STUDIES ON THE PLACENTA OF THE SHEEP  
*(OVIS ARIES L.)*

PLACENTAL SIZE

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**Summary.** A positive correlation is reported between birth weight and the weight of intact cotyledons and of foetal cotyledons. At equal cotyledon weight, single foetuses are heavier than twins, male foetuses are heavier than females and those of older ewes tend to be heavier than those of younger ones. There is great variability in the total weight of cotyledons per ewe, even within groups of similar ewes.

The relationship between foetal weight and cotyledon weight appears to be curvilinear near parturition, increases in foetal weight being associated with increasingly large increments in cotyledon weight. Foetal weights of about 1·5 kg are achieved at very low cotyledon weight.

There is only slight correlation between foetal weight and number of cotyledons, because of a compensatory increase in the weight of individual cotyledons when they are few in number. This involves both foetal and maternal tissue.

The number of cotyledons per foetus varies with breed and strain of sheep, litter size, age (or parity) of ewe, sex of lamb and with environmental conditions and the number of caruncles in the uterus. There is a marked tendency for a constant proportion of the caruncles (70 to 80%) to develop into cotyledons. Fine and medium wooled Australian Merinos have fewer cotyledons than any other breed for which data are available.

In the sheep, as in man, a number of very small foetuses, with very small placentae, reach full term.

**INTRODUCTION**

That the likelihood of survival of the new-born is closely related to birth weight has been demonstrated in the sheep (Moule, Jackson & Young, 1956) and in some other species, including man (Record, Gibson & McKcown, 1952).

It is reasonable to suppose that placental function is among the factors which determine birth weight, and there is some evidence, based on visual assessment of the placenta in man, that placental insufficiency may lead to low birth weight and poor vigour (see Warkany, Monroe & Sutherland, 1961, for discussion). There is now a considerable amount of information about the placental
transfer of nutrients for foetal growth (see Barcroft, 1946; Huggett & Hammond, 1952) but, as Huckabee (1962) has indicated, a quantitative assessment of overall placental efficiency cannot yet be made; it is therefore not yet possible to relate foetal growth and development to placental efficiency. There is, however, indirect evidence that birth weight is in part determined by placental size. Thus, birth weight and the weight of the complete placenta are positively correlated in man (see McKeown & Record, 1953a) and in the guinea-pig (Ibsen, 1928; Eckstein, McKeown & Record, 1955) and birth weight is correlated with the weight of the foetal placenta in the rabbit (Hammond, 1935). Barcroft (1946) and Wallace (1948) could find no such relation in the sheep, but the reports of McKenzie & Bogart (1934), Thomson & Thomson (1949) and Foote, Pope, Chapman & Casida (1959) suggest a positive correlation between birth weight and the size of the cotyledonary placenta.

In the present paper the relationship between birth weight and placental size in the sheep is examined further, together with factors that affect placental size. Only the cotyledonary part of the placenta is considered here since the maternal and foetal circulations are in their most intimate contact within the cotyledons, which hence provide the most favourable region for nutrient exchange.

MATERIALS AND METHODS

SOURCE OF MATERIAL

The material was collected from ewes in experiments not designed to provide data about the placenta. Some of the relationships that might be examined are thus confounded by age, breed and nutrition of the ewes and by sex and size of litter.

The ewes were mainly Merinos or Merino derivations (Corriedales and crossbreds). Most were mated to rams of the same breed (Table 1). Unless otherwise stated the ewes were adequately fed throughout pregnancy.

Two types of material were examined:

(i) 250 uteri from five series of ewes killed at various stages of pregnancy from about 90 days to 145 days.

(ii) 440 ‘afterbirths’, i.e. foetal placentae, delivered at normal parturition in ewes forming eleven separate series. These were collected within 6 hr, usually within 1 hr, of shedding from ewes either under direct observation or individually penned. During part or all of the last 50 days of pregnancy some ewes were deliberately underfed so that they lost about 0-1 kg body wt per day.

Most of the tissues were fixed in 10% formalin and were stored for various periods before examination. Details of the material appear in Table 1.

