country, it is felt that recourse to such quotation is preferable to losing entirely a useful reference.

guidance of Dr. Laurence, Statistician, I would have experienced much difficulty in arriving at an accurate interpretation of the vast amount of data collected.

My thanks are due to Messrs. C. G. Walker and T. Meyer for the efficient manner in which they have dealt with the figures and plates, and to Mr. F. D. Horwell, technical assistant in the Section of Anatomy, for his valuable services throughout the course of the work.

Finally, it is a pleasure to record my appreciation of the assistance rendered by my wife, not only through her deep interest, at all times an inspiration, but also in typing the entire manuscript and assisting with the graphs and tables.

CHAPTER 2.—REVIEW OF LITERATURE.

A search of the literature has emphasised the meagreness of authoritative knowledge of prenatal growth of domesticated mammals. Much of the available knowledge is contained in works of which foetal development was not the primary consideration. In such cases the information, being merely incidental, received but scant attention. This literature is, to some extent, supplemented by studies on small laboratory mammals, in which the general principles of growth are apparently similar to those in larger mammals.

For the sake of clarity the literature will be treated under headings corresponding with those to be employed in the consideration of the experimental data.

(a) GENITAL TRACT AND PLACENTA.

1. *Vagina.*

Anatomists differ as to the nomenclature of that portion of the female genital tract situated posteriorly to the cervix. The English school refers to the portion between the cervix and the external urethral opening as the vagina. Everything posterior to this is called the vulva. In German works this latter part is further divided into the vestibulum vaginae, which constitutes the major portion, while the term vulva is restricted to the external opening of the urogenital sinus. In this work the German teaching will be followed, except for the fact that vestibulum and vagina will be taken together and will be referred to as " vagina ".

Sisson (1927) found that the vagina of the sheep has a length of between 10·5 and 11 cm. He states that the labiae of the vulva are thick and firm and that the ventral commissure is prominent.

From a consideration of the anatomical situation of the vagina it would appear that, after having served as a portal of entry for the sperm, this structure does not again participate in the reproductive process until the approach of parturition, when it dilates to allow of birth. On account of its relative unimportance this organ does not receive attention in embryological texts. However, it seems

obvious that during the period of pregnancy certain changes must occur and that the purport of these must be to render the vagina a larger and more easily distensible organ.

It has long been noted that with the approach of term there is an enlargement and a relaxation of the vulva. Craig (1912) and Williams (1917) refer to the soft and flabby nature of this organ prior to parturition. Hammond (1927) states that " just prior to parturition the vulva and the vagina become red and swollen." While studying parturition in the mare, Holzgruber (1925) observed that these changes are recognisable as long as three weeks prior to the birth of the foal.

Hammond (1927) finds that the vaginal pregnancy changes resemble closely those of the oestral cycle. They are mostly of a microscopic nature. In the anterior half of the vagina the changes are similar to those of the adjacent portion of the cervix. In this portion of the vagina is found a fair amount of sticky mucous material. Opperman (1922) states that during pregnancy the vaginal wall has a dry and sticky feel.

2. Cervix.

In the ruminant the cervix is an extremely well-developed structure, with walls that are thick, dense and inelastic (Ellenberger & Baum, 1921). The cervix of the sheep has a length of about 4 cm. and its lumen is practically obliterated by reciprocal prominences and depressions of the mucous membrane (Sisson, 1927). Trautmann (1917) also indicates that in this species the uterine seal is well-developed. The presence of such a seal during pregnancy is mentioned by many authors. For the cow, Williams (1917) gives details of the cranio-caudal development of the mucous plug. Woodman and Hammond (1925) show how in the bovine the actual quantity of mucous material increases steadily throughout pregnancy. There can be no doubt that this accumulation of mucus effectively closes the uterine canal during the major part of the gestation period.

In dealing with the physiology of parturition Craig (1912) states that there is a softening and a loosening of the cervical walls " a few days prior to parturition." Zeiger (1908) shows that there is also an enlargement of the cervical canal towards the end of pregnancy. In the bovine he finds that by the end of the seventh month the canal is sufficiently open to allow of the introduction of one finger. Hammond (1927) is of opinion that, apart from sealing off the uterus, the steady accumulation of mucus in the cervix assists in the dilation of this structure.

3. Uterine Body and Horns.

The uterus of the sheep is bicornuate. According to Sisson (1927) each horn has a length of from 10 to 12 cm. As a result of the gradual tapering of the anterior extremities of the cornua there is no definitely discernible external landmark indicative of the exact point of junction of the horn with the Fallopian tube. Posteriorly the two horns unite to form the body. The arrangement of the peritoneal covering makes this undivided portion appear longer than it is. The approximate length of the body is 2 cm.

Roux (1936) gives the weight of the uterus of the non-gravid Merino sheep as varying between 15·5 and 77·5 gm. He deals with eight groups of sheep, some of which, having been fed on low level rations, were in very poor condition. In these latter sheep the extremely low uterine weights were encountered. Although he does not find it possible to demonstrate a definite correlation between the weight of the ewe and the weight of its uterus, Roux nevertheless concludes that nutrition does exert an influence on uterine development. It is not possible to determine to what extent previous pregnancies might have affected the figures given by him.

Favilli (1928) gives the weight of the ovine uterus as varying between 40 gm. in virgin ewes, and 65 gm. in previously pregnant sheep. His observations, having been made in the slaughter-house, are not likely to represent poor conditioned sheep. This might explain the absence of figures as low as those mentioned by Roux. Favilli gives the weight of the uterus at term as 1,010 gm. This figure appears somewhat high and in a later publication (Favilli, 1929), it is stated that such a figure is obtained in cases of twin pregnancies. With a single foetus the figure is about 600 gm. Favilli (1928) quotes figures to show that similar increases in uterine weight occur in the bovine. Malan and Curson (1937) present figures for the weight of the empty uterus at the end of each month of pregnancy. These show that there is a steady increase in the weight of the organ from about 60 gm. up to about 700 gm.

Rorik (1907) shows that in the bovine there is a definite increase in the weight of the uterus during pregnancy. During the first half of pregnancy this increase is most marked. What few figures are given by Hilty (1908) seem to support this view. Hammond (1927) obtains his figures from heifers pregnant for the first time, thus eliminating the effects of previous gestations. These figures show that by the end of the period the non-gravid weight of the uterus has increased almost twentyfold.

Figures for the pig (Stegmann, 1923) show that by the end of the thirteenth week of pregnancy the non-gravid weight of the uterus has been doubled.

Draper (1920) gives a complete series of weights for the guinea-pig uterus throughout pregnancy. These figures indicate that the growth curve of the uterus is of double parabolic nature, i.e. first slow, then increasing rapidly and later slowing down again. This type of curve had already been suggested by the figures quoted above for the bovine and the sheep.

In all these cases it is the weight of the empty uterus that has been considered. This figure is the sum of the weights of the uterine wall and of the placenta. As will be seen later, the placenta undergoes a great deal of growth during pregnancy and it exhibits its own type of curve. It is evident, therefore, that the weight of the empty uterus is influenced greatly by the inclusion of the placenta. In the ruminant it is an easy matter to separate the placenta from the uterine wall and thus obtain the weight of the latter alone. However, no such figures appear to be available.

As might be expected, the increase in weight of the uterus is accompanied by an increase in size. For the sheep Favilli (1928) shows that the area of the uterus increases from less than 100 sq. cm. in the non-gravid state to almost 1,600 sq. cm. at the end of gestation. He also demonstrates (1929) that there is a steady daily increase of the volume of the uterus. Stegmann (1923) shows that in the pig the length of the uterine horn becomes increased during pregnancy.

Favilli (1928) maintains that the increase in size of the uterus is brought about not only by a multiplication of muscle fibres, but by the enlargement of existing fibres. He gives measurements in support of this view. Hammond (1927) also mentions this point and indicates that during pregnancy the uterine muscle appears sparsely nucleated- an indication of the enormous increase in the cytoplasm of the cells.

Favilli (1929) draws attention to the decrease of the thickness of the uterine wall during pregnancy. From the fact that the greatest reduction occurs fairly early during gestation he concludes that passive stretching is not the sole cause of the thinning. He states that, due to the even distribution of pressure in the liquid contents of the uterus, the thinning is spread evenly throughout the uterine wall. In the goat the non-gravid thickness of 8 mm., is reduced to 1·5 mm. near term (Zeiger, 1908). This author maintains that the reduction is greater in the pregnant horn " where the pressure is higher." Hilty (1908) demonstrates similar reductions in the case of the bovine.

Further information regarding the effects of pregnancy upon the uterus may be obtained from comparisons between virgin and involuted uteri. For the sheep Behne (1929) finds that the effect of pregnancy is to increase enormously the blood and lymph vessels. Not only is there an increase in size, but also in number. The elastic coats of there vessels are greatly thickened. For the goat similar results are reported by Hackeschmidt (1920). The changes in the structure of the bovine uterus, observed by Kraft (1923), resemble closely those of the sheep (Behne). Kraft finds that the microscopic picture of the involuted uterus is so typical as to allow of immediate diagnosis of a previous pregnancy. Sommer (1912), also dealing with the bovine, makes the statement that after pregnancy the uterus never regains its virgin state. The weight of the uterus becomes permanently increased in the approximate ratio of 2 : 1. He maintains that as a result of previous pregnancy there may be an inequality in the size of the two uterine horns. In the pig Stegmann (1923) gives the weight ratio between virgin and involuted uteri as 1 : 2. Richter (1936) agrees that this is so when average figures are considered, but he maintains that in individual cases there is much overlapping. He finds that a definite diagnosis of previous pregnancy can be made from the enormous thickening of the *elastica interna* of the mucosal arteries, from the tortuous course of the uterine rami of the middle uterine artery and from the thickened, translucent appearance of the *ligamenta lata*.

A further important uterine change associated with pregnancy is the increase in the blood supply. The increase in the vascular bed of the uterus has already been mentioned. Barcroft and Rothschild

(1932) have determined the actual amounts of blood present in the rabbit uterus at different stages of gestation. In the resting state the organ contains about 2 c.cm. of blood. From the fifth day the uterus shows an increased vascularity and by mid-term the content has reached 10 c.cm. From now onwards the embryos begin to grow rapidly and the blood content of the uterus increases at a similar rate until the maximum of 30 c.cm. is reached at the 27th day. Just prior to parturition there is a rapid diminution in the blood content of the uterus. That this increase in vascular content is not due to simple stagnation of blood is shown by Barcroft, Herkel and Hill (1933). They prove that there is a corresponding increase in blood-flow to the uterus and that this process anticipates foetal growth. The increase in size and pulsation of the middle uterine artery, mentioned by Williams (1921), is well known as a means of diagnosing pregnancy in the cow. Hammond (1927) also mentions these points, although his observation was limited to one cow well advanced in pregnancy.

From the above it is evident that during pregnancy the weight of the uterus is greatly increased due to the presence of more muscular tissue, enlarged and multiplied vascular tissue and increased blood-content. In addition it is seen that in part these changes persist after parturition, and that any resting uterus can on morphological grounds be assigned to one of two groups—virgin or involuted. In comparing uteri the existence of these two classes must be borne in mind. Especially in the non-gravid and early pregnant stages will the differences be of importance. Later in pregnancy these class differences will tend to be obscured by the changes induced by the existing pregnancy.

Some workers have considered the weight of the gravid uterus. This is of interest in that it gives the weight of the entire foetal system. Knowledge of the growth in weight of this system is useful in the study of growth or fattening of pregnant animals—it allows of a correction being made for foetal material. In the bovine Bartlett (1926b) has studied this matter from a different angle and has constructed a table giving the weight of the cow at each stage of pregnancy relative to her farrow weight.

For the sheep Curson and Malan (1936) tabulate the weight of the gravid uterus from 3 to 149 days. The increase is from 163 gm. to 3,700 gm. It must be pointed out that the high value at 3 days is due to the authors having included under " gravid uterus " the vagina, vulva, tubes, ligaments and ovaries. In a further study (Malan and Curson, 1937) the weight at 30 days is found to be approximately 130 gm., while the weight near term is given as 5,320 and 6,058 gm. The authors express the weight of the gravid uterus as a percentage of the nett body weight (i.e. total body weight of ewe less the weight of the gravid uterus) of the ewe, and then these values are plotted against gestation age. The result is a parabolic curve for which a fairly simple formula is given.

Bergmann's (1922) figures indicate that in the bovine the weight of the gravid uterus increases fifty-fold between the second and the tenth months. The increase is more rapid in the later months, hence the data would fit a curve similar to that given for the sheep.

Bergmann makes the statement that from the weight of the gravid uterus, foetal age may be determined with as much accuracy as from the weight or the length of the foetus itself. According to Hammond's (1927) figures for the bovine the increase of the gravid uterus from the beginning of pregnancy till the approach of parturition is one hundred-fold.

Brodermann (1921), dealing with the sow, maintains that the weight of the gravid uterus is very variable. Contributing causes to this variability are previous pregnancies, the number of foetuses and the amount of fluid present.

For the guinea-pig Draper (1920) presents figures which indicate that the position here is approximately similar to that already described for the sheep.

4. *Placenta.*

The sheep has a cotyledonary placenta—*semiplacenta multiplex*—(Zeitschmann, 1923). The cotyledons are made up of two parts, maternal and foetal. In the sheep the maternal cotyledon is concave and encloses the foetal component. In the non-gravid uterus the cotyledons are exceedingly small and can be recognised merely as small circular elevations on the surface of the uterine mucosa. During oestrum they appear to be somewhat more prominent. Ellenberger and Baum (1921) state the total number of cotyledons in the sheep to be between 88 and 96, arranged in each horn in four longitudinal rows.

By Assheton (1905) the diameter of the ovine cotyledon at 60 days' pregnancy is given as about 2 cm., while at full term the figure lies between 2·5 and 3 cm. Natural-size illustrations of the sheep's cotyledon at mid-term given by Zeitschmann (1923) indicate that at this stage the diameter lies between 2 and 3 cm. It is evident that the small cotyledon of the non-gravid uterus must undergo enormous growth during pregnancy. These few figures suggest that the major portion of this growth occurs during the first half of gestation. In the bovine the growth of the placenta has received some attention. Rorik (1907) records the weight of the placenta at 7 weeks as 12 gm. and at 26 weeks as 5,200 gm. From his figures there does not seem to be any further increase after this stage. He shows how the ratio of foetal to placental weight widens with the advance of gestation. The area of the placental surface increases from 0·04 sq. metres at 7 weeks to 45·25 sq. metres at 31 weeks. Hilty (1908) traces the increase in size of the largest cotyledon. The diameter increases from 0·6 cm. in the virgin uterus to 7·8 cm. at the 26th week, after which the increase, if any, is only very slight. Similar results are reported for the increase in height of the cotyledon and in the length of its peduncle.

The idea of rapid enlargement of the placenta during the first part of pregnancy, followed by a period of little or no change, is supported by the figures for the rabbit given by Lochhead and Cramer (1908). Here placental weight is shown to increase rapidly up to the 22nd day, after which it remains stationary apart from a slight decrease at the approach of term. For the guinea-pig Draper (1920) supplies some data. Unfortunately foetal membranes and

placenta are grouped together, hence the use of the figures here is open to criticism. Nevertheless, Draper's graph shows a period of rapid increase followed by a period of little development.

In the bovine the number of functional cotyledons has been considered. Rorik (1907) reports wide variations in the total number of cotyledons— 70 to 120. He shows that the number in the horn bearing the foetus is always in excess of that in the other horn, sometimes by as much as 150 per cent. He encountered accessory cotyledons in one-third of his cases. The presence of these was not necessarily associated with a diminished number of true cotyledons. Bergmann (1922) also reports a preponderance of functional cotyledons in the gravid horn. By counting the numbers of cotyledonary attachments he concludes that in the gravid horn no more attachments occur after the second month, whereas in the non-gravid horn these continue to be formed until the end of the third month.

The mode of attachment of foetal to maternal cotyledons has been studied by Assheton (1905). In the sheep the first attack upon the maternal uterine mucosa occurs between the 17th and 18th days *postcoitum*. At this stage the attachment is most frail, the membranes being held in position mainly by the pressure of the foetal fluids. At about the 30th day villi appear on the chorionic surface, resulting in a closer attachment. At the 44th day the foetal cotyledons are visible and have convex free surface which fit into the concavities of the maternal cotyledons. Assheton states that by the 78th day the general character of the fully formed placenta is apparent and that from this time the only change is a slight increase in size. He also mentions the presence of large reddish-black coagula in the centres of the fully formed cotyledons.

5. *Fallopian Tubes.*

In the sheep the uterine tube has a length of 14 to 16 cm. (Ellenberger and Baum, 1921). The gradual merging of the tube with the horn of the uterus has already been mentioned. Lee (1929) maintains that in most mammals there is a more-or-less well-developed valve at this junction. The sheep is not specifically mentioned, nor is it implied that the junction is always readily recognised from the exterior.

In none of the standard embryological or obstetrical textbooks is any mention made of specific pregnancy changes in the Fallopian tubes. Bergmann (1922) concludes that pregnancy has no influence on the length of the tubes. Lambert (1928) states that in the horse, the bovine, the cat and the dog he has found, associated with gestation, extensive vascularization of the tubes accompanied by hypertrophy of connective and muscular tissue and a distinct increase of elastic tissue.

(*b*) Foetal Membranes and Fluids.

1. *Membranes.*

The formation and the appearance of the foetal membranes of the ruminant are well described by Zeitschmann (1923). He shows that there are two sacs, the inner being the allanto-amnion and the

outer the allanto-chorion. Like Craig (1912), Zeitschmann states that early in pregnancy the outer sac increases rapidly and soon comes to fill the entire uterine cavity. Meanwhile the inner sac has remained relatively small, investing the foetus closely. Later on the amniotic sac expands rapidly and soon it occupies a large portion of the chorionic space, practically obliterating the central part of the sac. At this stage the concentric arrangement of the two sacs is not very evident and the appearance is more that of a large amnio-allanto-chorionic sac with two allanto-chorionic appendages—one at either pole. These latter two sacs retain their communication with each other and with the umbilical infundibulum. This relationship is well indicated in a sketch given by Bailey and Miller (1921).

The appearance of the ovine foetal sacs is described by Assheton (1905). He shows how at 14 days *post-coitum* the blastocyst has extended the full length of both uterine horns. At this stage the membrane is very frail, is transparent and absolutely colourless. Unfortunately he does not trace the further development of the membranes to show at what rate these delicate sacs grow into the relatively large structures which come away as the afterbirth of the ewe. Malan and Curson (1937) give figures for the weight of the membranes during pregnancy. These show that the total weight increases steadily throughout the period, although this increase is not shared equally by the two components. The allanto-chorion is shown to undergo little or no increase after the second month, while the allanto-amnion increases steadily for four months, after which the rate of increase declines.

The weights of the membranes at different stages of gestation in the bovine are recorded by Rorik (1907) and by Hammond (1927). From these sources it is seen that just prior to parturition the membranes have attained more than 1,500 times their weight at the end of the first month of pregnancy. In addition Hammond's figures show that the membranes grow at a steady rate throughout the period.

The fact that Draper (1920) has included the weight of the placenta in his figures for the membranes of the guinea-pig, renders these data rather unsatisfactory. However, if it is assumed that during the second half of pregnancy the increase in weight of the placenta becomes negligible, then Draper's graph would tend to indicate a steady growth of membranes throughout pregnancy. Hammond (1937) deals with the foetal placenta and membranes of the rabbit. His graph shows that there is considerable increase in these structures and that a fairly steady rate of growth is maintained throughout the period of gestation.

2. *Fluids.*

Needham (1931) gives a summary of most of the available knowledge on the foetal fluids. Most of the researches quoted by him are spasmodic investigations on isolated chemical constituents of the fluids. For the purpose of an inquiry into the origin of the foetal fluids Paton, Watson and Kerr (1907) selected the sheep as their subject " because, in this species, both fluids are present in relatively large quantities throughout the entire foetal period ". The figures

supplied by the authors bear out this statement. Moreover, they show that both fluids undergo changes in physical and chemical properties with the advance of gestation, while at the same stage of pregnancy the two fluids differ. Malan and Curson (1937) show that, in the sheep, the total amount of fluid increases steadily with advancing foetal age. They find that the allantoic fluid shows little increase during the first three months, to which period the increase in the amount of amniotic fluid is mainly confined. Zeitschmann (1923) gives the amount of fluid at parturition as between 50 and 450 cc. for the allantoic and between 150 and 400 cc. for the amniotic.

For the bovine Bergmann (1922) shows that the quantity of foetal fluid increases rapidly during the first half of pregnancy, while later this increase is much less. Hammond (1927) indicates that during the first half of pregnancy the increase in weight of the foetal system is due mainly to the accumulation of fluid, whereas towards the end of the term it is accounted for almost exclusively by foetal growth. Bergmann finds that what increase does occur after the fifth month concerns mainly the allantoic fluid. The latter fluid is present in greater amount than the amniotic. Zeitschmann (1923) asserts that the ratio at full term is 3:1 in favour of the amniotic fluid.

Hammond (1927) draws attention to the fact that cessation of increase of the foetal fluids and inception of secretory activity of the mammary gland both occur at about the fifth foetal month. He suggests that this may be significant, and no mere coincidence. In the case of the rabbit (Hammond, 1937) it is also seen that there is a steady increase in the amount of fluid up to the 24th day, after which there is absorption of fluid, so that at birth only a few drops remain. The absorption is active on the part of the foetus itself, but the decrease in amount tends to indicate a cessation of fluid formation. If this is so, then in this species too there is apparently some correlation between mammary secretory activity and inhibition of foetal fluid formation.

In the guinea-pig Draper finds that the amount of amniotic fluid when plotted against gestation time gives a double curve, indicating at first an increasing rate of formation and later a decrease in this rate. Until the middle of the period of gestation the amniotic fluid weight exceeds that of the foetus. Afterwards the foetal weight is the greater, and the ratio between it and the amniotic weight becomes wider with the advance of pregnancy.

(c) The Foetus.

1. *Foetal Age.*

Although MacDowell *et al* (1927) speak of foetal age as distinct from age of the zygote, most workers use the term as indicative of the total age of the organism, from the time of fertilization of the ovum until the moment of death or examination of the foetus (Arey, 1931). In most cases it can be assumed that the time of death of the foetus coincides with that of the expiry of the mother. Especially is this the case where the mother is perfectly healthy right up to the time of slaughter. In the human being, where

available knowledge is not sufficient to allow of accurate determination of the time of fertilization, such terms as "copulation age" and "menstrual age" have been introduced. Neither of these gives the true foetal age.

In order to be able to determine the exact time of fertilization it is essential to know the time of ovulation, the rate at which the sperm traverses the female genital tract and the vitality of both ovum and sperm in the uterine passages. As a result of the investigations of Quinlan and his co-workers (1931 and 1932) a detailed knowledge of all these factors in the Merino sheep is available. They have shown that ovulation occurs at about the 30th hour after the onset of oestrum, and that the unfertilized ovum soon loses its vitality. The male sex cell reaches the Fallopian tube, in which fertilization occurs, within six hours of copulation, while it retains its vitality for from 12 to about 36 hours. If, therefore, service has been allowed at such times during oestrum as to ensure a large number of active sperms being available in the tube at the time of ovulation, then it may be taken that fertilization occurs without delay i.e. at about the 30th hour after the onset of "heat".

The methods of determining the onset of oestrum and of calculating foetal age will be considered in the following chapter.

2. *Factors Affecting Growth of the Foetus.*

In dealing with foetal growth it is essential that due consideration be given those factors which may influence development of the foetus. Where possible these factors should be controlled. Failing this they should at least be borne in mind in the interpretation of results. On consulting the literature one finds that in nearly all cases birth-weight has been the criterion as to whether a factor has influenced foetal growth. Under these circumstances, therefore, even after the influence of a certain factor has been established, there is still the difficulty of not knowing whether this influence was exerted with equal force throughout the entire prenatal period or whether it had been intensified at certain stages.

The painstaking efforts of MacDowell and his collaborators to ensure pure strains and absolute accuracy in ageing have failed to eliminate considerable variation in the weights of foetuses of identical age. No doubt the causes of such variations are highly complex and only by the most extensive research could they be checked with accuracy. Even to attempt a detailed discussion of this problem is far beyond the scope of this work, hence but a few of the more obvious points will be mentioned.

Firstly, *material influences* may be considered:

Breed and Strain.

To elaborate on the differences in size and conformation encountered in different breeds and strains of the same species, would be superfluous. It is shown by Fitch, McGilliard and Drumm (1924), that not only is there a difference in birth-weight in four milk breeds, but that the ratio of calf to maternal weight also differs. This

indicates that the birth-weight differences cannot be regarded merely as proportional increases or decreases according to maternal size. In a detailed study of two closely related strains of albino rats Freudenberg (1932) shows that apart from differences in body weight, there are present, even at birth, distinct differences in organ weight-ratio.

These few comments should suffice to demonstrate that in studies on prenatal growth it is essential that material be drawn not only from the same breed but also, as far as is possible, from the same strain.

Size and Condition.

His observations on both pure- and cross-bred sheep lead Russel (1919) to state that in nearly every case is size of the lamb determined by size of the ewe. Humphrey and Kleinhenz (1908) maintain that large ewes produce lambs heavier than those from small ewes. Hammond (1932) mentions a crossing experiment at the University College, North Wales. Welsh ewes were bred to Border-Leicester and to Southdown rams. Although the former rams are large animals, ewes served by them lambed with only very little more trouble than did those bred to the Southdowns. For the rat, King (1915) states that as the weight of the mother increases, so does the birth-weight of her young become greater. However, as in the rat weight and age are correlated, it is possible that the greater maturity of the heavier females played some part in her investigations.

Hammond (1932) points out that the maternal influence over foetal development is twofold—genetic and nutritional. He shows that in a favourable season, during which the ewes were in good condition prior to lambing, the birth-weights were high. Further, when ewes were divided according to condition into good, medium and poor groups, it was found that the average birth-weights followed the same order. The difference between the first two groups was much less than that between the medium and the poor groups. It appears that improvement in condition beyond a certain stage is not reflected in the birth-weights of the offspring from such ewes. In fact it is found by Quinlan and Roux (1936) that extremely fat cows produce very light calves. This finding is in accordance with the results of Eckles (1919 and 1920). The latter concludes that, in the cow, nutrition of the mother has but little influence on the size of the calf at birth. Donald and McLean (1935) state that " increase in birth-weight as the lambing season advances is due to improvement in the condition of the ewes ". Apparently they contend that an improvement in the condition of the mother is rapidly communicated to the foetus. Hammond (1932) stresses the necessity for providing pregnant ewes with good feeding for six weeks prior to lambing. He may, however, be more concerned with procuring a good milk-supply subsequent to parturition than with actually producing a heavier lamb at birth.

Eckles (1919 and 20) and Fitch et al (1924) maintain that cows in quite poor condition produce calves of average weight. The latter authors show that only when nutrition is severely depressed is calf

weight affected. Even then the effect is not proportional to the loss of condition of the mother. This is demonstrated by the decrease in the calf-mother ratio. In their work on the breeding of cattle on pastures deficient in mineral content, du Toit and Bischop (1929) show that, between their " bone-meal " and their " control " herds, in spite of marked differences in condition, vigour and resistance to disease, there is no significant difference in birth-weight. Groenewald (1935) also fails to find a reduction of birth-size in calves born of mothers fed on rations deficient in various mineral substances. Barry (1920) maintains that severe inanition in pregnant rats causes a decrease in body weight of the resulting litter. The different organs of the foetus are not all affected to the same extent, hence a change in the weight-ratio of the organs results. Quinlan and Roux (1936) find that in cows subject to severe restriction of exercise and sunlight there is no change in either size or degree of development of calves, provided that this treatment does not result in excessive deposition of fat, when low birth-weights will be encountered.

Age.

According to age, breeding stock may be divided into three classes—immature, mature and aged. In practical animal husbandry the breeding ability and production of animals in each of these classes have received much attention. All this work is of great interest here, for it brings out one essential fact—that both as a productive and as a reproductive machine the mature female exhibits an efficiency far superior to that of the female in either of the other two classes.

Hammond (1932) refers to the inability of shearlings to produce lambs with a birth-weight equal to that of the offspring of mature ewes. Donald and McLean (1935) conclude that the influence of age of the ewe is sufficiently strong to obscure the effects of sex differences in the lambs.

Fitch *et al* (1924) find that mature cows produce calves larger than those immature cows. Eckles (1919-20) states that first calves are smaller than later ones, and that the maximum weights occur at the third to the sixth calving. Later in life, in the aged group, there is a decrease in birth-weight. It is well known that milk production increases in the second and third lactations and declines in aged cows.

King (1915) states that birth-weight in the rat increases with the age of the mother. Donaldson (1924) mentions that the albino rat usually produces its largest litters at the second to the eight gestations. Long (1906) states that the average weight per pigling in the case of year-old sows is about 800 gm., and that this increases with age until at 5 years the figure is 1,300 gm. Carmichael and Rice (1920) show that, in addition to this increase in the size of the individual pigling, there is also an increase in the number per litter as the age of the sow increases. These changes are evident up to the 4th litter. Schneider (1936), studying German pigs, reports similar results.

431

A further indication of the greater reproductive ability of the more mature female is the fact that in sheep multiple births occur with the greatest frequency in the most mature age-groups (Jones and Rouse, 1920).

In the foregoing reference has continually been made to the number of gestations as an index of maturity. In most cases this will also be indicative of age. But that maturity is actually a matter of age, and is not dependent on previous breeding, is shown by Joubert (1936). He finds that virgin ewes of mature age will produce lambs equal in weight to those of ewes of the same age which had previously been bred. Donaldson (1924) shows that if the female rat is prevented from breeding until she is four months of age, then the first litter will be as large as any of the subsequent ones.

To sum up the various maternal influences it may be stated that breed, strain and maturity are of importance in foetal development, whereas the state of nutrition, unless grossly beyond normal limits, does not exert much influence.

Paternal Influences.

It is quite evident that the influence of the ram on the foetal size is limited to the genetic sphere. Mumford (1901) declares that the sire does not exert any influence on the size of its offspring. Humphrey and Kleinheuz (1908) find that paternal influence is rather limited. Their method of investigation, however, was not calculated to allow for differences in age and breed.

Hammond (1932) cannot agree that the ram has no influence on the size of its lambs. He draws attention to the fact that cross-bred lambs often differ in size from the pure breed. This difference must be induced by the sire.

In dairy cattle Fitch *et al* (1924) find that the sire does exert an influence upon calf-weight but that the degree of such influence is somewhat limited. Eckles (1919-20) shows that the calf produced by a Jersey cow mated to a Fries bull is much heavier at birth than is a pure-bred Jersey calf. Quinlan and Roux (1936) also find that the bull has an influence on calf-size.

Additional factors which should be considered are:—

Number of Foetuses.

It has been shown by Murray (1921) and by Hammond (1932) that each member of a set of twins is lighter than a single foetus of similar age. The total weight of the set, however, exceeds that of the single individual. Between twins and triplets similar differences exist. These results are confirmed by numerous other workers. Schneider (1936) points out that in pigs the size of the individual pigling at birth decreases with the increase of the number per litter. King (1915) says that the same applies in the albino rat.

Zuntz (1909) shows that when rats are kept on unbalanced rations, the frequency of gestation and the number per litter are reduced much more than is the size of each individual foetus. Evidently, where nutriment is limited, reduction in number safe-guards the size of the young.

Sex of the Foetus.

That the birth-weight of the male exceeds that of the female has been established for the human being (Jackson, 1909), the sheep (Hammond, 1932; Murray, 1921; Donald and McLean, 1935), the bovine (Fitch *et al*, 1924), the rat (King, 1915; Donaldson, 1924; Freudenberg 1932) and the pig (Schneider, 1936).

This difference in weight is not merely the result of an enlargement in all dimensions in the male. For the bovine it has been demonstrated by Keller (1920) and by Beer (1925) that somatic differences dependent upon sex do exist. These are detectable as early as the second month of prenatal life. The male is much more plump and is better muscled, especially in the cervical and the shoulder regions. The female is more slender and has a long, narrow head. The results of Keller were well controlled in that he used sets of twins of like and of unlike sex. Although variations were found in both groups he concluded that there was evidence of a definite inherent somatic sex-difference.

For the pig similar results are reported by Smestisko (1925). Jackson (1909) refers to sex-differences in human foetuses, and Scammon (1922) shows that in the height-weight index of new-born babies this difference is also discernible.

In addition to the above influences there is still to be considered the effect of the time of the year at which birth occurs.

Seasonal Influences.

Hammond (1932) and Donald and McLean (1935) find that birth-weight increases with advance of the lambing season. Apparently, however, they consider that this is the reflection of an improvement in the condition of the ewes. Carmichael and Rice (1920) find no regular birth-weight differences due to season, although they do state that piglings farrowed outside the regular seasons have smaller weights than usual. Schneider (1936) finds that the birth-weight of piglings farrowed in winter exceeds by 70 gm. per foetus that of similar young born in summer.

3. *Growth in Weight.*

Colin (1888) appears to have been the first investigator to study the growth in weight of the ovine foetus. A big gap in his figures between the ages of 57 and 120 days makes it impossible to determine the shape of the growth curve. Due to the absence of age figures the series of foetal weights given by Paton, Watson and Kerr (1905) is also useless for this purpose. These weights indicate only the range to be expected. Craig (1912) mentions 4 Kg. as the weight of the lamb at birth.

Curson and Malan (1935) give detailed figures for a whole series of Merino foetuses. In general their figures are lower than those of Colin. This may be due to their having worked upon a different breed. They show by means of a chart that when weight is plotted against age the result is a logarithmic curve. The latter

shows much fluctuation, especially after the age of 100 days. The authors state that " these fluctuations are well understood, for the series of lambs was taken at random, and there is considerable variation in the weight of individuals of the same age ". In a subsequent paper [Malan and Curson, 1936 (a)] additional weights are recorded, and although these are not plotted against age, it appears that the curve so obtained would follow a course similar to that of the curve given in the earlier article. Further weights are given by Malan and Curson (1937), and these only serve to strengthen the previous findings of these authors.

For the bovine foetus a fair amount of data are available. Rorik (1907) and Bergman (1922) tabulate a considerable number of weights for foetuses of various ages. Craig gives the average weight of the calf at birth as $32 \cdot 5$ Kg. Hammond (1927) mentions the weights of eight accurately aged foetuses. In spite of many overlappings, and the fact that breed differences must be considerable, these figures all indicate that the age-weight curve for the bovine foetus is similar to that described by Curson and Malan for the sheep.

The same type of curve has been established for other mammals. e.g. the human being (Jackson, 1909), the guinea-pig (Draper, 1920), the rat (Stotsenberg, 1915) and the mouse (MacDowell et al, 1927). Apparently the same type of curve holds good for other mammals, and it appears possible to express all foetal growth-rates by the same general formula, the constants of which have merely to be adjusted for each species.

So far only absolute growth has come under consideration. When relative or percentage growth curves are considered there is a remarkable lack of unanimity amongst workers. When it is noticed that Brody [1927 (a)] using the identical data employed by Minot (1908), obtains radically different rates, then it is realised that the real problem here is of mathematical nature.

Brody [1927 (b)] presents graphs with which he attempts to show that the relative growth curve is not of a steadily decreasing nature, but that it is made up of a series of straight segments. MacDowell et al (1927) do not agree with this. They demonstrate an even and regularly decreasing curve. They criticise the data upon which Brody relied for his determinations, and they also show that any curve may be approximated by a number of straight lines.

4. *Growth in Length.*

In the introduction to this paper was mentioned the failure of investigators to define with accuracy their procedure. Nowhere is this failure more apparent than in the case of length of foetuses. By determining increase in length an idea of skeletal growth, rather than of mass increase, is obtained. Later it will be seen that these two factors are actually correlated. It is evident that in each case the identical dimension should be measured. This can be done only when the extremities of the line are clearly defined. If the line

is not straight then its course between the two fixed points should be indicated. Unfortunately in the literature the term " length of foetus " is often given without further amplification.

For the human foetus certain fixed measurements are in general use. These are described by Mall (1910). Commonest of these are sitting-height (crown-rump), standing-height (crown-heel), and vertebral column length. In the veterinary field most workers have, no doubt, made use of a dimension akin to the human sitting-height or crown-rump measurement. That the fixed points have varied is indicated by the few instances in which an attempt at defining " length " has been made. Hammond (1927) refers to a poll-base-of-tail measurement, while Curson and Malan (1935) use the forehead and the base of the tail as landmarks. For rats the nose-anus dimension is in general use (Donaldson, 1924). Bergmann (1922) refers to a muzzle-rump measurement employed by some investigators. He concludes that this is inferior to the poll-rump measurement.

In all these cases attempts have been made to indicate the length of the foetal body. All the measurements are of straight-line dimensions. Although " crown-rump " appears to be a most useful measurement in the human being, its value in an animal with a relatively long and flexible neck is greatly reduced by the possibility of large errors due to changes in posture. In the sheep foetus, even when it is laid out flat with the neck perfectly straight, relatively large variations may be introduced simply by flexing and extending the head on the neck. In making use of such dimensions it is essential that the position of the foetus as well as the landmarks be standardised and defined with care.

Malan and Curson [1936 (a)] express the opinion that a measurement along the back of the foetus, in preference to a straight line, should assist in eliminating the effect of posture. It was their intention to make use of Mall's (1910) " length of vertebral column " dimension. They employed as an anterior landmark for their " curved crown-rump " length, the midpoint of the line joining the medial canthi of the eyes. The posterior landmark was the root of the tail (Curson, 1937). The authors do not find that the use of this measurement improves the fit of the data in a weight-length curve. From this it would appear that the curved line is no more accurate than a straight measurement. The line used is obviously not the equivalent of that referred to by Mall, and it remains to be seen whether Mall's line—length of the vertebral column—would produce better results. The difficulty in making this measurement is that there is nothing to indicate that, in any species other than the human being, the extension of the eye-ear line indicates the point of origin of the vertebral column.

Lengths of sheep foetuses from the third to the twenty-second week are given by Gurlt (1847). His figures show no close similarity to those of other workers. Better figures are those of Assheton (1905). These are limited to the very young stages. A large amount of data concerning accurately aged Merino foetuses are presented by Curson and Malan (1935). On plotting these data against age the authors obtain a curve which resembles an elongated " S ". This

curve differs from the weight-age curve previously described. Due to the small number of foetuses used the data show much variability, but this cannot obscure the fact that there is a definite length-age curve for foetal growth. Apart from additional figures for straight crown-rump length, Malan and Curson [1936 (a)] also tabulate their " curved crown-rump " measurements. Although the latter figures are not plotted, it appears that this would result in a curve of roughly the same shape as that obtained with the straight-line dimension. Galpin (1935) indicates the lengths of a number of accurately aged Romney foetuses. The measurement taken appears to have been somewhat akin to Malan and Curson's [1936 (a)] curved crown-rump dimension. If the figures represent straight crown-rump lengths then a Romney foetus must be considerably larger than a Merino foetus of identical age.

Craig (1912), who simply speaks of " length of the foetus ", gives figures for the horse, the bovine, the sheep, the pig and the dog. As he mentions merely the average length for each of the seven periods into which he divides the gestation period, no conclusions as to the nature of the growth curve may be drawn. His data for the sheep fit in well with the figures recorded by Curson and Malan. For the length of the lamb at birth Craig gives 18 inches, which is considerably higher than the figure mentioned for a 145 day foetus by Curson and Malan (1935). If Craig's figure is a good average one then the distinct flattening of the last part of Curson and Malan's curve should disappear and the curve should be much more even. This would then produce a curve resembling that given by Draper (1920) in his work on the guinea-pig. His curve also has a double nature, but the second change of direction is very gradual. Draper concludes that from the 15th day of foetal life till the 64th day the percentage rate of growth in length decreases steadily. Hammond (1927) indicates the measurements of eight bovine foetuses, ranging in age from 1 to 8 months. The figures would apparently fit a curve such as that under discussion.

Already it has been shown that there is a correlation between the age and the length of a foetus. In the human foetus this relationship has received a fair amount of attention. Simple empirical formulae for expressing each of these characteristics in terms of the other are given by Scammon and Calkins (1923). Noback (1922) demonstrates the simple relationship between sitting-height and standing-height. Scammon (1937) shows that the simple relationship between these two lineal dimensions may be extended to embrace numerous other external measurements of the foetus. Noback (1925) demonstrates that in the lineal growth of a single system such as the respiratory the age-length correlation is still present.

Curson and Malan (1935) consider the correlation between weight and length. In addition to presenting a graph for these two characteristics plotted one against the other, they show that relative increases in length and in weight may be plotted to a straight line. This is confirmed in a later article [Malan and Curson, 1936 (a)]. It is also found that the " fit " is not improved by the use of the

curved crown-rump measurement. They conclude that increase in length is a function of body weight, and that the reverse also holds good. They observe that the logarithmic values of weight and of crown-rump length are equally variable. Hammond (1927) states that " weight is more variable than length ". Curson and Malan (1935) show that when Arey's (1934) data for the human foetus are plotted, the same length-weight relationship becomes evident. According to Scammon (1922) there is a definite height-weight index for babies at birth.

Workers on post-natal growth have not been content with measuring one dimension. Instead they make use of a number of representative measurements, some of which are mentioned and illustrated by Ragsdale, Elting and Brody (1926). It will be seen that there are two classes—straight and curved or circumferential. The purpose of all this is to obtain an idea of growth in all three dimensions, rather than to be limited to a " one-dimensional " conception of the process. It appears that by an extension of this idea to the study of foetal growth a much clearer and more accurate visualization of the changing proportions of the developing foetus may be attained. It is conceivable that ratios between dimensions may prove to be better indices of age than the absolute measurements themselves.

5. *Growth in Surface Area.*

Not only weight and linear dimensions but also surface area may be used as a measure of size. In the living animal, especially after birth, surface area assumes an even greater importance, for it is intimately associated with heat regulation and metabolism. In the sheep foetus this aspect of growth has been studied by Malan and Curson [1936 (*b*)]. They find that the curve for surface area against age is similar to that for weight and age. Relative increases in area and in weight may be plotted to a straight line.

Owing to the desirability of preserving intact the foetuses used in the present work, surface area has not been considered.

6. *Changes in External Appearance.*

It is not intended to discuss here the earliest developmental stages of the sheep embryo and foetus. Rather is it the object to commence at the stage at which in textbooks the subject is usually left, and to consider the manner in which the early common mammalian type is moulded into the Merino lamb delivered at parturition. The changes which take place will be correlated with age so as to arrive at something similar to Arey's (1934) " Table of Correlated Human Development ".

Assheton (1905) intimates that the development of the sheep up till the 10th day has been worked out by him (1903), and that the further development has been fully described by Bonnet (1895 and 1898). Unfortunately neither of these papers was available to me.

For the bovine Hammond (1927) presents a table which, in spite of the small number of foetuses available for its compilation, is a useful guide in age-determination. An interesting feature of this

table is the use that is made of the stage of pigmentation of the eye in the early foetuses. Attention is also directed to the value of the limb-bud and the development of the hoofs in ageing foetuses. Some further details concerning the external form of bovine foetuses in the early stages of gestation are mentioned by Kupfer (1936).