TERMINOLOGY

The generally accepted modern terminology (Amoroso, 1952) is used herein; ‘cotyledon’ refers to the composite foetal–maternal structure and ‘foetal cotyledon’ and ‘maternal cotyledon’ are distinguished; unattached maternal endometrial thickenings are referred to as ‘cotyledonary burrs’, and the term ‘caruncles’ is used collectively for cotyledonary burrs together with cotyledons.
**Table 1**

**SUMMARIZED DESCRIPTION OF MATERIAL EXAMINED**

<table>
<thead>
<tr>
<th>Series No.</th>
<th>Breed and strain of ewe*</th>
<th>Age of ewes (years)</th>
<th>Stage of pregnancy (days ± 3)</th>
<th>Nutrition during last 50 days of pregnancy</th>
<th>Fixed or fresh material</th>
<th>No. ewes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Monotocous</td>
<td>Ditocous</td>
</tr>
<tr>
<td>Intact placenta</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Merino—various (medium wool)</td>
<td>2 to 8</td>
<td>146</td>
<td>Adequate</td>
<td>Fixed</td>
<td>17</td>
</tr>
<tr>
<td>2</td>
<td>Merino—various (medium wool)</td>
<td>3 to 6</td>
<td>146</td>
<td>Adequate</td>
<td>Fresh</td>
<td>16</td>
</tr>
<tr>
<td>3</td>
<td>Merino—Peppin (medium wool)</td>
<td>7 to 8</td>
<td>95, 114, 135</td>
<td>Adequate</td>
<td>Fixed</td>
<td>34</td>
</tr>
<tr>
<td>4</td>
<td>Merino—five distinct strains†</td>
<td>7</td>
<td>90</td>
<td>Adequate</td>
<td>Fixed</td>
<td>58</td>
</tr>
<tr>
<td>5</td>
<td>Merino—five distinct strains†</td>
<td>7</td>
<td>90</td>
<td>Adequate</td>
<td>Fixed</td>
<td>57</td>
</tr>
<tr>
<td>Foetal placenta</td>
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<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Merino—various</td>
<td>2 to 6</td>
<td>Term</td>
<td>Adequate</td>
<td>Fixed</td>
<td>26</td>
</tr>
<tr>
<td>7</td>
<td>Merino—Peppin (medium wool)</td>
<td>6</td>
<td>Term</td>
<td>Adequate</td>
<td>Fixed</td>
<td>21</td>
</tr>
<tr>
<td>8</td>
<td>Merino—non-Peppin (fine wool)</td>
<td>2 to 6</td>
<td>Term</td>
<td>Adequate</td>
<td>Fixed</td>
<td>84</td>
</tr>
<tr>
<td>9</td>
<td>Merino—various</td>
<td>Various</td>
<td>Term</td>
<td>Adequate</td>
<td>Fixed</td>
<td>7</td>
</tr>
<tr>
<td>10</td>
<td>Merino—non-Peppin (fine wool)</td>
<td>2 to 7</td>
<td>Term</td>
<td>High and low</td>
<td>Fixed</td>
<td>34</td>
</tr>
<tr>
<td>11</td>
<td>Merino—various</td>
<td>3 to 10</td>
<td>Term</td>
<td>High, intermediate and low</td>
<td>Fixed</td>
<td>66</td>
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<td>12</td>
<td>Corriedales</td>
<td>6</td>
<td>Term</td>
<td>Normal</td>
<td>Fixed</td>
<td>41</td>
</tr>
<tr>
<td>13</td>
<td>Merino—Peppin (medium wool)</td>
<td>6</td>
<td>Term</td>
<td>Normal</td>
<td>Fixed</td>
<td>16</td>
</tr>
<tr>
<td>14</td>
<td>Merino—various</td>
<td>5 to 7</td>
<td>Term</td>
<td>High and restricted for various periods</td>
<td>Fixed</td>
<td>26</td>
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<tr>
<td>15</td>
<td>Border Leicester × Merino</td>
<td>4 to 5</td>
<td>Term</td>
<td>Various</td>
<td>Fresh</td>
<td>11</td>
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<tr>
<td>16</td>
<td>Merino—various</td>
<td>5</td>
<td>Term</td>
<td>Adequate</td>
<td>Fresh</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>Merino—non-Peppin (strong wool)</td>
<td>2 to 5</td>
<td>Term</td>
<td>Adequate</td>
<td>Fresh</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>Southdown</td>
<td>2 to 4</td>
<td>Term</td>
<td>Adequate</td>
<td>Fresh</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>Southdown × Merino</td>
<td>2 to 4</td>
<td>Term</td>
<td>Adequate</td>
<td>Fresh</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>English Leicester</td>
<td>7</td>
<td>Term</td>
<td>Adequate</td>
<td>Fresh</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>English Leicester × Merino</td>
<td>2 to 4</td>
<td>Term</td>
<td>Adequate</td>
<td>Fresh</td>
<td>5</td>
</tr>
</tbody>
</table>

* Rams were of the same breed except in Series 15 where a Southdown ram was used. Merino ewes in most series were mated to medium wool rams.