The appearance and distribution of hair on the foetus has long been used as a criterion of age. The spread of hair over the Merino foetus is described by Duerden and Ritchie (1924). The time of the first appearance of hair is placed towards the end of the 2nd month. To this exception is taken by Curson and Malan (1935), who maintain that hair is first visible on the forty-second day of prenatal life. Galpin (1935) describes the development of hair in the New Zealand Romney foetus. She draws a distinction between tactile hairs and body hairs. The former start to appear on about the 76th day, whereas the latter are first seen on the 90th day. These times refer to the actual emergence of the hairs above the surface of the epidermis. The hair follicles, of course, are distinguishable long before this. Like Duerden and Ritchie, she finds that the development of hair is furthest advanced on the most anterior parts of the body. The backward spread takes place in accordance with a plan so regular that it appears to offer an excellent means of age-determination.

Craig (1912), Bergmann (1922) and Hammond (1927) give details for hair development in the bovine. When due allowance is made for the increased length of the developmental period in this species, it is seen that the figures mentioned correspond closely to those obtained for the sheep.

Other external features do not appear to have received much attention.

(d) The Maternal Mammary Gland.

Probably on account of the relative unimportance of the sheep as a milk producer, its udder does not appear to have excited scientific interest. That milk production in the sheep is of economic importance, especially in crossing for fat lamb production, has been shown by Joubert (1936). From the above it must not be assumed that in other species the causes of mammary development and milk secretion have not been the subject of scientific investigation. On the contrary, much work has been done in this connection.

Early in the present century Lane-Claypon and Starling (1907) studied with inconclusive results the effects on mammary development of various foetal placental extracts. Loeb and Hesselmann [1917 (a) and (b)] show that mammary activity is correlated with the oestral cycle as well as with pregnancy. The changes occurring with each oestral cycle resemble closely those of the early part of gestation.

A close study of the whole problem has been made by Turner. This author (1930) traces the foetal development of the udder, indicating the stage attained at birth. In a later paper (1934) development in the post-natal period is followed up and it is shown that, excepting for the deposition of fat, there is no change between

birth and the onset of puberty. From this time onwards, with each recurring oestral cycle, there is more and more growth and branching of the duct system and the gland tissue comes to resemble a tree without leaves. With the onset of pregnancy there is still more branching activity, followed by alveolar development. At mid-term the leafless tree picture has been altered to resemble a bunch of grapes, the ducts being the stalks and the alveoli the grapes. Now secretion commences and it is the accumulation of the secretory product which results in the visible filling out of the mammary gland. Hammond's (1927) results are in agreement with these views. Woodman and Hammond (1923) and Asdell (1925) find that the watery secretion which, in virgin heifers, may be withdrawn from the udder, begins at mid-term to undergo qualitative and quantitative changes which foreshadow the formation of the cholostral milk. The above results appear to explain why workers such as Ragsdale *et al* (1924) have found that in lactating cows a subsequent pregnancy does not have any effect upon milk yield until the end of the fifth month of gestation.

At the time of fertilization, therefore, mammary development is already under way. The further development of the udder occurs in a slow and orderly manner and is spread out over the entire period of gestation. It is incorrect to regard milk secretion as being initiated rapidly a short while prior to parturition.

This internal development and the accumulation of fluid must make itself evident in an increase in size and weight of the gland. Hammond (1927) shows that in heifers in their initial gestation period, the weight of the udder increases from 2,200 gm. at the end of the first month to about 3,000 gm. at mid-term, and to between 6,000 and 9,000 gm. shortly before parturition. Thus the major portion of the increase in weight occurs in the second half of pregnancy. Hammond further indicates that in considering udder weights, especially in dry cows, the age and the condition of the subject should receive due attention, as both these factors have an influence on the weight of the organ.

(c) The Maternal Endocrine Glands.

1. *The Pituitary.*

This gland is most intimately associated with all the sexual functions of the animal body. A sex-difference in pituitary size has been demonstrated in man (Rasmussen and Herrick, 1922), in the albino rat (Jackson, 1913; Hatai, 1913; and Addison and Adams, 1926) and in the pigeon and the dove (Riddle and Nussmann, 1933). In each case it is the female that has the larger pituitary, and in this sex the gland also has a steeper growth curve. Addison and Adams show that 95 *per cent* of the excess weight is accounted for by the anterior lobe of the hypophysis while the remaining portions are but slightly heavier than in the male. This is in agreement with the conclusions drawn by Rasmussen and Herrick from a study of a few human pituitaries. Working with albino rats, Andersen (1933) shows that at different times in the sexual cycle the hypophyseal weight varies. The maximum weight is encountered during oestrum. From then onwards there is a gradual decrease to the minimum

weight which is reached twenty-four hours prior to onset of the next heat period. In the fowl it has been shown that during the laying period the pituitary is heavier than during the moulting stage (Marza and Blinov, 1936). For the mare Schopf (1935) gives pituitary weights at different stages of gestation. He states that due to differences in the size of his subjects, no conclusions may be drawn from the absolute weights. When relative weight is the criterion then it appears that pregnancy is accompanied by an increase in pituitary weight. Whether Schopf is justified in assuming that pituitary size is proportional to body weight is questionable. Upon this point no direct evidence is available, yet it must be admitted that in the results of Addison and Adams (1926) some correlation between body weight and pituitary weight is evident.

From a study on the albino rat, Herring (1920) concludes that the effect of pregnancy is to reduce the weight of the pituitary. Hammond (1927) considers the oestral cycle to be the homologue of the reproductive cycle with dioestrum corresponding to the gestatory period. If this is the case, Herring's view receives confirmation from Andersen's (1933) findings.

In the bitch (Wolf, Cleveland and Campbell, 1933), in the sow (Cleveland and Wolf, 1933), in the mare (Schopf, 1935) and in the sheep (Warbritton and MacKenzie, 1937) it has been shown that widely differing histological pictures are associated with the various phases of sexual activity. The changes take the form of qualitive as well as quantitive variations in the chromophil cells of the anterior lobe. The changes described in the bitch and the sow suggest that during pregnancy a decrease in the size of this lobe is to be anticipated.

2. *The Adrenal.*

In the albino rat the sex differences and the growth curves of the adrenals resemble closely those of the pituitary (Jackson, 1913; Hatai, 1913). Barker (1937) finds that the adrenal of the dog is lighter than that of the bitch. Mature females have heavier adrenals than immature bitches. In mice Deanesley (1928) demonstrates histological differences between male and female adrenals.

Andersen and Kennedy [1933 (b)] show that in female albino rats adrenal weight is very variable, and that factors such as age, body weight, sexual season and the presence of infection must receive attention. They contend that bacterial infections of the lungs make for increased adrenal weight. Motow (1937) does not agree with this. Rosenbluth and Gayet (1932) conclude that the adrenal is relatively stable, both in weight and in adrenin content, to changes in diet.

Andersen and Kennedy (1932) show that during oestrum the rat adrenal is heavier than in dioestrum. This increase during oestrum is associated with definite histological changes, the cortex being increased in thickness at this stage of the cycle. Nahm and McKenzie (1937) demonstrate variations in the adrenal of the ewe.

Deanesley (1928) finds that in the mouse there is associated with pregnancy a degeneration of the inner portion of the cortex, described by her as the " inner dark staining zone ".

Herring (1920) maintains that during pregnancy the adrenals of the rat are slightly hypertrophied. This finding is criticised by Andersen and Kennedy [1933 (a)], who maintain that the figures do not show any significant changes. The latter authors, after reviewing all the available literature, conclude that the case for pregnancy hypertrophy of the adrenal has not been establised. They admit that there is a possibility that all species may not react in an identical manner. After careful consideration of all the available data they maintain that in the case of the human being, the rat, the mouse, the rabbit and the cat, the balance of evidence is against such a hypertrophy. Their own results, in which relative adrenal weight is employed, indicate that during pregnancy the adrenals resemble those of the dioestrus period, which again are lighter than the adrenals of the oestrus period. During lactation a significant rise in adrenal weight is evident.

Mutow (1937) finds the adrenals to be heavier at oestrus than during dioestrus. When he considers a combined group of pregnant and lactating rats, a weight slightly above the dioestral standard is obtained. This increase may be accounted for entirely by the lactating rats in the group.

3. *The Thyroid.*

Andersen (1933) states that the great variability in weight of this organ is due partly to the difficulty of dissecting it free from the surrounding tissue. Jackson (1913) shows that during growth the relative weight of the thyroid changes, being highest at birth. Zimmermann (1933) states that with increasing age the thyroid undergoes regressive changes, the isthmus in particular being affected. Orywall (1933) finds that there is a definite weight difference between thyroids from female rats at Leipzig and from similar rats in Stuttgart. This may be a strain difference or it may be of dietary origin.

With all these possible sources of variation, only exceedingly large changes in the size of the thyroid would be of significance. It is not surprising to find that Andersen (1935) concludes that she is unable to demonstrate changes due to sexual season.

Guggisberg (1933) maintains that in the pregnant woman the thyroid is enlarged to such an extent as to render the increase clinically detectable. He states that although there is hypersecretion from the thyroid, this cannot be termed hyperthyroidism because the thyroxin content of the maternal blood is not raised. The surplus secretion is for the use of the developing foetus, which, until late in foetal life, is devoid of its own supply.

Herring (1920) claims to have shown that in the rat there is a decrease in thyroid size during pregnancy.

4. *Corpus Luteum.*

It is now agreed that this body should be regarded as a temporary endocrine gland. Its function has been studied in great detail. With this aspect the present paper is not concerned. Only its macroscopic appearance will be considered here. The appearance of the ovine

corpus luteum, both in the oestral cycle and in pregnancy, has been well described and illustrated by Quinlan and Mare (1931). The microscopic appearances have been reported upon by Marais (1936). From these works it is learned that the corpus luteum of the sheep maintains its structure for a long time. The corpus luteum of menstruation shows its first signs of deterioration shortly prior to the next oestrum, while that of pregnancy remains unchanged until the approach of parturition. The latter corpus luteum tends to sink deep into the ovarian tissue, especially during the latter half of gestation. This fact may be responsible for the statements of certain observers to the effect that the size of the corpus becomes reduced during the second half of pregnancy.

From actual weights of bovine corpora lutea, Bergmann (1922) concludes that there is no decrease in size throughout the period of pregnancy. Kaltner (1923) shows that during the entire term the bovine corpus luteum conforms closely to the average weight of 4·26 gm. Even at parturition he could not detect macroscopic changes in the size and appearance of this structure. In twin pregnancies he finds that each of the two corpora is smaller than the average referred to above. He maintains that the presence of dead, mummified or macerated foetuses does not prevent degeneration of the corpus nor even the approach of a new ovulation. Hammond (1927) also finds that there is no change in the size of the corpus luteum during the course of pregnancy. He mentions that during gestation follicular atrophy is much more severe than during the sexual cycle. This tends to cause a decrease of ovarian weight, especially in the case of the ovary not containing the corpus luteum.

5. *The Pineal.*

No reference to the macroscopic appearance of this organ during pregnancy has been encountered.

(f) GENERAL.

1. *The Situation of the Foetus.*

From a consideration of the mode of liberation of the ovum and of fertilization it will be evident that the foetus should in most cases be situated on the same side of the body as the corpus luteum. From the figures of Kupfer (1923) and those of Curson (1934) it is seen that this is the case. In a small number of cases, however, " migration " occurs. This may take place via the abdominal route (Leopold, 1888) or it may occur by the internal or uterine route (Warwick, 1926). This latter type of migration is not possible in mammals which have a uterus duplex—e.g. the rabbit.

When more than one foetus is carried, the corpora lutea may be in one or in both ovaries. Irrespective of the distribution of the corpora between the ovaries, the foetuses are usually spaced evenly along the entire tract (Warwick, 1926). Even in a case of uniovular twins, Henning (1937) found the foetuses lying one in each horn.

In cattle it has been found that the foetus is carried in the right horn more often than in the left (Keefer, 1937). Clark (1936) shows that this is not due to more frequent ovulation from the right ovary. It appears, therefore, that in the bovine migration usually occurs from the left to the right horn. Clark failed to find justification for the belief that there is usually a relationship between the sex of the calf and the horn in which it is carried.

In the sheep, due to the prolonged persistence of the corpus luteum, it is a simple matter to determine whether ovulation has occurred from alternate ovaries or not. Quinlan and Mare (1931) find that usually there is alternation between the two ovaries, but that on occasion ovulation may occur twice or even three times in succession from the same ovary. Ruhl (1925) mentions the fact that in the human being after unilateral ovariotomy the length of the sexual cycle is not doubled. This, he states, indicates that there is no inherent mechanism preventing successive ovulations from the same ovary.

2. *Effect of Gestation on Maternal Weight.*

In view of the scarcity of data on prenatal growth, some investigators (e.g. Ragsdale, Elting and Brody, 1926) have attempted to gauge foetal development by noting the increases in weight of pregnant cows. This method at best can but give an indication of the growth of the whole foetal system, which is neither equal nor proportional to foetal growth.

Bartlett [1926 (*b*)] states that in experimental work on pregnant cows it is often necessary to be able to apply a correction for foetal weight. He is of opinion that the only increase in weight of mature pregnant cows is that due to the foetal system, while in immature cows there is actual growth up to the fourth month of gestation. The former class will regain its service weight after parturition, while the farrow weight of the immature cow is the same as that shown at the fourth month of pregnancy.

Reference has already been made to Curson and Malan's (1936) treatment of the weight of the gravid uterus as a percentage of the nett live weight of the ewe.

CHAPTER 3. PLAN OF INVESTIGATION.

(*a*) MATERIAL.

Apart from a few references to some of the material already reported upon by Curzon and Malan, the data for this paper are derived entirely from observations upon a series of ewes slaughtered at Onderstepoort during the period April to November, 1937. These sheep, typical Merinos, were drafted from the available flock on the research station at Ermelo, Transvaal. The methods of sheep husbandry practised on this farm are described by Hoffman (1935) and Roux and Hoffman (1935). Information regarding the physiography of the station and its effect on Merino sheep is given by Roux (1936).

The known sexual history of the ewes has been arranged in tabular form (Appendix—A). As the ewes had not been kept under constant supervision from the time of attaining sexual maturity, it is certain that many occurrences of oestrum are not recorded. However, the information concerning previous services and pregnancies is complete and accurate.

Regarding the service of the ewes, the " controlled " method (Roux and Hoffman, 1935) was practised. " Teasing " was carried out twice daily and service was allowed soon after the detection of oestrum, and again twelve hours later. Where an ewe was still willing to stand for the ram at the next testing (i.e. 24 hours after the onset of oestrum) a third service was allowed.

Four rams were employed to serve the ewes. All were typical specimens of the Merino breed, of mature age (about six years) and of fairly uniform size (around 140 lb. live weight). The rams were used at random, no attempt being made to mate a certain ram to any particular ewe, nor were the two consecutive services of any ewe necessarily given by the same ram.

Most of the ewes were tested daily for 22 days following service, and where oestrum reappeared it was assumed that the ewe had failed to conceive. In the case of those ewes required for the study of the earliest stages of pregnancy this testing was impracticable. When slaughtered, many of these ewes proved to be non-pregnant.

Soon after completion of the testing (or, in cases where no testing was carried out, soon after service) the ewes were railed to Onderstepoort, where they were placed in a small camp (100 × 50 ft.) in which a good water-supply was always available. They were allowed the ration* usually fed to sheep at this Institute.

At first each sheep was weighed once only, i.e. just prior to slaughter. However, it was later decided to weigh the ewes at weekly intervals, this taking place at 11 a.m. on Monday mornings. The sheep were not starved before being weighed.

Although the ewes were selected at random, they were nevertheless drafted from a flock of fairly uniform size and age. As a result the ewes of the experimental group showed but slight variations in these respects.

Had the weights of the ewes at the time of service been available, it would have been an easy matter to test the group for uniformity of weight. As there were differences in condition (fatness) it cannot be claimed that the weights would have been a true index of skeletal size—the factor which is of importance in the determination of size of the foetus.

It was decided to make use of brain weight as an indication of size. It must at once be pointed out that there is no definite authority for this procedure, and that no undue importance should

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* This is as follows:—Roughage always available in racks: ¾ lb. of maize per sheep per day. Green feed or, when this is not procurable, lucerne hay two or three times per week.

be attached to it. However, in the human being it is known that the brain reaches its fullest development relatively early in life (Scammon and Dunn, 1922; Kappers, 1936).

By comparison of the respective life-cycles and the ages of sexual maturity, it is estimated that in the sheep brain weight is likely to have reached its maximum at the age of $2\frac{1}{2}$ years. Therefore, in sheep older than 3 years, but not yet " aged ", brain weight should not be affected by small differences in age. Further, it has been shown in the rat that the coefficient of variation of the brain is considerably lower than that of most organs or of the body as a whole (Jackson, 1913). It is evident that from day to day the brain weight of an individual is not likely to change in the same proportion as the body weight which, especially in the ruminant [Bartlett, 1926 (a)], is subject to large daily variations. Kappers (1936) states that in the human being body weight and brain weight are correlated. It is felt that these facts go far to justify the use of brain weight as an index of body size.

In Table 1 are given the mean brain weights of the ewes when placed in monthly groups according to the stages of pregnancy reached at the time of slaughter.

When tested by Fisher's (1936) " Analysis of Variance " method these figures show no significant differences.

TABLE 1.

Brain Weights of Ewes.

GROUPS OF EWES.		No. of Ewes.	Mean Brain Weight.*
No.	Class.		
1	Non-preg	11	107·73
2	1st month	12	104·42
3	2nd month	8	103·25
4	3rd month	6	106·17
5	4th month	7	107·43
6	5th month	5	104·60

* Weight in Grams.

In Table 2 the conception ages of the same groups of ewes are treated in a similar manner. Again the differences are insignificant. From these tests it is concluded that, although the ewes did show variations both as regards size and age, the effect of the random selection was such as to spread the differences evenly throughout the six groups, there being no " prejudice " in favour of any particular group.

All details regarding dates and times of service and of slaughter are to be found in tabular form in Appendix A.

445

TABLE 2.

Ages of Ewes.

GROUPS OF EWES.		No. of Ewes.	Mean age* of Group.
No.	Class.		
1....................	Non-preg....................	11	3·72
2....................	1st month....................	12	3·75
3....................	2nd month....................	8	3·60
4....................	3rd month....................	6	3·67
5....................	4th month....................	7	3·63
6....................	5th month....................	5	3·77

* Age in years.

(*b*) PROCEDURE.

(1) *Collection of Data.*

Immediately before slaughter the ewe was weighed and a clinical examination was made. Age was determined according to the teeth, while the degree of development of the mammae and of enlargement of the abdomen were determined both by visual inspection and by palpation. Then the animal was placed on its left side and was held firmly while its throat was cut and the spinal cord severed.

Immediately after death the mammary gland was removed; the skin was incised transversely in the region of the umbilicus and the gland was loosened from the abdominal wall; at its caudal border the mammary vessels were examined before being cut through. When the organ, together with its lymph glands, had been freed, the incision through the skin was continued along the circumference of the base. The gland, removed with its covering skin, was laid flat on its base, and left in a cool place until its adipose tissue had set.*

Meanwhile, avoiding rolling of the carcase, assistants had removed the skin and had inserted hooks into the loins, the withers and the neck. By means of these the carcase was supended in imitation of its natural standing posture.

Through slits in the flanks the positions of the rumen, the intestines, the uterus and the ovaries were observed. The gastro-intestinal tract was removed and a note was made of the appearance of the broad ligaments and uterine vessels. Then the entire tract (plus the anus and part of the rectum) was transferred to a mesh-covered frame which fitted accurately over an enamel tray. Various sizes of frame and tray were used, and the weight of each set was known.

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* In some instances it was necessary (for the purposes of a different investigation) to inject into the lactiferous sinus a solution of gelatine coloured with Giemsa. The injection was carried out immediately after removal of the gland. The quantity of fluid injected as well as its specific gravity was noted.

The adrenals and the thyroid gland were removed, cleaned of all adherent tissue and weighed. By this time an assistant had opened the skull. The brain was removed in its membranes and with the hypophysis intact. After the medulla oblongata had been severed transversely at the point of divergence of the restiform bodies, the membranes were carefully stripped off and the brain was weighed.* Both the pituitary and the pineal were removed, cleaned and weighed.
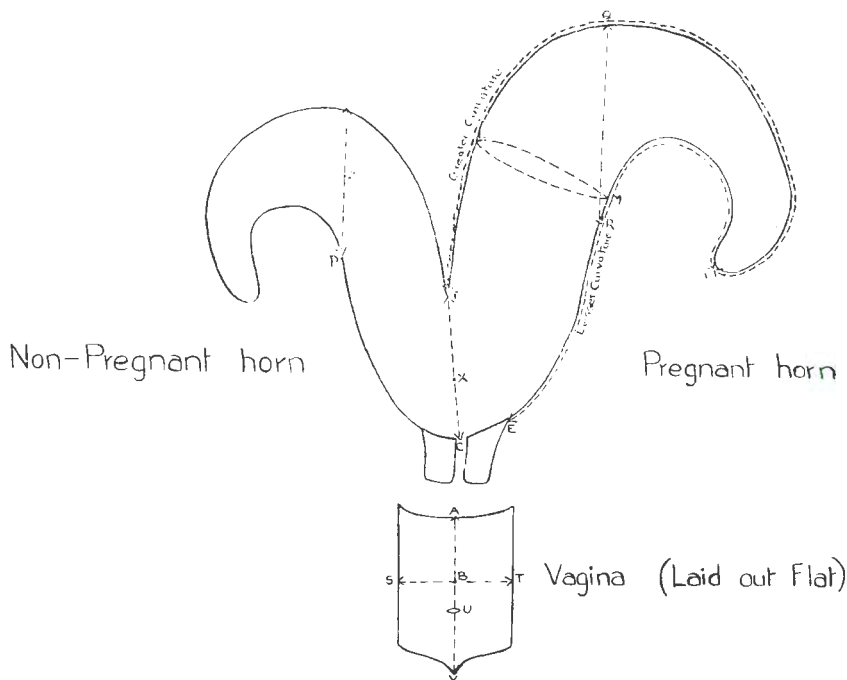
Plate I. Showing the method of measuring the uterus and the vagina.

After this the genital tract was again considered. The rectum, the bladder and all loose tissue were removed and the skin and muscular tissue around the vulva were trimmed, care being taken to ensure uniformity in all cases. The vagina and the Fallopian tubes, mesosalpinx and ovaries were detached, thus leaving the " dressed uterus ", the weight of which was then recorded. (In this, as in all similar procedures, the tray was placed on the scale, and the weight of its contents obtained by deducting from the recorded figure the weight of the tray and frame.)

The vagina was weighed and, having been cut longitudinally in the mid-dorsal line, was laid out flat and its length and width were measured as indicated in plate 1. In determining the thickness of the vaginal wall, which was measured at the middle of the length line, the loose adventitious tissue on its outer surface was not included.

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* Here the weight of the small piece of bone—dorsum sellae of the sphenoid—which lies between the brain and the hypophysis, was included; however, the relative error is negligible.

Each Fallopian tube, having been freed from its mesosalpinx, was weighed. The length was measured and at the middle of this the diameter was taken.

The ovaries were cleaned and were measured as follows:— Length (in a cranio-caudal direction), width (from side to side) and depth (in the sagittal plane). In each case the maximum measurement was recorded. The presence and appearance of Graafian follicles and corpora lutea were noted and then each ovary was weighed.

Attention was now transferred to the gravid uterus. The broad ligaments were severed in such a way as to allow of the organ being laid out as illustrated in outline in plate 1. Using a piece of twine the lengths of the greater and the lesser curvatures and the circumferences of the horns were measured. (See plate 1.)

Starting from the tip of each horn, and using small blunt-nosed scissors to avoid injury to the underlying sacs, the uterine wall was incised along the greater curvatures. Any strain on the membranes due to their cotyledonary attachments was relieved by cutting through the peduncles of the maternal cotyledons. In this way the uterus was opened completely, leaving the entire foetal system exposed.

At this juncture the situation and the posture of the foetus (visible through the membranes) were noted, as were details of the appearance of the allanto-chorion, the placenta and the uterine mucosa.

After collecting* some allantoic fluid for chemical analysis, the allanto-chorion was slit open and the fluid was allowed to escape. Then the loss of weight was determined and from this and the specific gravity (determined in connection with the chemical investigation) the volume of allantoic fluid was calculated. This was repeated with the amniotic fluid.

After a double ligature had been applied at its foetal extremity, the umbilical cord was severed and the foetus removed. After the adherent mucus and amniotic fluid had been rubbed off its coat the foetus was weighed. Then is was placed on its right side, with its neck and back in the same straight line and with the long axis of the head at right angles to this line.[1] Both the straight and the curved crown-rump lengths were measured (as shown in plate 2). After this the foetus was placed in a flat-bottomed dish in the posture described above and was covered with fixing fluid. For foetuses under the age of 45 days Professor Darts solution [2] was used, while for all others the fluid employed was a 5 *per cent.* solution of neutralized formalin. Foetuses were allowed to remain in this fluid for a few weeks.

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* In order to obtain fluid free from any contamination or admixture, it was withdrawn through a hypodermic needle inserted into the sac.

[1] In very young foetuses this was made impossible by the curvature of the body and neck. In these cases the maximum straight measurement was recorded as the straight crown-rump length.

[2] Neutralised formalin ... ... 300 ccm.
  Sodium chloride ... ... 75 gm.
  Tap water ... ... 9,700 ccm.

The neutralised formalin is prepared from commercial formaldehyde (40 per cent. strength) to 1,000 ccm. of which is added 2 ccm. of a N 10 solution of caustic potash. The precipitate is filtered off through pulp.

By replacement of the membranes upon the uterine wall the detached cotyledons were returned to their original situations. By severing their placental attachments flush with the surfaces of the maternal cotyledons, the membranes were removed and weighed and their volume was determined by the displacement method. It will be noticed that the umbilical cord was included with the foetal envelopes.

After the weight of the empty uterus (i.e. the weight of the uterine wall plus that of the placenta) had been determined, the number of cotyledons in each half of the uterus was counted. For this purpose the line C—J (plate 1) was taken as the division between the two halves.
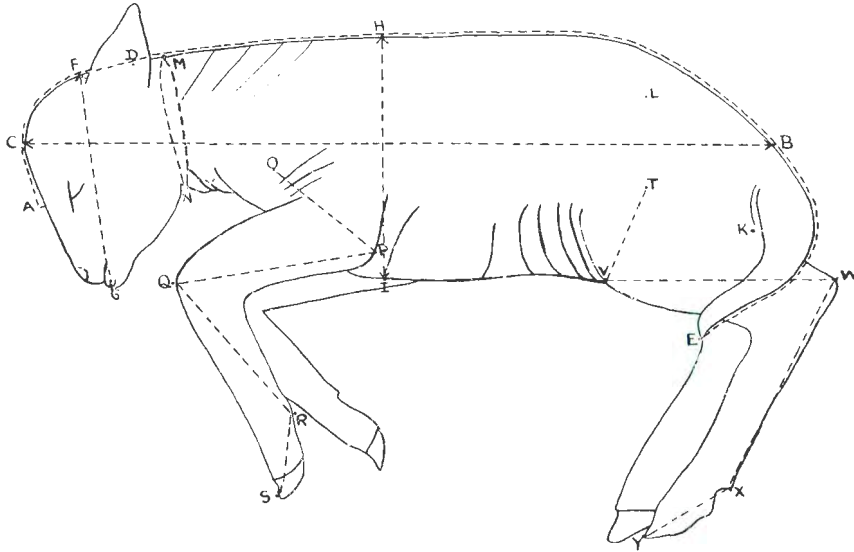
Plate II.—Illustrating the foetal dimensions measured.

The minute, very pale and non-functional cotyledons present in the tips of the cornua were not counted. In each horn the maximum diameter and height both of the largest and of an average-sized cotyledon were measured. In subjects pregnant for 59 days or more the cotyledons were removed from the uterine wall by cutting the mucosal penduncles, and their weight and volume* were determined. Then the uterine wall was weighed, after which it was again stretched out and the cervix was opened by a longitudinal incision. The procedure for determining the length, width and thickness of the cervix was similar to that described for the vagina. Notes were made of the amount and nature of the mucus contained in the cervical canal, while the appearance of the cut surface was also recorded. The length and thickness of the body and the thickness of the wall of each of the horns of the uterus were measured. All the points for measurement are indicated in Plate 1.

* By the displacement method.

By this time the adipose tissue in the mammary gland had set. In removing the covering skin those portions enclosing the teats were left in position. The weight of the gland was determined and where necessary this was corrected for the weight of injection fluid present in the lactiferous sinus.

Later, as time permitted, the preserved foetuses were studied. The crown-rump lengths were re-measured to determine whether shrinkage had occurred. In no instance was any appreciable difference observed. The dimensions listed and illustrated in Plate 2 were measured. Then the external appearance of the foetus was studied, special attention being devoted to the appearance of hair and the development of the appendages.

2. *Treatment of the Data.*

A glance at the tables in the appendix will indicate the variability of most of the data relating to the genitalia, the fluids and the endocrines. In order to minimize this and to arrive at simpler indications of the growth processes involved the data were grouped into six monthly groups according to the stage of pregnancy (the non-pregnant ewes being considered to be 0 months pregnant. The means for the groups were calculated, and these were then tested by Fisher's (1936) "Analysis of Variance" method, the Z-test being employed to determine the existence of significant differences, while the significantly differing groups were picked out by means of the *t*-test.

Here it must be noted that due to the continuous nature of growth the differences within groups are not purely of an experimental nature, hence the accuracy of the tests is impaired. However, as the effect of the error is to obscure significance (by increasing " SD for a single observation ") the results in the present work will not suffer adversely.

As standards for significance were taken the values of Z and t when $P = 0.05$ (i.e. 5 per cent. probability) and when $P = 0.01$ (i.e. 1 per cent. probability). A positive result at the former level (which already indicates definite significance) is indicated by $\times$, while a similar result at the higher level of significance is indicated by $\times$ $\times$.

The complete data are given in a series of tables in the appendix. In the text only the means for the groups are mentioned, these figures being employed in the graphs as well as the tables. In the latter are indicated the number of each group, the class (or description), and the number of ewes in the group. Then the mean for the group is stated, and this is followed by the results of the tests for significance. The latter are given in two columns, the first showing the results when each group is tested against the non-pregnant group, while the second indicates the results of testing each group against the one immediately preceding it.

In that section dealing with the foetus the procedure outlined above was not followed. Details of the treatment in this, as well as in a few other special instances, are given at the appropriate places in the text.

450

The last point to be considered is the method of estimating the age of the foetus. As testing for " heat " was carried out once every twelve hours, the " most probable " time of onset of oestrum was taken to be six hours earlier than the time at which its presence was detected. As the services were regulated so as to ensure the presence of large numbers of active sperms in the Fallopian tubes at the time of ovulation, fertilization was presumed to occur at the thirtieth hour after the onset of heat. It will be seen that this time is determined with greater ease by simply adding twenty-four hours to the time of detection of " heat ". The age of the foetus was calculated from the time of fertilization until the time of death of the mother, and this figure was then approximated to the nearest full day.

CHAPTER 4.—OBSERVATIONS.

(a) GENITAL TRACT AND PLACENTA.
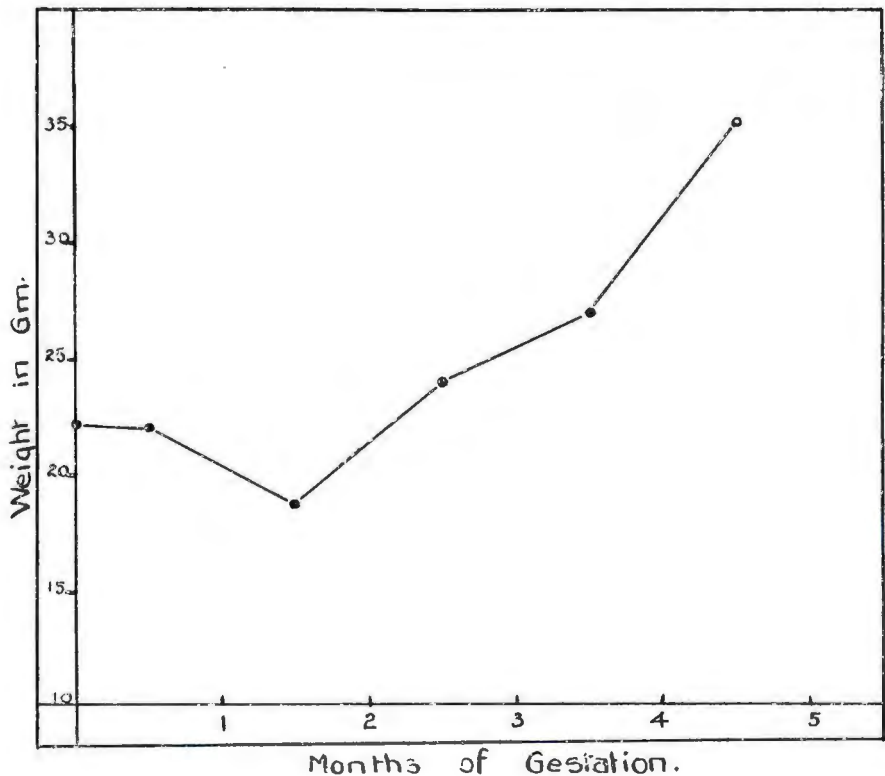
1. *Vagina.*

In order to determine whether, during the course of pregnancy, there is definite growth of the vagina, the weight of this organ must be considered. The mean vagina weights in each of the six monthly groups are presented both in tabular and in graphic form (Table 3 and Fig. 1).

Weight of Vagina.

GROUPS OF EWES.		No. of Ewes.	Mean Vagina Weight.	SIGNIFICANCE TESTS.	
No.	Class.			W. Group 1.	W. Preced. Group.
			Gram.		
1	Non-preg..........	11	22·45	—	—
2	1st month.........	12	22·25	—	—
3	2nd month.........	8	18·88	—	—
4	3rd month.........	6	24·83	—	—
5	4th month.........	7	27·29	—	—
6	5th month.........	5	35·20	XX	X

The most striking feature of the table is that, apart from the increase in the last month, all the variations are insignificant and may be due entirely to chance. Thus no importance is to be attached to the drop in weight in the second month. When this is disregarded it appears that during the first half of pregnancy the weight of the vagina remains unchanged. From the third month onwards there is to be detected an upward trend which, however, becomes definitely significant in the last month only. By the end of gestation the mean vagina weight has reached a level 50 *per cent.* above that of the non-pregnant group. Of this increase almost 70 *per cent.* is added during the last month, the remainder being accounted for by the small but cumulative increases of the third and fourth months.

Fig. 1.—Weight of Vagina.

Having demonstrated an increase in mass, one may now consider the dimensions of the vagina with a view to determining the manner in which the additional substance is distributed. Length is considered in Table 4 and Fig. 2.

TABLE 4.

Length of Vagina.

GROUPS OF EWES.		No. of Ewes.	Mean Vagina Length.	SIGNIFICANCE TESTS.	
No.	Class.			W. Group 1.	W. Preced. Group.
			Cm.		
1	Non-preg..........	11	10·73	—	—
2	1st month.........	12	11·08	—	—
3	2nd month.........	8	11·60	—	—
4	3rd month.........	6	13·33	XX	X
5	4th month.........	7	13·43	XX	—
6	5th month.........	5	15·60	XX	XX

452

Apart from the absence of a drop in the second month, the general trend of this graph is similar to that of Fig. 1. Again there is little or no change in the first three groups, followed by a definite increase in the following months. However, in its earlier stages the upward trend here is fairly definite, with the result that the total increase, far from being practically confined to the last group, is spread more or less evenly over the last three months.

Fig. 2.—Length of Vagina.
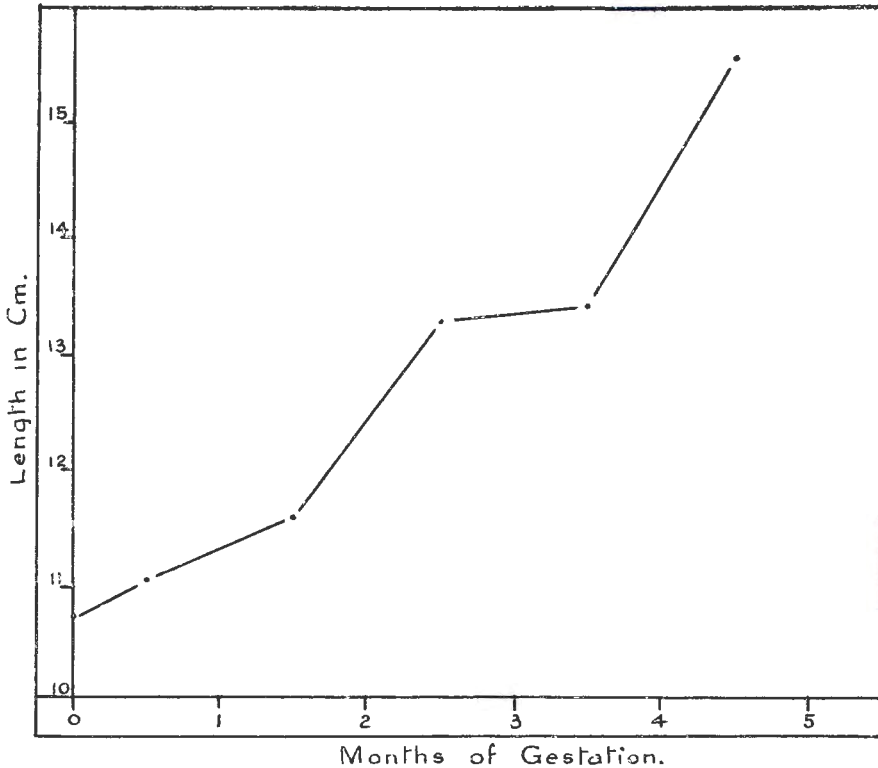
When each group is compared with the one immediately preceding it, it is found that Group 5 does not show significance, whereas both Groups 4 and 6 do. This is to be expected from the marked flattening of the graph between the third and fourth months. For this no reason is apparent, and it might be that it is a discrepancy due to the manner of grouping. It is quite possible that with different grouping the shape of the graph between its two extremities would be altered.

The next dimension to be considered is the width (Table 5 and Fig. 3).

TABLE 5.

Width of Vagina.

GROUPS OF EWES.		No. of Ewes.	Mean Vagina Width.	SIGNIFICANCE TESTS.	
No.	Class.			W. Group 1.	W. Preced. Group.
			Cm.		
1	Non-preg..........	11	3·47	—	—
2	1st month.........	12	3·39	—	—
3	2nd month.........	8	2·92	—	—
4	3rd month.........	6	4·00	—	X
5	4th month.........	7	4·30	X	—
6	5th month.........	5	6·40	XX	XX

Fig. 3.—Width of Vagina.
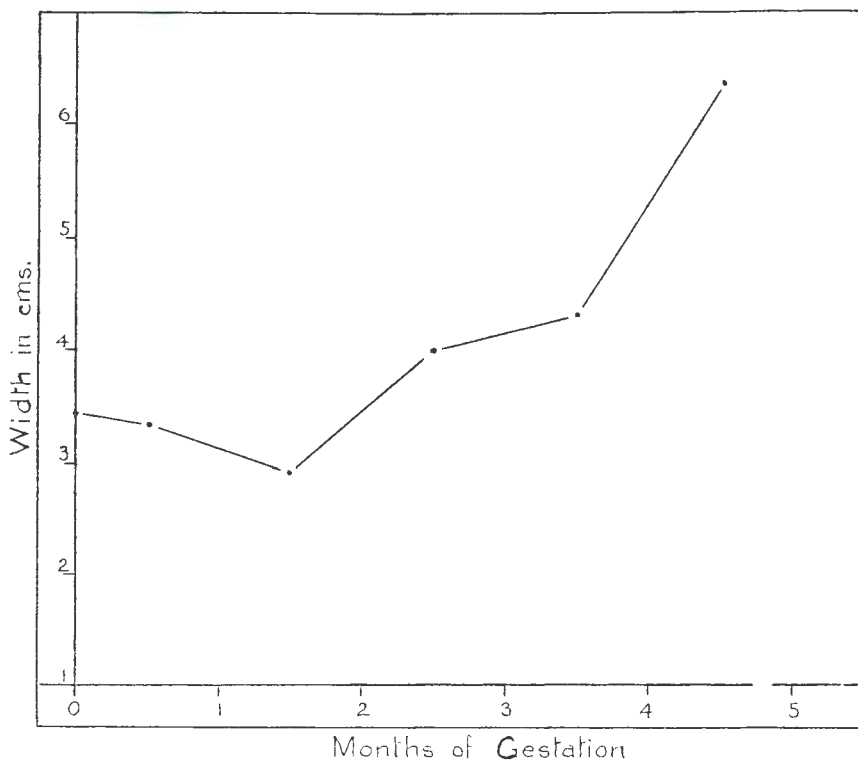
The shape of this graph bears a close resemblance to that of the weight (Fig. 1). The increase in width becomes significant in the last two months only. The positive result obtained when Group 4 is tested against Group 3 is undoubtedly due to the accident of

a low figure in the second month, rather than to a marked increase in the third. As a result of the very steep rise during the last month, the total increase in width amounts to almost 100 *per cent.* of the width of the non-pregnant group.

Fig. 4.—Thickness of Vagina.
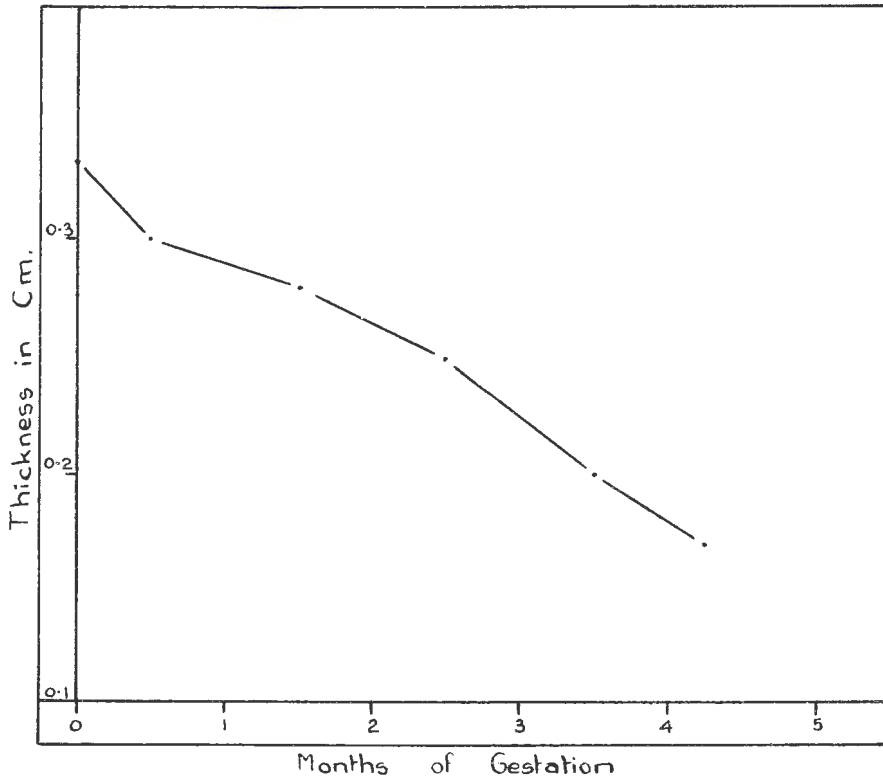
Figure 4 (thickness of vaginal wall) gives the impression that during pregnancy the vaginal wall is reduced steadily to half its original thickness. However, in Table 6 it is seen that not one of the variations is significant, and that all may be due to chance alone. This finding is due to the large degree of variation in the original data (see Appendix), in which consecutive figures in the same group are seen to differ by as much as 400 *per cent.* These widely differing figures are comparatively few. However, under the circumstances, all that can be said is that there is apparently a slight tendency for the thickness of the vaginal wall to be reduced during gestation.