† For description see Dunlop (1962). These ewes were mated to similar rams.
EXAMINATION OF MATERIAL

All material from any one series was examined at about the same time. Uteri were examined within 6 months of collection, but some 'afterbirths' were stored for 6 years. Fixed material was washed briefly in tap water just before examination.

Uteri were opened along the antimesometrial surfaces and the chorion was trimmed off flush with the surface of the cotyledons. These were removed by severing from the pedicle as close to the cotyledon as possible. Cotyledons were blotted dry, then counted and weighed. Cotyledons from the right and left horn were usually treated separately as were those from individual twin foetuses. Unfortunately, twin foetuses were not always identified with the horn from which they came. Cotyledonary burrs were also counted. In Series 1 and 2, cotyledons were weighed individually and their position within the uterus was plotted.

Separation of maternal and foetal cotyledons was attempted in Series 2 in which the uteri were examined fresh. It was accomplished by gentle traction applied with forceps, and was accepted as satisfactory if the foetal cotyledon slipped easily from the maternal portion and was of distinct colour and texture; the foetal tissue was darker and the surface was smoother and shinier than the maternal. Unpublished histological studies have shown that under these conditions the chorionic villi come away completely and without maternal crypt tissue. The interposing layer of epithelial cells (presumably mostly of foetal origin—Assheton, 1906; Wimsatt, 1950) did not come away cleanly, but appeared to partition evenly. Its contribution to errors in the weight of the maternal and foetal portions is unknown.

'Afterbirths' were examined in detail only if the chorion was complete. The foetal cotyledons were trimmed from it, blotted dry, counted and weighed together. Twin placentae were treated separately, but it was not possible to distinguish the parts of the placenta lying in the right and left horns. Agreement was good between the number of foetal cotyledons on the afterbirth and the number of attachment sites in the uteri of thirteen ewes killed within 2 or 3 hr after parturition. The small discrepancies of the order of two or three could be accounted for by extremely small foetal cotyledons (<0.1 g) escaping detection or by fragmentation of one or two foetal cotyledons at the site of rupture of the membranes during parturition.

STATISTICAL COMPARISONS AND RELATIONSHIPS

Statistical analyses were made by methods described in Dixon & Massey (1957). Complete statistical examination of each series for each variate was not attempted because of the heterogeneity of the ewes, particularly in some of the 'afterbirth' series, in respect of age, nutrition, strain and breed, sex of lamb and litter size, and because of small numbers in these categories. Instead, analyses were confined to groups of ewes homogeneous except for the variate under examination, or, where ewe numbers were small, were confined to groups not obviously biased by unbalanced distribution of the other variates. Thus any series may contain several groups. To minimize possible bias within series, analyses were
based where possible on the mean values from the various series, and the means of these means were compared. Simple regression analysis was used to give quantitative information about relationships, but the finding of significant regression does not necessarily mean that the true relationship is a simple linear one.

Since fixation and storage may affect tissue weight, no comparisons between series were made of the weight of fixed tissue. In the results below, all the series relevant to the various comparisons are mentioned, provided there is no obvious bias due to unbalanced distribution of variates.

RESULTS

RELATIONSHIP BETWEEN FOETAL WEIGHT AND NUMBER OF COTYLEDONS

Foetal weight was not well correlated with the number of cotyledons. For example, in the foetal placentae the coefficients ranged from $-0.46$ to $+0.49$ (mean $+0.15$) and only three of the eleven were significant; they were all less than the corresponding correlation coefficients between birth weight and weight of cotyledons.

RELATIONSHIP BETWEEN FOETAL WEIGHT AND WEIGHT OF COTYLEDONS

The weights of the foetus and of the complete cotyledons were positively correlated in all six groups examined at 114 days of pregnancy or later. Two groups of ditocous ewes in Series 3 are included. These coefficients ranged from $+0.36$ to $+0.87$ and four of the six were significant ($P<0.05$). A small, though significant, correlation ($+0.35$) was found as early as the 90th day of pregnancy in Series 5, but in general correlations were weaker and were less consistent in groups examined at the earlier stages. In Series 2, where foetal and maternal cotyledons were separated, the correlation of foetal weight with weight of intact cotyledons and with the weight of the maternal and foetal portions was $+0.84$, $+0.82$ and $+0.54$ respectively ($P<0.05$ in each); these coefficients were not significantly different.