Discussion.

From a consideration of the length and the width of the vagina it becomes evident that, with the advance of pregnancy, the surface area of this organ is increased. This must mean that the lumen of the vagina is enlarged. To a certain extent the reduction in

455

thickness contributes towards this increase in surface area. However, the main factor is undoubtedly the actual increase of vaginal substance. As there is no internal pressure in the vagina the thinning cannot be due to passive stretching. There must be active re-distribution of the tissue-elements. These processes all indicate a slow preparation of the vagina for the stretching due to occur at parturition. It is presumed that the individual muscle fibres increase in size and become re-distributed to form a thinner layer.

TABLE 6.

Vagina Thickness.

Groups of Ewes.		No. of Ewes.	Mean Vaginal Thickness.	Significance Tests.	
No.	Class.			W. Group 1.	W. Preced. Group.
			Cm.		
1	Non-preg..........	11	0·33	—	—
2	1st month.........	12	0·30	—	—
3	2nd month.........	8	0·28	—	—
4	3rd month.........	6	0·25	—	—
5	4th month.........	7	0·20	—	—
6	5th month.........	5	0·17	—	—

All these changes are recognisable upon macroscopic inspection. The vagina of the non-pregnant ewe is small and compact and ordinarily its lateral walls are in apposition. The labia of the vulva are firm and the opening between them is a mere slit, while the whole of the vaginal lumen is merely a potential space. The mucosa is smooth, only slightly moist and is usually pale. Posterior to the external urethral opening it often has a pinkish-brown colour. During oestrum the mucosa becomes hyperaemic and some clear mucus is present on its surface, while there is a suggestion of increased turgidity of the vulval labia.

For the greater part of the first month of gestation the picture resembles that of the oestral period. Probably this is the effect of the last " heat " period rather than of pregnancy. By the end of the first month the appearance is that of the anoestral period. This return to the less vascular " inactive " state probably accounts for the slight drops during the second month in Figs. 1 and 3.

Not until the 110th day of gestation is it possible to observe definite departures from this state. At this stage the vagina has become loose and flabby, the labia of the vulva are soft and are no longer in close apposition. From now on these changes become more marked until at the approach of parturition the whole organ is soft and flabby and the lumen is large and fairly patent. In its anterior half a fair amount of white, cloudy and viscid mucus is present, while the mucosa is pale and smooth.

2. *Cervix.*

As the weight of this organ was not recorded separately, it is not possible to determine directly the changes in mass that may occur during the course of gestation. However, these may be deduced from a study of the dimensions of the cervix.

Length is considered in Table 7 and Fig. 5.

TABLE 7.
Length of Cervix.

GROUPS OF EWES.		No. of Ewes.	Mean Length of Cervix.	SIGNIFICANCE TESTS.	
No.	Class.			W. Group 1.	W. Preced. Group.
			Cm.		
1	Non-preg..........	11	5·14	—	—
2	1st month.........	12	5·23	—	—
3	2nd month........	8	5·94	X	—(¹)
4	3rd month........	6	6·87	XX	X
5	4th month........	7	7·93	XX	X
6	5th month........	5	8·80	XX	—(¹)

(¹) Here significance at P = 0·05 is just missed.

Fig. 5.—Length of Cervix.

Months of Gestation.
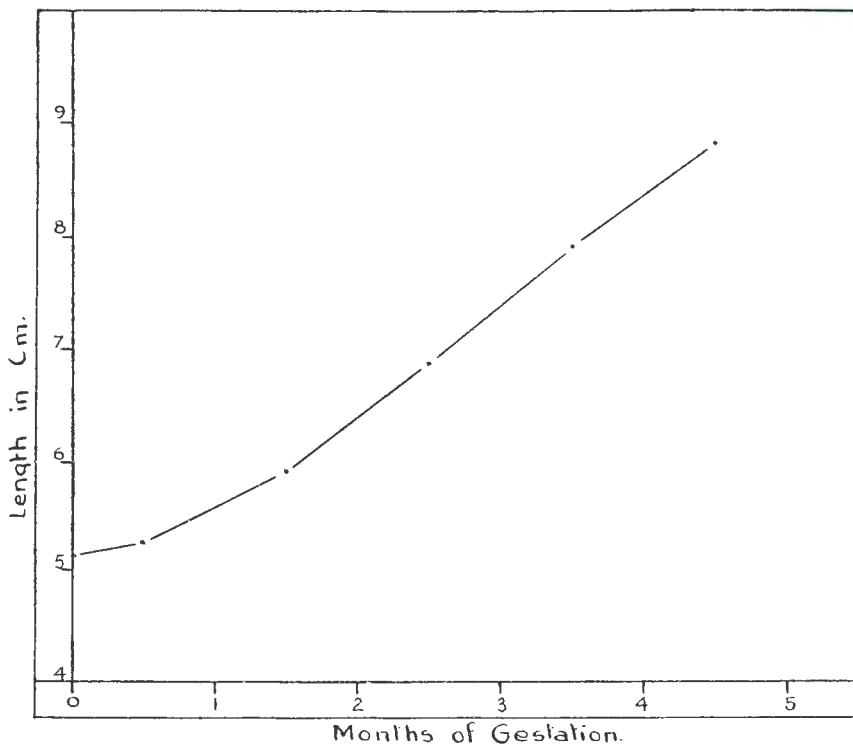
It is seen that there is a definite increase, which is distributed evenly over the entire period of pregnancy. By the second month the increase has become significant and from now on during each month approximately equal amounts are added. This is indicated by the straight line which this portion of the graph assumes.

TABLE 8.

Width of Cervix.

GROUPS OF EWES.		No. of Ewes.	Mean Width of Cervix.	SIGNIFICANCE TESTS.	
No.	Class.			W. Group 1.	W. Preced. Group.
			Cm.		
1	Non-preg..........	11	1·75	—	—
2	1st month.........	12	2·12	X	X
3	2nd month.........	8	1·68	—	X
4	3rd month.........	6	1·92	—	—
5	4th month.........	7	2·29	X	—
6	5th month.........	5	2·98	XX	XX

Fig. 6.—Width of Cervix.

Months of Gestation.
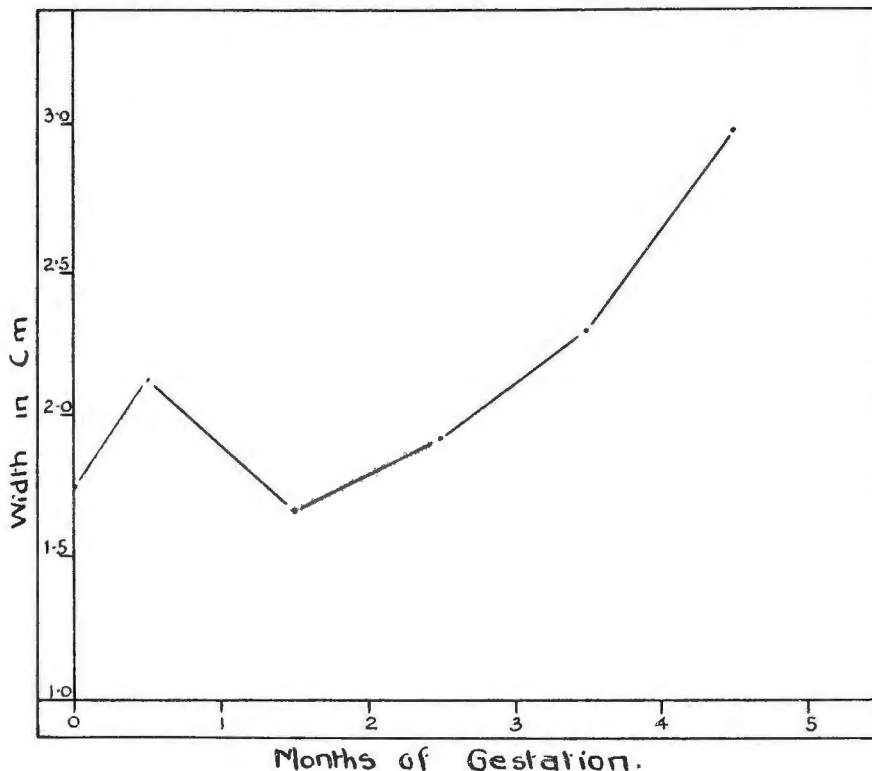
The width (Table 8 and Fig. 6) does not exhibit this same regular growth. The peak in the first month is definitely significant. This is followed by a drop in the second month, the figure here being

458

slightly lower than that of group 1. From here onward there is an increase in width, the increment for each succeeding group being larger than that of the preceding one. By the fourth month the width is again significantly larger than that of the non-pregnant group.

TABLE 9.

Thickness of Wall of Cervix.

GROUPS OF EWES.		No. of Ewes.	Mean Cervical Thickness.	SIGNIFICANCE TESTS.	
No.	Class.			W. Group 1.	W. Preced. Group.
1	Non-preg..........	11	0·39	—	—
2	1st month.........	12	0·35	XX	XX
3	2nd month.........	8	0·34	XX	—
4	3rd month.........	6	0·28	XX	XX
5	4th month.........	7	0·33	XX	XX
6	5th month.........	5	0·55	XX	XX

Fig. 7.—Thickness of Cervical Wall.
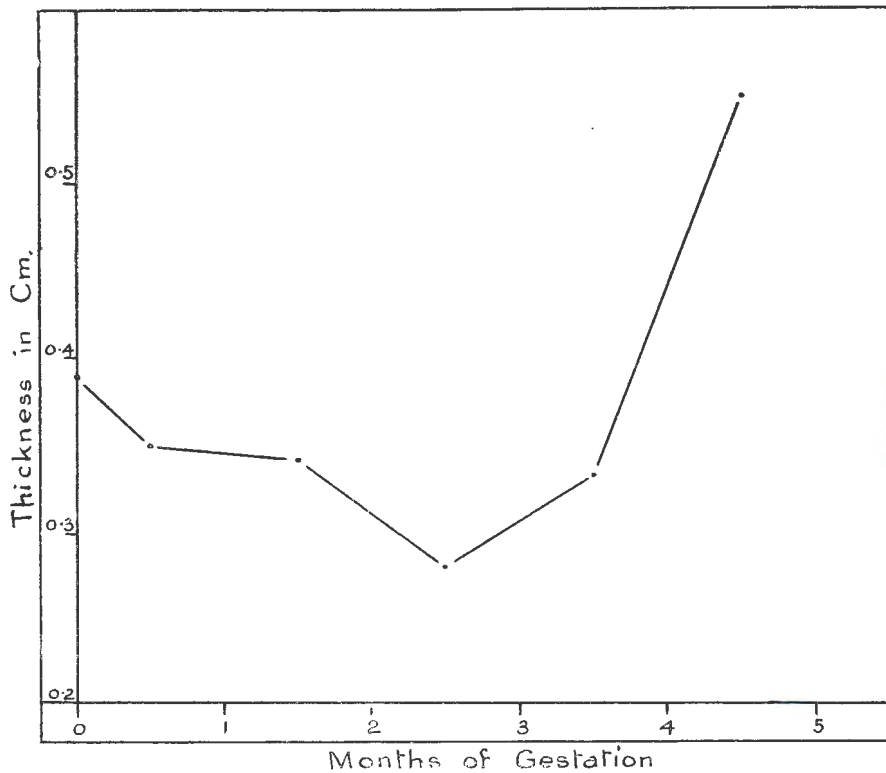
In Table 9 and Fig. 7 are given details of the thickness of the wall of the cervix. During the first three months of gestation this dimension decreases until during the third month the lowest level is

reached. In the following two months there are definite increases, that for the last month being very large indeed. This part of the graph rises almost vertically. When compared with the figure of the non-pregnant group all these variations are found to be significant, and in the last column of the table, Group 3 only fails to give a positive result: note the flattening of the graph at this point.

Discussion.

Before discussing these results it is necessary to consider some details concerning the uterine seal, the development of which appears to be closely associated with the changes in the wall of the cervix. Only once (ewe No. 44326 at 130 days) was anything resembling a definite " plug " seen, hence the term " seal " is preferred, as it describes more accurately the appearance and distribution of the mucous secretion in the cervix. By the end of the first month of pregnancy small accumulations of mucus may be detected in the depressions between the cervical folds. This is more noticeable in the cranial portions of the organ. As the amount of mucus increases it appears to " glue " together the adjacent surfaces of the folds, and by the 45th day of pregnancy this sealing is complete and a small amount of free mucus is seen in the lumen of the cervix. This tends to collect in the more cranial portions of the organ, where the tissue is less tense and where the folds are small. Gradually the quantity of this cloudy, white, sticky mucus increases, and as this happens the free mucus is no longer found only in the cranial portion, but spreads in a caudal direction. At no stage of pregnancy was the mucus seal found to protrude at the external uterine os.

Whereas the length of the cervix undergoes a steady increase, the width displays a sharp peak, followed firstly by a drop and then by a gradual increase. The first rise may be the result of dilatation of the cervix caused by an increased secretion of mucus during oestrum. As this effect passes off the organ returns to its contracted, dioestral state. Later the effects of pregnancy bring about a steady increase in the width of the cervix. This theory receives support from the fact that in the first month of gestation the thickness of the wall of the cervix is greatly reduced—just what would be expected to result from stretching of the organ caused by pressure in its lumen. As the seal substance accumulates in the cervix the stretching is intensified, hence during the third month there is a further decrease in thickness. After mid-term there is active growth of the organ, with a consequent increase in all dimensions. The thickened wall loses its firmness and becomes soft and slightly oedematous. These changes are first detectable at the 89th day of pregnancy. By this time the accumulation of mucus has increased the size of the lumen, and the whole organ appears enlarged, soft and spongy. At the 110th day these changes are more noticeable and due to the stretching and the accumulation of mucus the folds are well separated. Nine days later the canal is still more dilated and at this stage it is an easy matter to insert a probe into the cervix. At the 130th day the lumen has a diameter of 0·5 cm. and at the 140th day this has increased to 1·0 cm. The whole organ is extremely soft and spongy and it appears markedly oedematous. The tense cervix of the non-pregnant sheep has now become loose and elastic and is capable of extreme dilation.

3. *Body and Cornua.*

From a consideration of its curvatures an indication of the growth in length of the uterus is obtained. In Table 10 and Fig. 8 are to be found details concerning the length of the greater curvatures of both horns.*

TABLE 10.

Greater Curvatures of Cornua.

GROUPS OF EWES.		No. of Ewes.	Mean Length Preg- nant Horn.	Significance Test.		Mean Length Non- preg- nant. Horn.	Significance Test.	
No.	Class.			W. Group. 1.	W. Preced. Group.		W. Group. 1.	W. Preced. Group.
			cm.			cm.		
1	Non-preg......	11	15·59	—	—	15·18	—	—
2	1st month.....	12	19·12	—	—	17·58	—	—
3	2nd month.....	8	38·06	X X	X X	34·25	X X	X X
4	3rd month.....	6	62·67	X X	X X	50·17	X X	X X
5	4th month.....	7	74·86	X X	X*	54·57	X X	—
6	5th month.....	5	100·00	X X	X X	62·60	X X	X

* Significance at 1 per cent. level is just missed.

Fig. 8.– Greater Curvatures of Cornua.

——•——•—— Pregnant horn
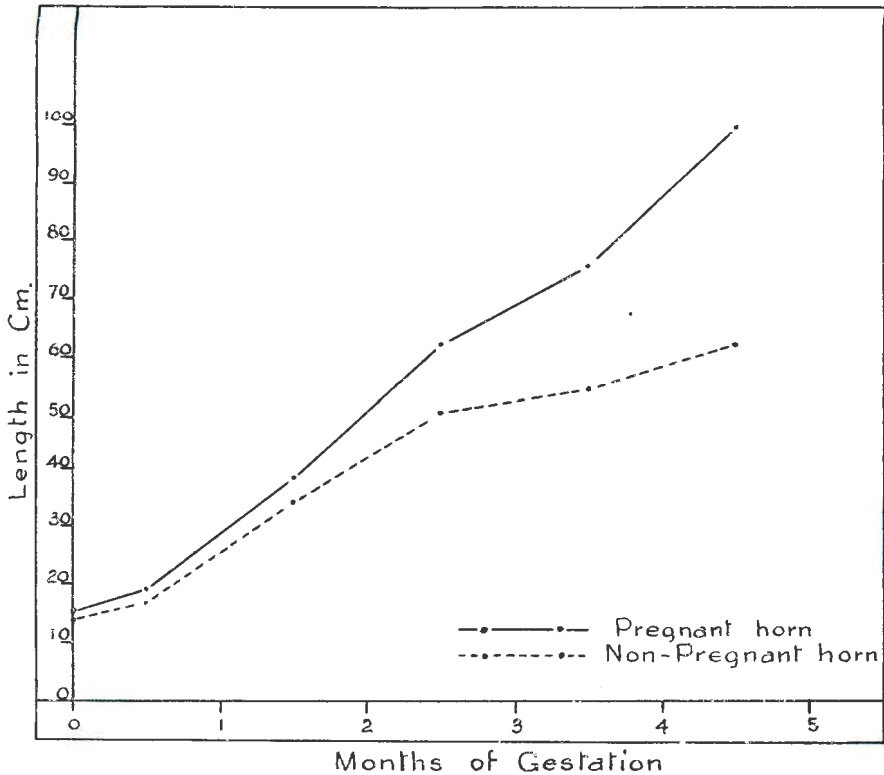
-------•------•------ Non-Pregnant horn

* Here, and in all similar cases, in Group 1 the left horn has been classed as the gravid one (pregnant 0 days), while the right horn is regarded as being non-gravid at 0 days.

In the pregnant horn there is a steady increase throughout pregnancy. Significance is reached in the second month. The greater curvature of the non-pregnant horn also undergoes much elongation, although its graph always remains below that of the gravid horn. For the first three months the general trend of the two lines is very similar, although from their gradual divergence it is evident that the non-pregnant horn grows at the slower rate. In the last two months the latter curve flattens out considerably, making for much greater dissimilarity between the two curves. The increments for the last two months are much more significant in the gravid horn than in the opposite one (see Table 10 —second columns of significance tests).

Table 11 and Fig. 9 show that, regarding the lesser curvatures, there are no marked differences between the two horns. The two graphs assume similar courses, with that of the non-gravid horn always just below the other. The amount of divergence, even at its maximum (in the fourth month), is comparatively small. As is the case with the greater curvatures, here again significance is reached early on in pregnancy, i.e. in the second month.

Fig. 9.—Lesser Curvatures of Cornua.

TABLE 11.

Lesser Curvatures of Cornua.

GROUPS OF EWES.		No. of Ewes.	Mean Length Pregnant Horn.	Significance Test.		Mean Length Non-pregnant Horn.	Significance Test.	
No.	Class.			W. Group. 1.	W. Preced. Group.		W. Group 1.	W. Preced. Group.
			cm.			cm.		
1	Non-preg......	11	11·59	—	—	11·18	—	—
2	1st month.....	12	13·96	—	—	13·63	—	—
3	2nd month.....	8	22·81	XX	XX	22·50	XX	XX
4	3rd month.....	6	33·17	XX	XX	32·83	XX	XX
5	4th month.....	7	36·86	XX	—	31·71	XX	—
6	5th month.....	5	43·80	XX	XX	39·80	XX	XX

Fig. 10.—Circumferences of Cornua.
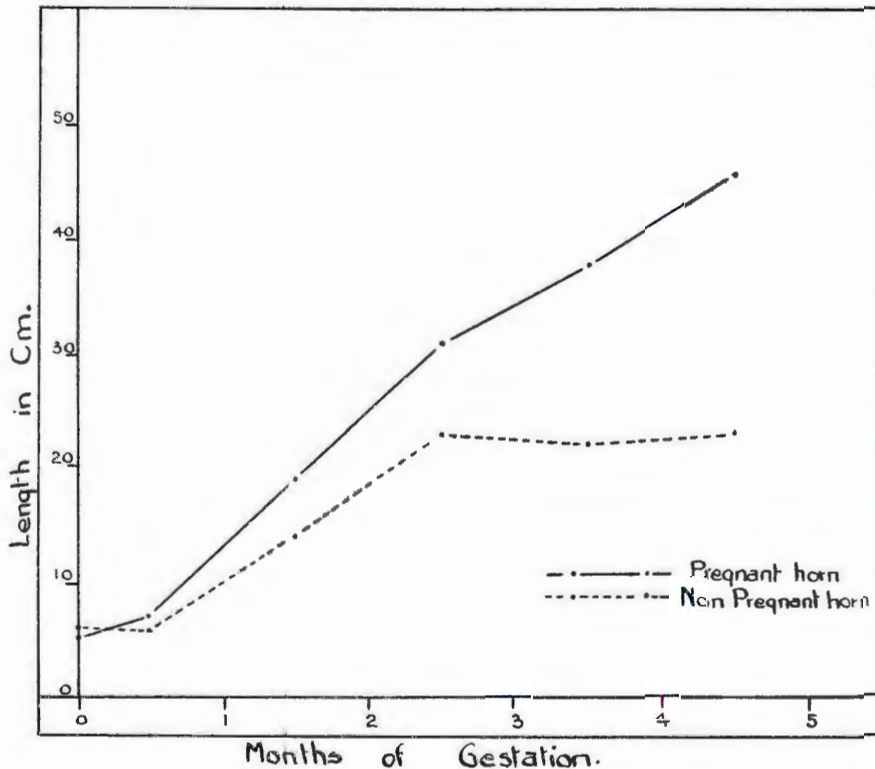
It appears that in both horns there is considerable growth in length. That there is uneven growth on the two sides of the uterus is evident from the different slopes of the curves in Figs. 8 and 9 (drawn to the same scale). Here it is seen that in each horn the

463

greater curvature outgrows the lesser, this being particularly noticeable in the last two months. The larger size of the gravid horn is due almost entirely to more extensive development of the greater curvature.

A peculiar feature of both these graphs is the distinct flattening between the third and fourth months. Further, it will be noticed that only in the case of the greater curvature of the pregnant horn is Group 5 significantly larger than Group 4, and even in this instance the level of significance is lower than that of the immediately preceding and following groups. An apparent explanation for this anomalous behaviour will suggest itself later, when the volumes of the foetal fluids are studied.

There is a fairly close resemblance between the graphs of the circumferences (Fig. 10) and those of the greater curvatures (Fig. 8). Again the line for the non-gravid horn lies below the other and diverges from it, slowly up to the third month and then more rapidly. After the third month the circumference of the non-pregnant horn does not increase at all, whereas during the same time the monthly increments of the gravid horn remain definitely significant (Table 12). Here too the peculiar flattening of the curve, referred to above, is evident. In the pregnant horn it is of only slight degree, yet in the other horn it is most marked.
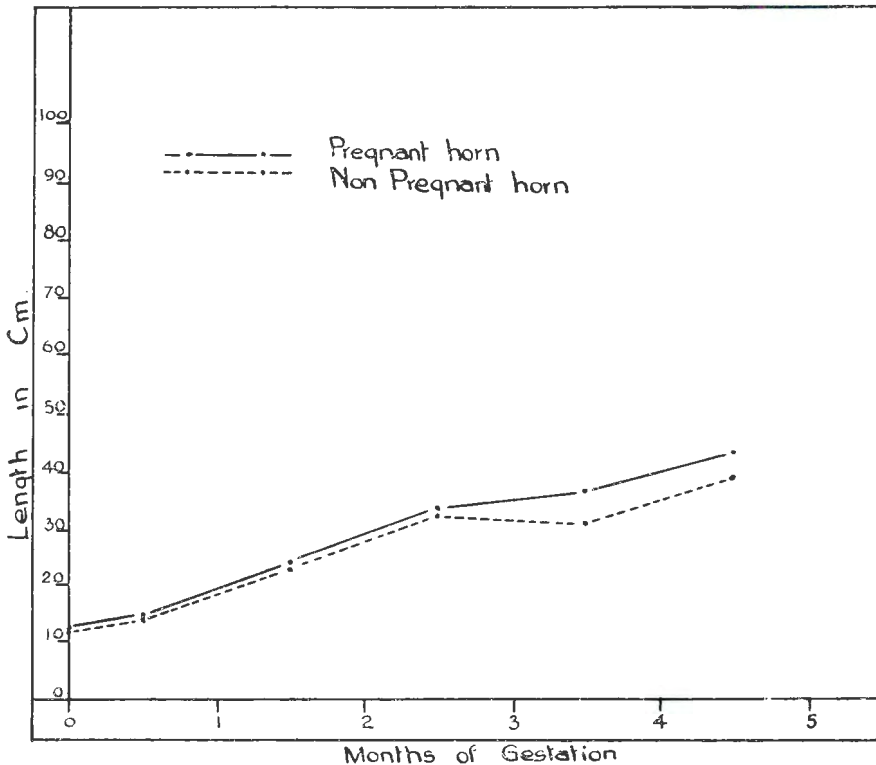
TABLE 12.

Circumferences of Cornua.

GROUPS OF EWES.		No. of Ewes.	Mean Length Pregnant Horn.	Significance Test.		Mean Length Non-pregnant Horn.	Significance Test.	
No.	Class.			W. Group. 1.	W. Preced. Group.		W. Group. 1.	W. Preced. Group.
			cm.			cm.		
1	Non-preg......	11	5·05	—	—	5·23	—	—
2	1st month.....	12	6·87	—	—	5·73	—	—
3	2nd month.....	8	18·75	XX	XX	14·00	XX	XX
4	3rd month.....	6	31·08	XX	XX	23·00	XX	XX
5	4th month.....	7	37·57	XX	XX	22·00	XX	—
6	5th month.....	5	45·49	XX	XX	23·00	XX	—

Discussion.

It is only to be expected that with the advance of pregnancy there will be an increase in the size of the uterine horns. Here it is seen that during the first half of gestation both horns increase fairly steadily, with but a slight difference in favour of the pregnant horn. However, during the second half of pregnancy the horn in which the foetus lies completely outgrows the other, this being noticeable in both curvatures (not marked in the lesser) as well as in circumference. As a result of the uneven distribution of the

increase, the enlargement of the horns is accompanied by a change in shape. All these changes are well illustrated in the photographic plates presented by Curson and Quinlan (1934) and Curson and Mare (1934).

Fig. 11.—Weights of Dressed Uterus, Empty Uterus and Uterine Wall.
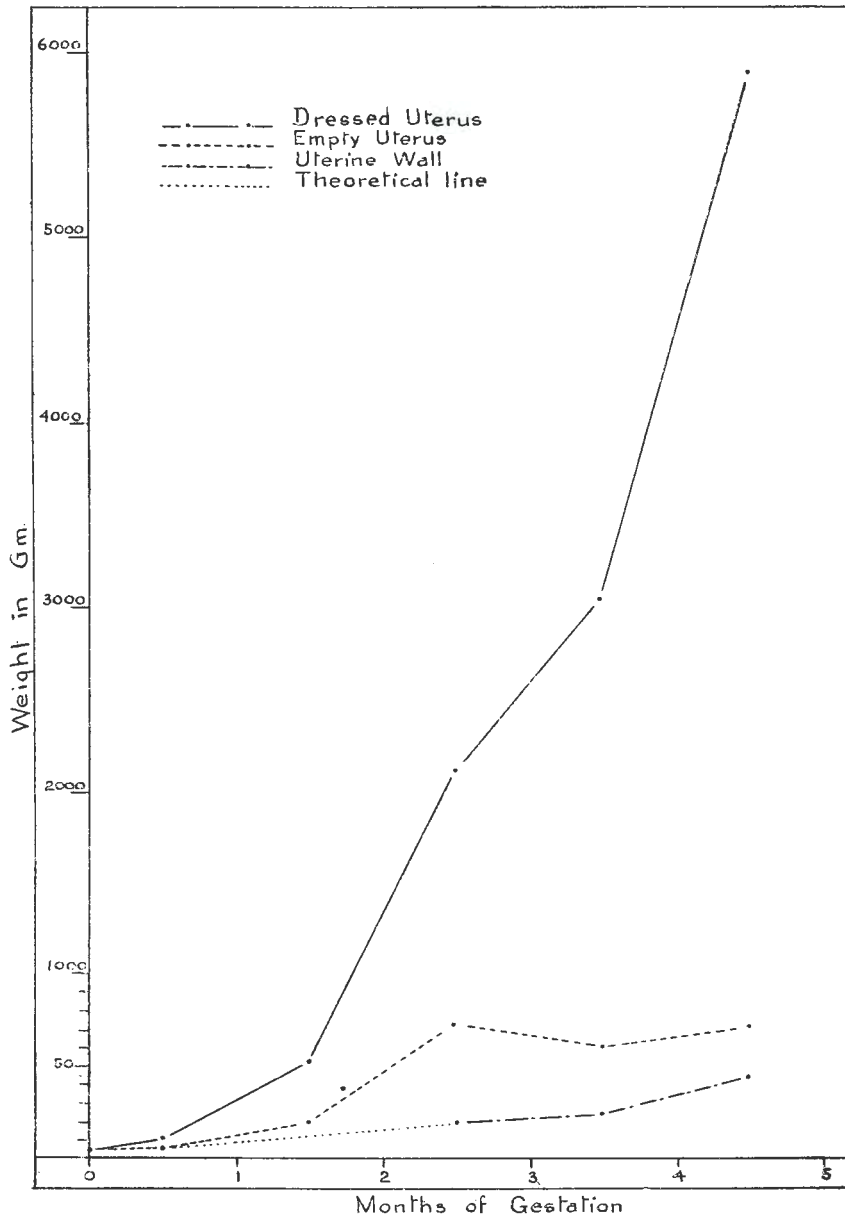
The growth of the uterus in weight is considered in Table 13 and Fig. 11. By the end of pregnancy the " dressed " uterus has reached the relatively enormous weight of approximately 6 Kg.

TABLE 13.

Weights of Dressed Uterus, Empty Uterus and Uterine Wall.

Groups of Ewes.		No. of Ewes.	Mean Weight Dressed Uterus.	Significance Test.		Mean Weight Empty Uterus.	Significance Test.		Mean Weight Uterine Wall.	Significance Test.	
No.	Class.			W. Gr. 1.	W. Prec. Gr.		W. Gr. 1.	W. Prec. Gr.		W. Gr. 1.	W. Prec. Gr.
			Gm.			Gm.			Gm.		
1	Non-preg.	11	42·73	—	—	42·73	—	—	42·73	—	—
2	1st month.	12	65·92	—	—	39·67	—	—	?	?	?
3	2nd month.	8	519·75	X	X	292·75	XX	XX	?	?	?
4	3rd month.	6	2,128·67	XX	XX	740·17	XX	XX	221·00	XX	?
5	4th month.	7	3,026·57	XX	XX	597·43	XX	X	248·00	XX	—
6	5th month.	5	5,880·80	XX	XX	708·80	XX	—	442·00	XX	XX

Fig. 12.—Weight of Placenta.
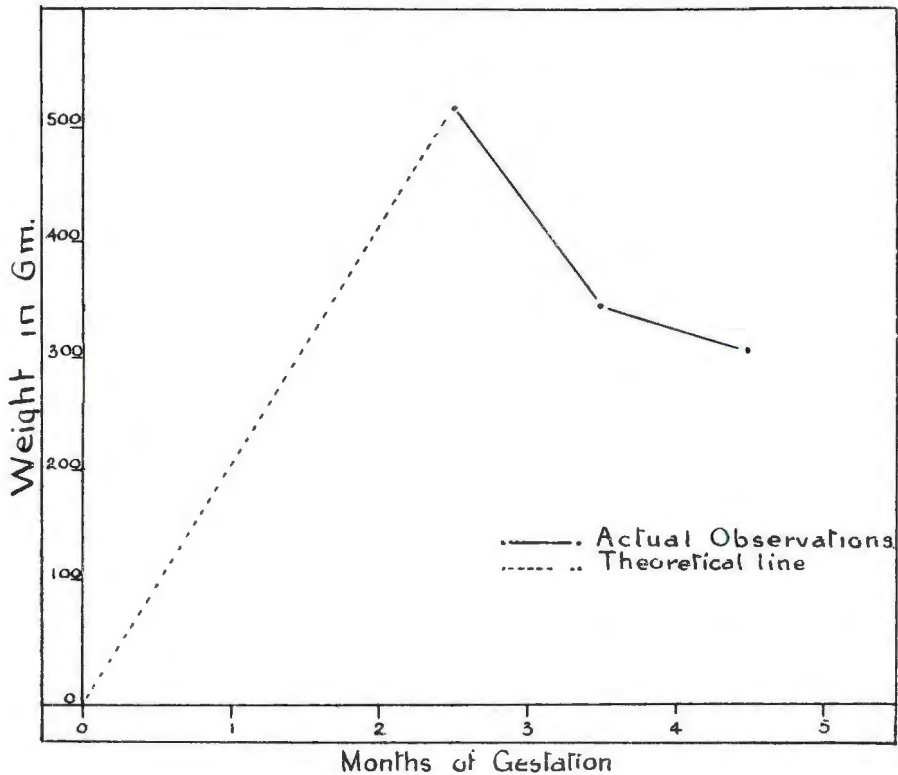
The graph rises but little in the first month, more in the second (at this stage the increase becomes significant) and then assumes an exceedingly steep slope. Between the third and fourth months there is a distinct flattening of the curve. Later it will be seen that this is caused in part by a decrease in the total volume of foetal fluid and in part by a drop in placental weight.

The weight of the empty uterus is the sum of the weights of the uterine wall and the placenta. Details of the latter are presented in Table 14 and Fig. 12. For the first four months of pregnancy the course of the graph of the empty uterus is governed mainly by the weight of the placenta. Until the end of the second month there is a gradual rise, and then in the third month there is a sudden peak, followed by a drop. Meanwhile the weight of the uterine wall has increased steadily, but this has not been sufficient to influence to any great extent the weight of the empty uterus. In the last month there is a highly significant increase in the weight of the wall and as a result of this the graph of the empty uterus rises again to the level of the third month, in spite of the fact that at this stage the placental weight undergoes a further (but insignificant) reduction.
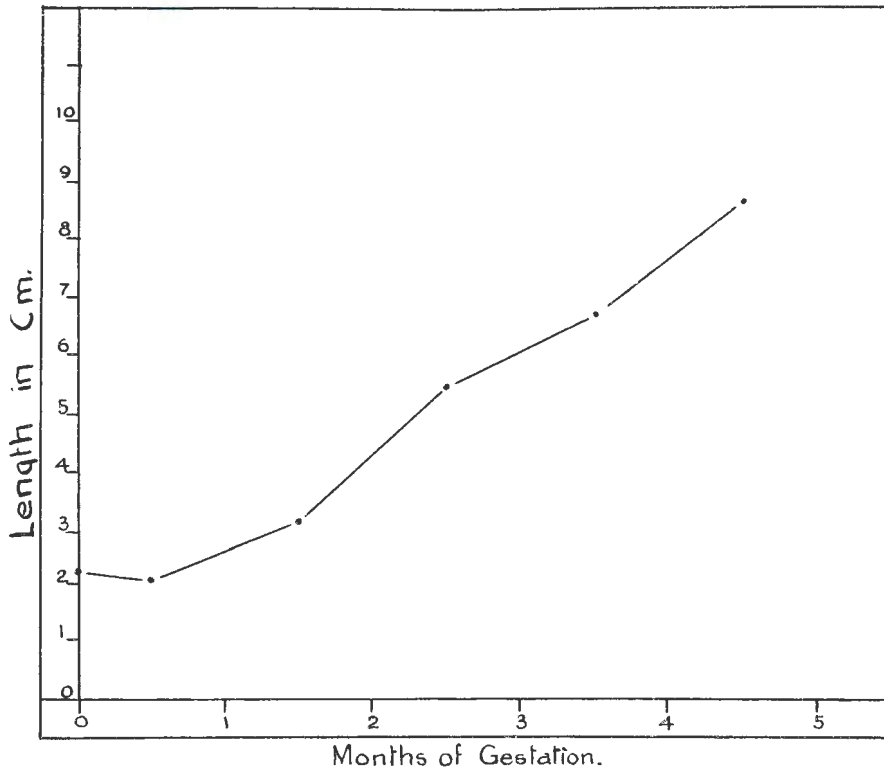
Fig. 13.—Length of Body of Uterus.

TABLE 14.

Weight of Placenta.

GROUPS OF EWES.		No. of Ewes.	Mean Weight of Placenta.	SIGNIFICANCE TESTS.	
No.	Class.			W. Group 1.	W. Preced. Group.
1	Non-preg..........	11	Nil	—	—
2	1st month.........	12	?	—	—
3	2nd month.........	8	?	—	—
4	3rd month.........	6	519 gm.	XX	?
5	4th month.........	7	349 gm.	XX	X
6	5th month.........	5	307 gm.	XX	—

The increase in size of the uterus is not limited to its horns, but is also evident in *the body*. When it is remembered that the body is the portal through which the cornua communicate with each other, it is not surprising to find that in its increase in length (Table 15 and Fig. 13) this portion of the uterus follows a course closely resembling, and more or less intermediate between, those observed in connection with the greater curvatures of the cornua. Here too a slight flattening of the curve in the fourth month of pregnancy, is observed.

TABLE 15.

Length of Body of Uterus.

GROUPS OF EWES.		No. of Ewes.	Mean Length of Body.	SIGNIFICANCE TESTS.	
No.	Class.			W. Group 1.	W. Preced. Group.
			Cm.		
1	Non-preg..........	11	2·16	—	—
2	1st month.........	12	2·07	—	—
3	2nd month.........	8	3·19	*	X
4	3rd month.........	6	5·50	XX	XX
5	4th month.........	7	6·71	XX	XX
6	5th month.........	5	8·70	XX	XX

* Just misses significance at 5 per cent. level.

From the fact that by the end of the second month of gestation the body is not yet* significantly longer than in the non-pregnant group. it is concluded that in this part of the uterus growth is initiated later than in the horns. In this connection it is to be

* By how little significance is missed is indicated by the fact that Group 3 is significantly larger than Group 2, which is but slightly lower than Group 1.

observed that during the first month there is no change from the non-pregnant state, in fact the mean of Group 2 is slightly (but not significantly) lower than that of Group 1.

In each of the three portions of the uterus (the two horns and the body) the *thickness of the wall* was measured. Details are presented in Table 16. In the column headed " Total Groups " are tabulated the means of all the measurements recorded in each month, while in the last line of the table are given the " Averages " for all the readings (throughout pregnancy) at each point.

Fig. 14.- Thickness of Uterine Wall.
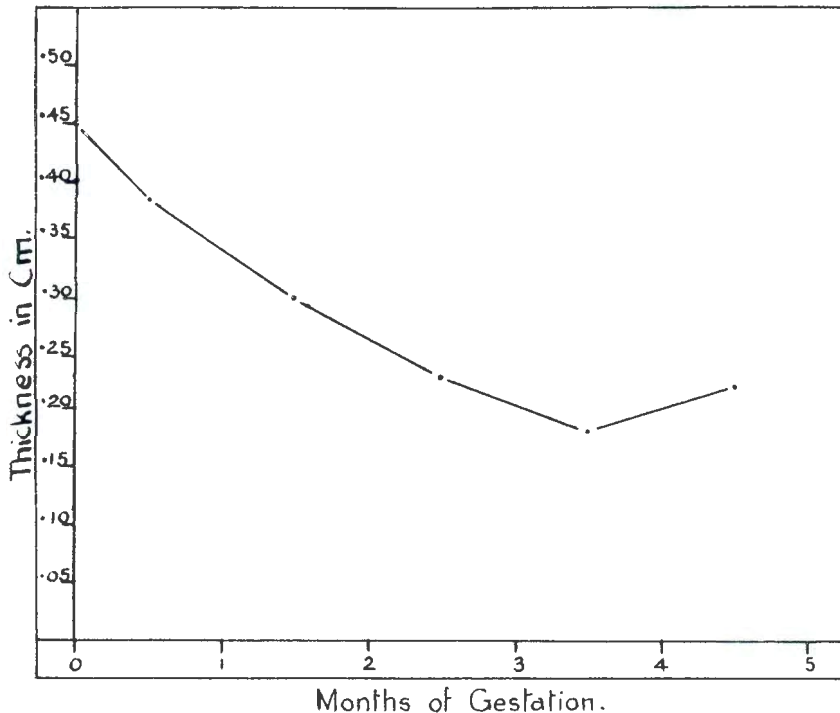
When these data are analysed, by means of Fisher's Z-test, to show the effects of (*a*) stage of gestation (total groups), (*b*) position (in pregnant or non-pregnant horn or in body), and (*c*) interaction between stage of gestation and position, a positive result is obtained in the first instance only. From this it is concluded that the effect of pregnancy is to cause a reduction in the thickness of the uterine wall, that this influence is exerted evenly over the whole wall, and that in their response to this effect all three parts behave identically.

The significance tests referred to in Table 16 are those concerning the " total groups ". These indicate that as early as the first month of gestation there is a significant reduction of the thickness of the uterine wall. In each of the following three months a further significant decrease occurs, so that by the end of the fourth month the

39   469

uterine wall has been reduced to one-third of its original (non-pregnant) thickness. In the last month there is an insignificant increase in thickness. This is caused solely by the high figures obtained at the 147th day, resulting from the general oedematous condition of all the genitalia at this stage.

TABLE 16.

Thickness of Uterine Wall.

Groups of Ewes.		No. of Ewes.	Thickness of Uterine Wall.			Total Groups.	Significance Test.	
No.	Class.		Body.	Preg. Horn.	Non-preg. Horn.		W. Gr. 1.	W. Preced.
			Cm.	Cm.	Cm.	Cm.		
1	Non-preg......	11	·45	·45	·46	·45	—	—
2	1st month.....	12	·39	·38	·38	·38	XX	XX
3	2nd month....	8	·29	·30	·30	·30	XX	XX
4	3rd month....	6	·23	·22	·23	·23	XX	XX
5	4th month.....	7	·20	·17	·18	·18	XX	X
6	5th month.....	5	·27	·18	·22	·22	XX	—
	AVERAGE......	·33	·31	·32				

Discussion.

The small, pale and contracted uterus of the non-pregnant ewe changes during pregnancy. By the eighteenth day the horns appear slightly puffy and by its slightly larger size it is possible to distinguish the horn in which the ovum has become implanted. At about this time the colour of the organ changes to a distinct pink and numerous very tortuous subserous bloodvessels become visible. As gestation progresses so these vessels become larger and less tortuous, the colour becomes redder, and the size-difference between the horns becomes much more distinct. After the 38th day there is also a change in the shape of the horns, this being the result of more rapid growth along the greater curvatures. Seven days later the uterine wall has a bluish-grey colour and the subserous vessels are large and pursue an almost straight course. The largest are to be found along the lesser curvatures, and from these branches run across the uterine horns at right angles to their long axes. By this time the walls have become markedly thinner and through them the outlines of the cotyledons may be distinguished. From the middle of gestation the pregnant horn grows much more rapidly than its fellow and the discrepancy in size becomes so marked that towards the end of the fourth month the non-gravid horn has become merely an appendage of the other. At this stage the walls have become very thin and vascularity is pronounced throughout the organ. This state is maintained until the approach of parturition. At the 147th day the thickness of the wall is increased as a result of a general oedema of the genitalia.

470

In the non-pregnant uterus the mucosa is pale and but slightly moist. Early in pregnancy there is a slight reddening at the site of implantation and this spreads rapidly until at the 45th day the entire mucosa is markedly vascular. At this stage the epithelial lining appears to have been destroyed (at least the surface is not smooth and clear) and the surface is covered with a layer of dirty, greyish, sticky material. As a result of this the allanto-chorion is found to adhere to the inner surface of the uterine wall and in stripping it off care has to be exercised not to rupture the sac. This condition persists until midway through the fourth month, after which the epithelial lining appears to be restored, and with the disappearance of the slimy coating the mucosa is again smooth, fairly moist and of a light red colour. At this stage the amount of adherence between the uterus and the foetal sac is negligible.

4. *Placenta.*

It has been seen (Table 14 and Fig. 12) that the weight of the placenta increases to a peak in the third month, after which it drops fairly rapidly. In an attempt to determine the cause of this behaviour the numbers and the sizes of the cotyledons have been studied. Details of the former are presented in Table 17 and Fig. 15.
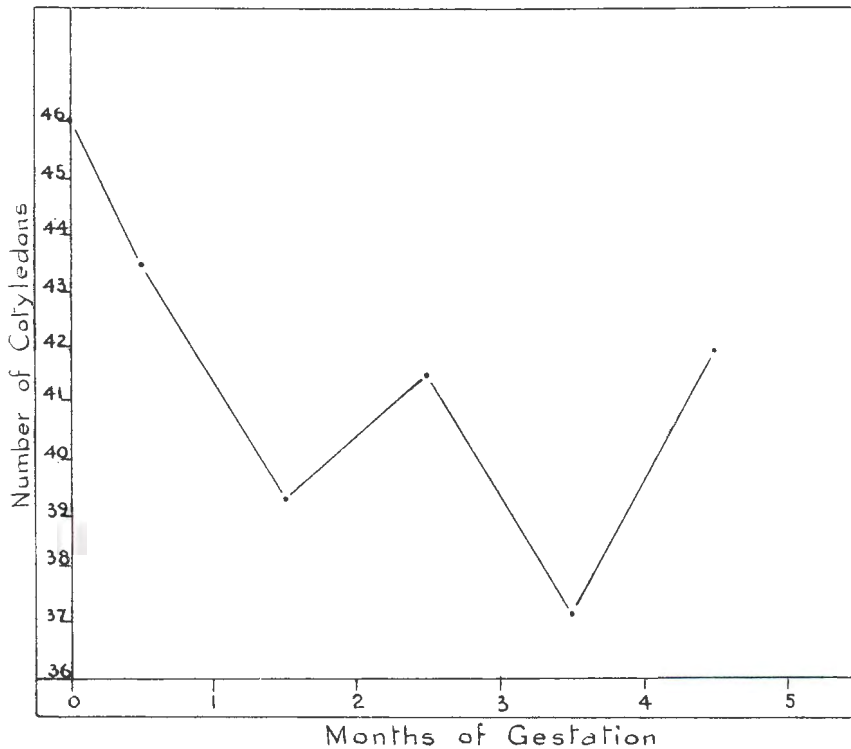
Fig. 15.—Number of Cotyledons.

TABLE 17.

Number of Cotyledons.

| Groups of Ewes. | | No. of Ewes. | Mean Number of Cotyledons. | | Total Groups. | Significance Tests. | |
No.	Class.		Pregnant Side.	Non-preg. Side.		W. Group, 1.	W. Preced. Group.
1	Non-preg.............	11	46·64	45·26	46·00	—	—
1	1st month.............	12	44·17	42·92	43·54	—	—
3	2nd month.............	8	42·13	37·63	39·38	X X	X
4	3rd month.............	6	43·83	39·33	41·58	X	—
5	4th month.............	7	37·71	36·57	37·14	X X	X
6	5th month.............	5	43·00	40·80	41·90		X
	AVERAGE.........	—	43·31	41·02	—	—	—

Fig. 16.—Diameter of Cotyledons.
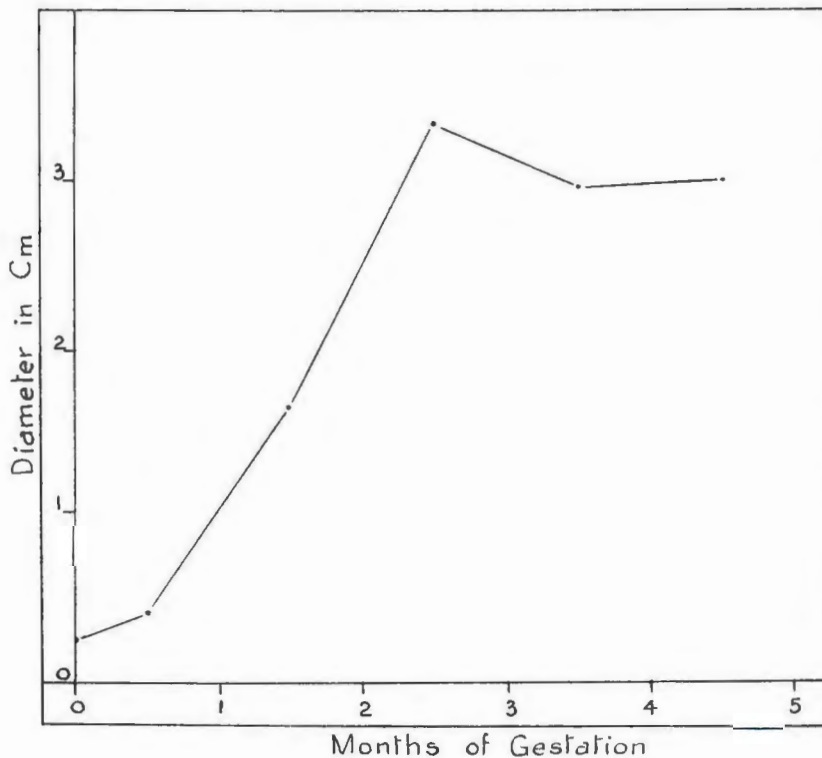
When these data are analysed in the manner described in connection with the thickness of the uterine wall, it is found that there is a significant difference between the pregnant and non-pregnant sides of the uterus. The excess in favour of the former side is never great.

and falls far short of the figure (50 per cent.) mentioned by Bergmann (1922) in his work on the bovine. From the tests it appears that there are significant differences at the various stages of pregnancy. Here it must be remembered that in the first two groups it was impossible to distinguish between functional and non-functional cotyledons, thus the figures given represent counts of all the cotyledons as compared with counts only of functional cotyledons in the remaining groups. Hence it is not surprising to find that the numbers for the first two groups are higher than those of any other group. As this method of testing is open to criticism, it is more satisfactory to employ Group No. 3 (the first in which functional cotyledons are easily recognisable) as the standard for comparison. When this is done it appears that in spite of a fair degree of variation in the last four groups, none of the differences are significant. Therefore, the differences observed may be ascribed to " unfair " counts in the first two groups, and it may be concluded that, whatever the true position regarding functional cotyledons in the first month (in Group 1 their number is nil), the observed variations in the weight of the placenta are not caused by variations in the numbers of cotyledons.

TABLE 18.

Diameter of Cotyledons.

			Mean Diameter of Cotyledons.			Significance Tests.	
No.	Class.	No. of Ewes.	Preg- nant Side.	Non- preg. Side.	Total Groups.	W. Group 1.	W. Preced. Group.
			Cm.	Cm.	Cm.		
1	Non-pregnant.........	11	·28	·30	·28	—	—
2	1st month............	12	·42	·39	·40	—	—
3	2nd month............	8	1·86	1·47	1·67	XX	XX
4	3rd month............	6	3·45	3·24	3·35	XX	XX
5	4th month............	7	3·04	2·94	2·99	XX	X
6	5th month............	5	3·00	3·00	3·00	XX	—
	AVERAGE.........	—	1·63	1·52	—	—	—

As a result of the above conclusion, it becomes necessary to consider the size of the cotyledons at the various stages of pregnancy. For this purpose two dimensions (diameter and height) have been studied. In each instance the average of the readings for the largest cotyledon in the horn and one representing the modal size of that horn, is employed. Details of these representative figures are presented in Tables 18 and 19 and Figs. 16 and 17. Both these dimensions increase rapidly (becoming significant in the second month) up to the end of the third month. After this their behaviour differs, the diameter dropping significantly in the fourth month and then maintaining its level in the fifth, whereas the height still increases (although insignificantly) in the fourth month and then undergoes a significant decrease in the last month of pregnancy. However, by

the end of gestation both dimensions are significantly lower than they were in the third month. It is clear that the placental weight is affected mainly by this change in the size of each individual cotyledon.
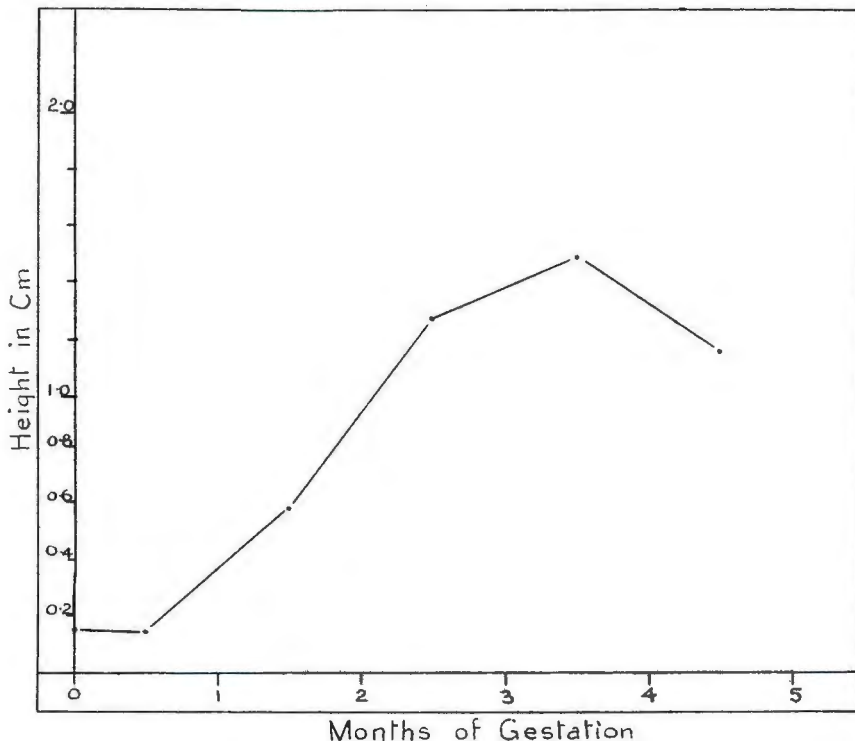
Fig. 17.—Height of Cotyledons.

TABLE 19.
Height of Cotyledons.

			Mean Height of Cotyledons.			Significance Tests.	
	Groups of Ewes.	No. of Ewes.	Preg-nant Side.	Non-preg. Side.	Total Groups.	W. Group 1.	W. Preced. Group.
No.	Class.						
			Cm.	Cm.	Cm.		
1	Non-pregnant..........	11	·15	·16	·16	—	—
2	1st month.............	12	·16	·15	·15	—	—
3	2nd month............	8	·63	·55	·59	XX	XX
4	3rd month.............	6	1·42	1·37	1·39	XX	XX
5	4th month.............	7	1·50	1·46	1·48	XX	—
6	5th month.............	5	1·16	1·16	1·16	XX	XX
	AVERAGE.........	—	·681	·656	—	—	—

Discussion.

It has been seen that during pregnancy the cotyledons increase enormously both in diameter and in height. Apart from this enlargement there are other changes which may be observed macroscopically. In the non-pregnant uterus the cotyledons appear to be arranged in four longitudinal rows. All are of the same size and are just visible as pinhead-sized brownish centres with pale peripheral zones. In dioestrum they are level with the surface of the mucosa, but during oestrum they become slightly swollen and elevated. Early in pregnancy a change is evident and by the 21st day both the size of the central portion and the elevation of the cotyledon are visibly increased. During the next few days the brownish centre, previously concave, becomes flat and numerous pin-point red spots appear all over its surface. On the 27th day the cotyledon has the form of a small nodule under the epithelial layer of the uterus. Four days later the central portion has a distinct red colour and a pitted appearance, while the periphery has become elevated to form a thin circular lip around the wide flat centre. From this time until the 80th day the cotyledon develops rapidly: it enlarges and the peripheral lip begins to curl inward, thus decreasing the size of the central cup and trapping the foetal cotyledon which has come to rest upon the surface of the cup. At the same time the base of the cotyledon becomes convex and detaches itself from the surrounding tissue to such an extent that finally the entire cotyledon is attached to the uterine wall merely by a thin mucosal peduncle.

At about the end of the third month the cotyledons appear to have reached their maximum development. The cups are small and the rolled-in edges resemble motor-tyres in miniature. In some instances the cups tend to lose their circular shape and become elongated slits. From this stage onward fairly large blood clots of long standing are visible in the cups of the cotyledons. Apart from a tendency to decrease somewhat in size (partly due to a tighter rolling in of the edges) the cotyledons do not undergo further change until about the 140th day, after which the turned-in periphery relaxes and the whole cotyledon opens out flat with the central portion partially everted and covered with clotted blood. By the 147th day most of the cotyledons resemble flattened reddish-black discs, while in a few the eversion has been so marked as to give the appearance of a mushroom (similar to the convex cotyledon of the bovine). At this stage the attachment of the membranes is not firm and they may be pulled away with ease. Usually the entire central portion of the cotyledon comes away, leaving an empty thin-walled cup.

In the above description only the general appearance of the cotyledons at each stage has been mentioned. However, at one and the same time a variety of phases of development may be encountered. This is well illustrated in the accompanying plate (No. III). Development is initiated at the site of implantation and throughout the progressive stage the largest and best developed cotyledons are to be found here. In the opposite horn growth is slower and the maximum is reached later. In both horns the largest cotyledons are found towards the centre, while the size decreases towards the apices and towards the body. In the latter only a few cotyledons are present and

these are but poorly developed. During the last two months there is practically no difference between the cotyledons of the pregnant and non-pregnant horns, although the differences at different situations in the horn persist until parturition. The smaller cotyledons, although slower in growth, appear to reach the same stage of differentiation as the largest ones.
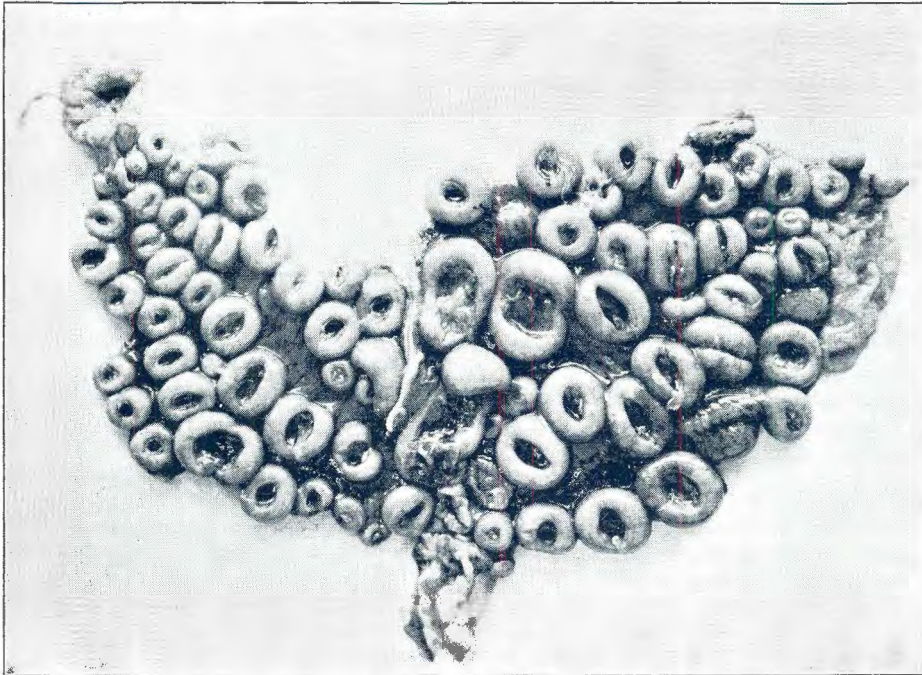

Plate III.—Gravid uterus laid open to show cotyledons, pregnant horn on the right and cervix towards the bottom.

The foetal part of the placenta also develops fairly early. At the 21st day of pregnancy dull white areas appear on the allanto-chorion wherever it touches the surface of the maternal cotyledons. A few days later these patches have become almost opaque and at the 31st day numerous closely grouped pin-point red spots mark out on these areas the size and shape of the central portions of the maternal cotyledons. At this time there is a very loose attachment of membrane to cotyledon. The red spots increase in size and coalesce, and small vessels can be distinguished running from these circumscribed areas to the umbilical cord. By the 45th day these areas have developed into thick discs firmly attached to the outer surface of the allanto-chorion. By this time there is a fair degree of adherence between the discs and the maternal cotyledons. From now on the edges of the latter curl inward to hold the foetal cotyledons firmly in position, thus completing the cotyledonary attachment.

476

5. *Fallopian Tubes.*

The tubes were considered from the point of view of length, diameter and weight. For each of these characteristics an analysis similar to that described in connection with the thickness of the uterine wall, has been undertaken and the results are presented in Tables 20-22.

TABLE 20.

Length of Fallopian Tubes.

Groups of Ewes.		No. of Ewes.	Mean Length.		Total Groups.	Significance Tests.	
No.	Class.		Preg-nant Side.	Non-preg. Side.		W. Group 1.	W. Preced. Group.
			Cm.	Cm.	Cm.		
1	Non-pregnant..........	11	15·41	15·05	15·23	—	—
2	1st month.............	12	15·55	15·88	15·72	—	—
3	2nd month............	8	18·00	17·13	17·56	X X	X X
4	3rd month............	6	17·17	17·08	17·13	X	—
5	4th month............	7	17·64	17·57	17·61	X X	—
6	5th month............	5	18·60	17·90	18·25	X X	—
	AVERAGE.........	—	16·73	16·35	—	—	—

TABLE 21.

Diameter of Fallopian Tubes.

Groups of Ewes.		No. of Ewes.	Mean Diameter.		Total Groups.	Significance Tests.	
No.	Class.		Preg-nant Side.	Non-preg-Side.		W. Group 1.	W. Preced. Group.
			Cm.	Cm.	Cm.		
1	Non-pregnant..........	11	·21	·21	·21	—	—
2	1st month.............	12	·19	·20	·20	—	—
3	2nd month............	8	·19	·19	·19	—	—
4	3rd month............	6	·19	·19	·19	—	—
5	4th month............	7	·19	·19	·19	—	—
6	5th month............	5	·20	·20	·20	—	—
	AVERAGE.........	—	·196	·196	—	—	—

TABLE 22.

Weight of Fallopian Tubes.

Groups of Ewes.		No. of Ewes.	Mean Weight.		Total Groups.	Significance Tests.	
No.	Class.		Preg-nant Side.	Non-preg. Side.		W. Group 1.	W. Preced. Group.
			Gm.	Gm.	Gm.		
1	Non-pregnant.........	11	0·5227	0·4773	0·5000	—	—
2	1st month............	12	0·4858	0·5042	0·4950	—	—
3	2nd month............	8	0·5438	0·5375	0·5406	—	—
4	3rd month............	6	0·4750	0·4617	0·4683	—	—
5	4th month............	7	0·5423	0·5100	0·5172	—	—
6	5th month............	5	0·5000	0·5200	0·5100	—	—
	AVERAGE.........	—	0·5092	0·5008	—	—	—

Fig. 18.—Length of Fallopian Tubes.
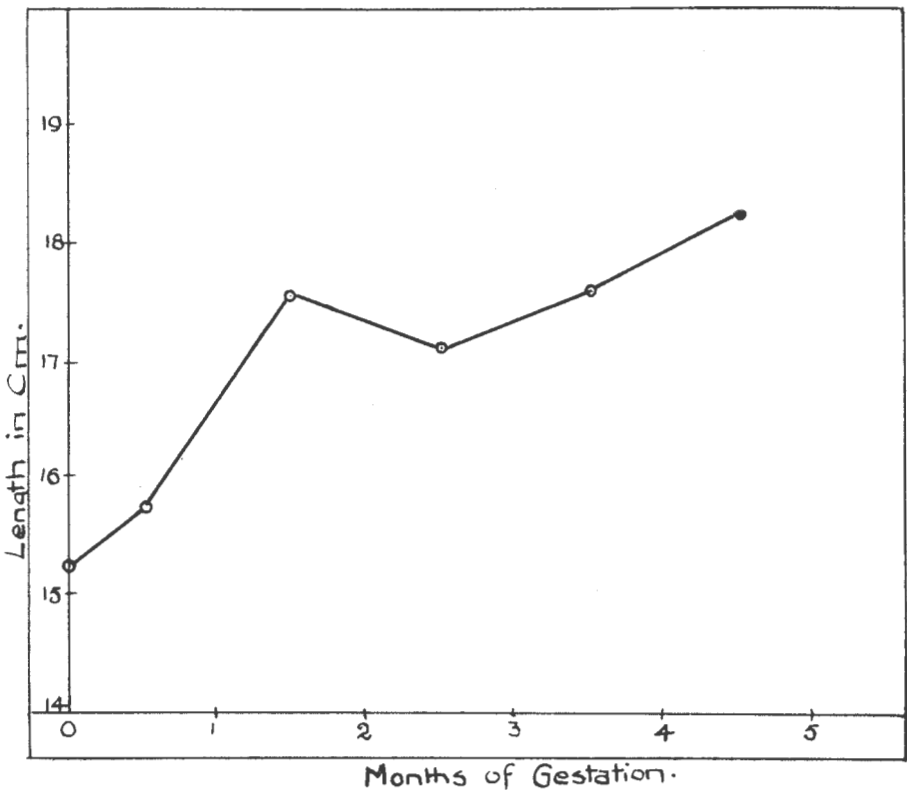
In no instance is it possible to demonstrate any difference between the tube from the pregnant side and the opposite one. However, when the total groups are considered, significant variations are encountered. Here the last four groups are significantly longer than the first two, but show no real differences among themselves. This is illustrated in Fig. 18. From the second month of gestation the tube from the pregnant side is always slightly (but not significantly) longer than the other.

Discussion.

As the increase in length is not accompanied by an increase in weight, actual growth cannot account for the elongation. It is suggested that a certain amount of stretching of the tube occurs early in pregnancy when both curvatures of the horn are growing steadily. The reason why this stretching does not appear to cause a decrease in the diameter of the tube may be that the latter was measured at the middle of the length, whereas the stretching is likely to occur mainly at the uterine end of the tube. If mechanical stretching is the cause of the elongation, then the latter cannot be regarded as a true change of pregnancy, and it must be concluded that it is not possible to demonstrate that gestation has any effect on the macroscopic appearance of the Fallopian tubes.

(b) Foetal Membranes and Fluids.

1. Membranes.

The weights tabulated in Table 23 are those of the two membranes combined. These data are presented graphically in Fig. 19. The reason for the omission of Group 1 is obvious.

The membranes grow rapidly until the end of the third month; in the following month there is hardly any increase in weight, and then in the last month the rate of increase exceeds that of any of the previous months. Attention is directed to the flattening of the graph during the fourth month, this being similar to what is observed in connection with the growth of the uterus.

TABLE 23.
Weight of Foetal Membranes.

GROUPS OF EWES.		No. of Ewes.	Mean Weight of Membranes.	SIGNIFICANCE TESTS.	
No.	Class.			W. Group 2.	W. Preced. Group.
			Gm.		
2	1st month.........	7	6·94	—	---
3	2nd month.........	8	60·70	X	X
4	3rd month.........	6	115·58	XX	X
5	4th month.........	7	128·57	XX	—
6	5th month.........	5	192·80	XX	X

As at all times the volume of the membranes is very similar to their weight, the data for the former have not been analyzed.

Fig. 19.—Weight of Foetal Membranes.
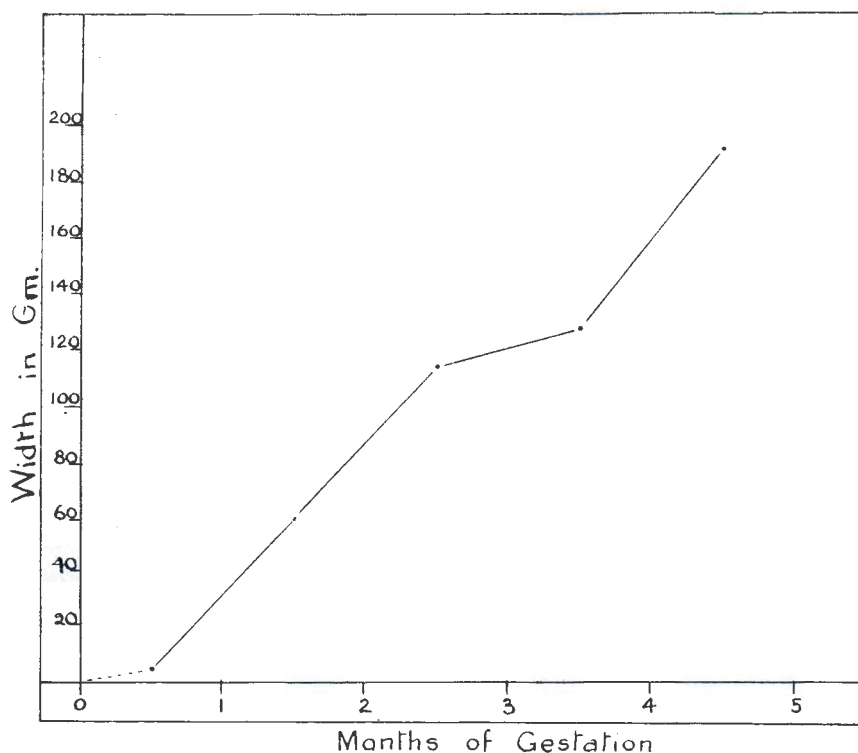
Discussion.

It has been seen that the membranes undergo an enormous increase in weight during pregnancy. However, the two membranes do not at all times contribute equally to this enlargement. At the 16th day of pregnancy an elongated pale membrane is seen in the pregnant horn, but it is not till the 18th day that the two sacs can be distinguished. At this time the allantois is distended with fluid and it extends almost the entire length of the gravid horn. The amnion is just visible as a narrow crescent-shaped membrane closely investing the embryo, which is situated towards the middle of the length of the allantoic sac. Two days later the latter sac has extended into the body of the uterus and some distance along the non-gravid horn. At this stage this sac appears to be composed of a loose, velvety outer layer and a clearer inner one in which the blood vessels seem to be located. Of the latter there are two main sets, extending from the umbilical region to each tip of the sac, and from these small branches run across the inner surface of the sac. By the 25th day the narrow, elongated allantoic sac has reached almost to the apices of both uterine horns. At this time the amnion begins to enlarge slightly, but it is not until the 30th day that it stands away clearly from the foetus.

480

From now on the membranes increase in size and also in thickness. The bloodvessels enlarge and their branches become more numerous. At the middle of the second month opaque white flakes begin to appear on the surface of the allantoic membrane. These are seen first in the region over the foetus, from where they spread towards the tips. They are never so dense as to impair seriously the transparency of the membrane. Now too the necrotic tips of the allanto-chorion are plainly visible. At the 66th day the amnion has enlarged to such an extent as to bring the central zone of its outer surface into direct contact with the overlying parts of the chorion. Thus the allantoic sac is divided into two compartments which communicate with each other and with the urachus by means of a narrow tube-like cavity.

Until the 100th day both sacs are well filled with fluid, hence the the membranes are tense. After this time the same degree of distension is apparently not maintained, so that by the end of the fourth month the sacs appear slightly collapsed. Especially is this the case with the amnion. The membranes are now relatively thick and fairly strong. Till the end of gestation they remain colourless and more-or-less transparent.

Fig. 20.—Volumes of Foetal Fluids.
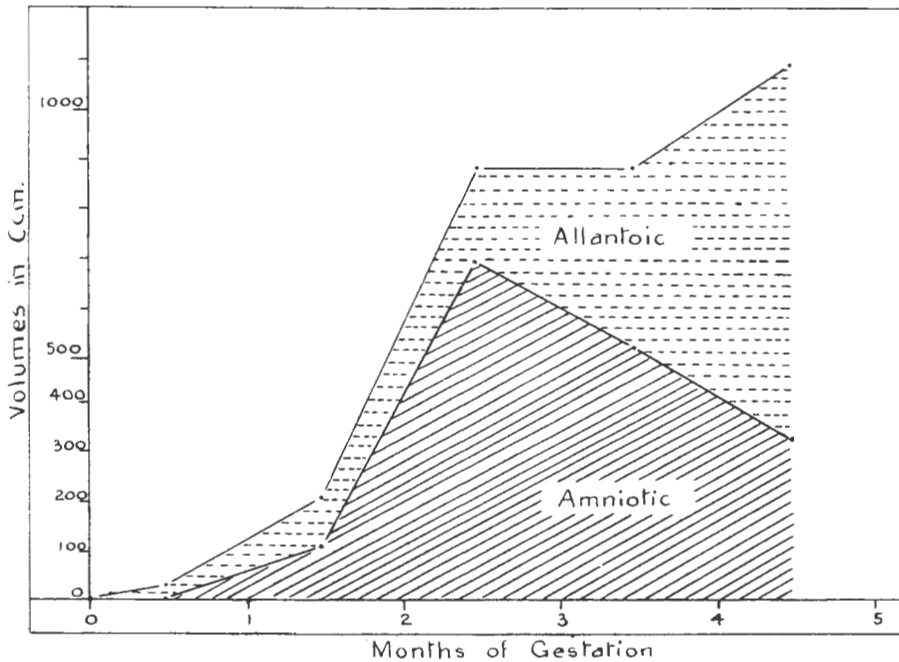
(NOTE.—Allantoic Volume is represented by the *vertical* distance between the two lines.)

2. *Foetal Fluids.*

Details of the volumes of both the allantoic and the amniotic fluids are presented in Table 24. In Fig. 20 the upper curve represents the total volume of fluid while the lower one indicates

the volume of amniotic fluid. The allantoic volume is represented by the portion lying between the two graphs. The total volume increases rapidly until the end of the third month. This is caused mainly by the amniotic fluid, which reaches its peak in the third month. Although the volume of allantoic fluid increases during this period, the rate is comparatively slow. However, in the fourth month this rate is accelerated to such an extent that the increase in the allantoic fluid is sufficient to neutralize the effect of a rapid drop in the amniotic volume, and thus in this month the total volume remains practically unchanged. In the last month the allantoic fluid is doubled and as a result of this the total volume shows a distinct rise, in spite of the continued decrease in amniotic fluid.

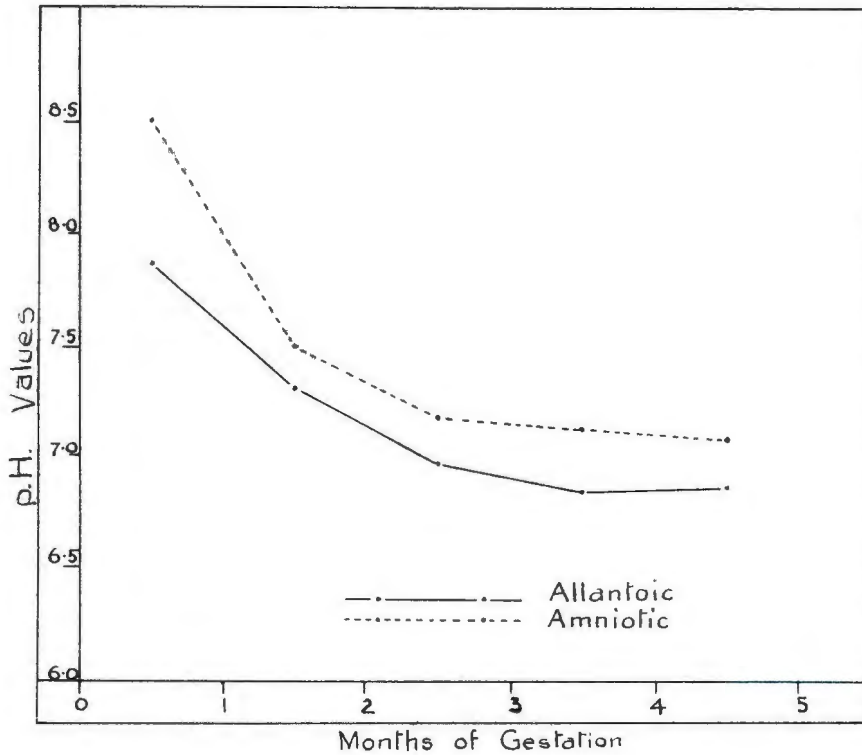TABLE 24.

Volumes of Foetal Fluids.

Groups of Ewes.		No. of Ewes.	Mean Vol. Allantoic.	Significance Tests.		Mean Vol. Amniotic.	Significance Tests.	
No.	Class.			W. Group 1.	W. Preced. Group.		W. Group 1.	W. Preced. Group.
			cc.			c.c		
2	1st month.....	9	24·17	—	—	1·5	—	—
3	2nd month.....	8	93·63	—	—	112·69	X	X
4	3rd month.....	5	185·20	X	—	698·00	XX	XX
5	4th month.....	7	347·29	XX	—	525·71	XX	XX
6	5th month.....	5	762·00	XX	XX	329·00	XX	XX

In Table 25 it is seen that, as regards hydrogen ion concentration, there are differences between the two fluids; also in each fluid this concentration decreases with the advance of gestation. However, in both fluids this reduction is significant only in the second month, after which the variations are slight. These facts are illustrated in Fig. 21.

TABLE 25.

Hydrogen Ion Concentration of Fluids.

Groups of Ewes.		No. of Ewes.	Mean pH. Allantoic.	Significance Tests.		Mean pH Amniotic.	Significance Tests.	
No.	Class.			W. Group 1.	W. Preced. Group.		W. Group 1.	W. Preced. Group.
2	1st month.....	6	7·867	—	—	8·500	—	—
3	2nd month.....	8	7·329	XX	XX	7·500	XX	XX
4	3rd month.....	5	6·960	XX	—	7·180	XX	—
5	4th month.....	7	6·829	XX	—	7·143	XX	—
6	5th month.....	5	6·860	XX	—	7·080	XX	—

Fig. 21.—Hydrogen Ion Concentration of Foetal Fluids.

The specific gravity of the fluids is considered in Table 26. Due to insufficient data for the first month, Group No. 2 had to be omitted. All that may be concluded from these figures is that the specific gravity of the allantoic fluid is always higher than that of the amniotic. All other variations, even the apparent steady downward trend in the case of the amniotic fluid, are insignificant.

TABLE 26.

Specific Gravities of Fluids.

GROUP OF EWES.		No. of Ewes.	MEAN SPECIFIC GRAVITY.	
No.	Class.		Allantoic.	Amniotic.
3	2nd month..............	6	1·0075	1·0045
4	3rd month..............	5	1·0123	1·0042
5	4th month..............	7	1·0127	1·0043
6	5th month..............	5	1·0114·	1·0026

Discussion.

In the failure of the total volume of foetal fluid to increase during the fourth month of pregnancy lies the explanation for the peculiar flattening observed in most of the curves dealing with growth of the uterus and its parts. Apparently to some extent enlargement of these parts is dependent upon the stretching effect of the foetal fluids. Moreover, at this time, the fluids constitute a very large proportion of the weight of the entire foetal system. These facts make it clear why in its growth the uterus should follow a course resembling closely that of the increase in volume of the fluids.

Both as regards hydrogen ion concentration and specific gravity the fluids differ one from the other. In other physical properties these differences are also recognizable. In the early stages of pregnancy both fluids are clear, colourless and watery. This is maintained until the 45th day, at which time the allantoic fluid turns slightly cloudy. During the following two weeks this turbidity becomes more pronounced and a lemon tinge appears in the previously colourless fluid. This becomes more intense and then gradually changes to amber. At the 81st day it is difficult to decide which of the two colours is present, but a week later there is no longer any doubt. The bright amber colour then seen darkens into a golden brown, which shade is encountered throughout the remainder of the period. The turbidity previously mentioned increases until the end of the third month, as does the viscosity. During the last two months the fluid again becomes watery, clear and practically transparent.

In the amniotic fluid the lemon tinge is visible at the 52nd day. Within a short time it has changed to amber, but the latter shade is never very deep. During the fourth month the amber colour disappears and the fluid acquires a very light green colour. In the meantime the viscosity and the turbidity have increased so that by the end of the fourth month the amniotic fluid is thick and "syrupy" and very cloudy. The colour varies from a pale green to a dirty white. During the later stages a fair amount of meconium is found in the amniotic fluid.

(c) FOETUS.

All details regarding age, weight and dimensions of the entire series of foetuses are presented in tabular form in Appendix A. The trends of the growth processes exhibited by these observations are best studied through the process of curve-fitting. In order not to complicate the text with statistical technicalities all details of the procedure adopted have been relegated to Appendix B.

1. Growth in Weight.

MacDowell *et al* (1927) maintain that when for a series of foetuses the natural logarithms of age are plotted against those of the corresponding weights, a straight line distribution results. However, in their work age is calculated not from the moment of

fertilization of the ovum, but from the time of formation of the
" embryo proper ".* So distinct is the linear trend of the data in
Fig. 22 that one is led to surmise that, in spite of the difference
in the method of computation of age, here too the general type
of MacDowell's formula will hold good. Moreover, it is possible
to fit such a line to the data and to obtain a result significant at
1 per cent. probability. Nevertheless, the points representing the
actual observations are not evenly scattered about this straight line.
When, by the use of antilogarithms, a weight-age curve is
constructed from the formula, it becomes quite evident that the
formula in question does not give a good representation of the trend
of the data. Towards the latter end of the period the curve rises
much too steeply and thus passes well to the left of all the actual
observations.

Fig. 22.— Natural Logarithms of Weight plotted against those of Age.
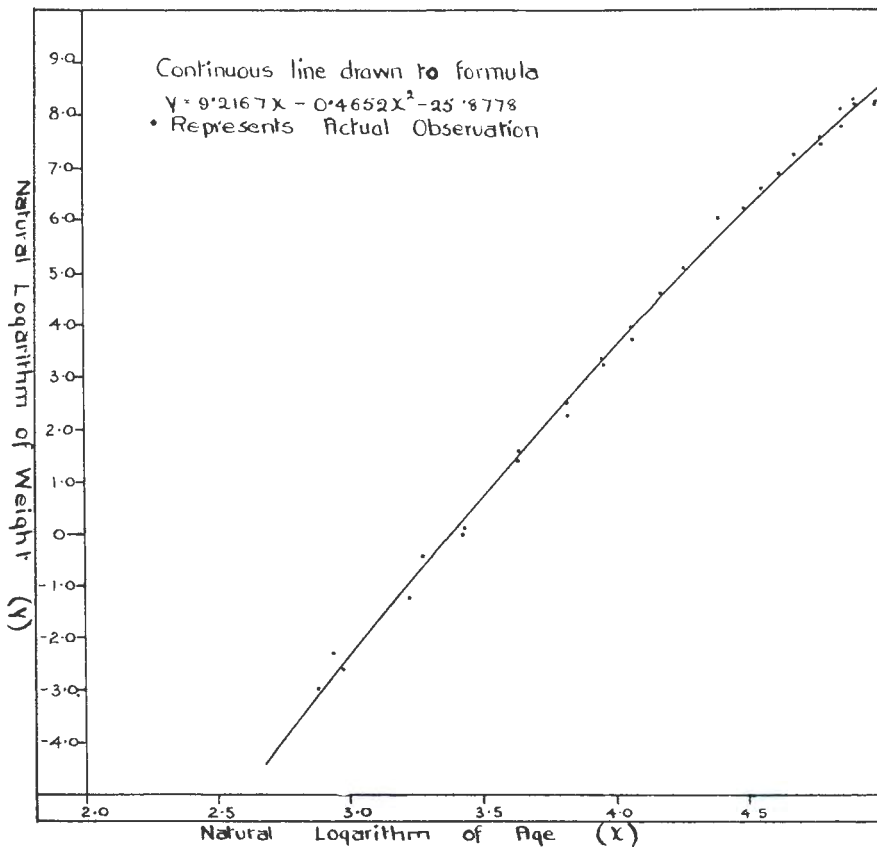
* Probably the stage with the primitive axis established and with the
formation of somites just commencing.

485

In view of the above it becomes necessary to test the " fit " of a parabola. The regression formula obtained is : —

$$\text{Log}_e \text{ Wt} \quad 9\cdot2167 \text{ Log}_e \text{ Age} \quad 0\cdot4652 \ (\text{Log}_e \text{ Age})^2 \quad 25\cdot8778,$$

and the continuous line in Fig. 22 is drawn from it. Both factors b_1 and b_2 (see Appendix B) are highly significant (at $P \ \cdot 01$), while the coefficient of variation is $20\cdot07$ per cent., which is 4 per cent. lower than that of the straight-line formula. Both in Fig. 22, and in the weight-age curve in Fig. 23, the actual data are well scattered around the curves, indicating that the formula from which these curves are constructed gives a true representation of the trend of the data. The figure for the coefficient of variation (20 per cent.) is still high, but, in view of the large variations in the original data (see differences in weights of foetuses of identical age) this is only to be expected. Fitting to a further degree will certainly not bring about any improvement and will only serve to complicate the formula.

It is concluded that the age and weight data obtained from the series of Merino foetuses are best fitted by a curve of the second degree. This type of formula produces a curve with two inflections, the first convex, and the second concave to the axis. In Fig. 23 the second inflection is not distinct. Nevertheless the retarding effect of the factor " $0\cdot4652 \ (\text{Log}_e \text{ Age})^2$ " is evident in the widening and flattening of the curve during the latter part of prenatal life.

The presence of the second inflection is of importance; nevertheless it has either not been recognised or its significance has not been appreciated and has thus not been stressed. It has been customary to look upon the prenatal growth curve as one continuously rising and joining on to the initial part of the postnatal curve without any significant break. Now, however, it becomes apparent that, at least in the case of the Merino foetus, the prenatal growth curve has both accelerated and retarded phases and that, if these two phases together constitute a " growth-cycle ", then it must be concluded that one such cycle is completed before the birth of the lamb.

It has been shown by Scammon and Calkins (1923) that growth in length of the human foetus is best represented by a curve of the second degree. This appears to support the above conclusions and thus suggests that the growth of all foetal dimensions follows a similar trend. This surmise will be tested later in this work.

Scammon (1922) maintains that there is no sudden change in growth at the time of birth, but that the transition is gradual. Kislovsky and Larchin (1931) state that in the bovine there is little difference between the relative growth-rates just before and just after birth. From the above it is to be concluded that there are no abrupt changes of direction of the curve at the time of birth. It is a feature of the curve described that the second inflection makes it possible to link up pre- and post-natal curves in a manner which satisfies the above conditions.

Fig. 23.—Growth in Weight.

Continuous line drawn to formula
$Log_e y = 9.2167 \ Log_e x - 0.4652 (Log_e x)^2 - 25.8778$

Weight in Grams (y)

Foetal Age in Days (x)