Similarly, in each of the eleven series of foetal placentae, the correlation coefficients between birth weight and weight of the foetal cotyledons ranged from $+0.35$ to $+0.79$ (mean $+0.56$) and nine were significant ($P<0.05$). Thus in this survey some 30% of the variation in birth weight, i.e. $100 \times (\text{the correlation coefficient})^2$, can be accounted for, statistically, by variations in the weight of the placenta.

The coefficients of linear regression of foetal weight on weight of intact cotyledons in monotocous ewes examined near term (Series 1 and 2, and ewes killed at 135 days in Series 3) were 2-8, 6-9 and 1-5 g of foetus per g of cotyledon, respectively. In homogeneous or unbiased groups of foetal placentae the values ranged from 5-8 to 22-2 g of foetus per g of foetal cotyledon. The coefficients appeared to be unrelated to sex, age and nutrition of the ewes, or to litter size. On extrapolation the regression lines intercepted the foetal weight axis considerably above the origin at about 2 kg foetal weight.
Effect of foetal sex

The regression line of foetal weight on cotyledon weight for male lambs was slightly above that for females (Text-fig. 1) in each of the nine relevant series of foetal placentae in which sex differences were not obviously biased by age, nutrition or strain differences. The difference is therefore real though small; it ranged from 0-1 to 0-4 kg at a given foetal placental weight. In the intact placentae the differences were in the same direction in Series 1 and 3 (0-7 and 0-4 kg; neither differed significantly from zero) but were in the reverse direction in Series 2 (0-1 kg).

![Graph](image)

**Text-fig.** 1. Relation between total weight of foetal cotyledons and birth weight: the effect of (a) foetal sex, (b) age of the ewe, (c) litter size, and (d) nutrition of the ewe. The results shown do not necessarily prove the existence of differences; they are presented for illustrative purposes only. The calculated slopes (regression coefficients) and their standard errors are expressed as g foetus per g cotyledon.

(a) Sex. Series 7: ○, male; ●, female; larger symbols represent means. Regression coefficients: upper line (males), \( b = 8.1 \pm 2.2 \); lower line (females), \( b = 7.3 \pm 1.1 \).

(b) Age. Series 8 (means only): □, males; ○, females; age of ewe in years shown within symbols. Regression coefficients: upper line (males), \( b = 7.6 \pm 2.0 \); lower line (females), \( b = 6.0 \pm 0.8 \). The slopes of the regression lines are the means of the slopes for each age group in each sex. Regression coefficients: upper line (single), \( b = 10.1 \pm 2.9 \); lower line (twins), \( b = 9.0 \pm 3.0 \). (d) Nutrition. Series 10: ○, high plane; ●, low plane; larger symbols represent means. Regression coefficients: upper line (high plane), \( b = 7.6 \pm 2.5 \); lower line (low plane), \( b = 4.9 \pm 2.2 \).

Effect of age and nutrition of ewe

In Series 6, 8 and 10, the regression relationship in the foetal placentae of older ewes tended to be above that in younger ewes (Text-fig. 1), but numbers were too small for significance tests. There were too few animals for age comparisons in the intact placenta. The line for lambs from well-fed ewes was also well above that of lambs from poorly-fed ewes in Series 10 (Text-fig. 1), 11 and 14.

Effect of litter size and strain of ewe

The regression line for single lambs was above that for twins in Series 3, 7, 12, 15 and 16 and the difference was obviously significant in Series 12 (Text-fig. 1).
Placental size in sheep

It is worth noting that the mean birth weight divided by the mean weight of foetal cotyledons was higher in twins than in single lambs in each of Series 7, 15 and 16. The results do not permit conclusions to be drawn about strain differences.

NUMBER OF CARUNCLES PRESENT

The average number of caruncles per uterus was about 100, and some of the differences between series were significant. The numbers were greater in Series 1 and 3 than in Series 2 (109, 106 and 96 respectively) and greater in Series 5 than in Series 4 (104 and 96). Differences within Series 4 and 5 were not significant. Since the same strains are represented in Series 4 and 5, the significant difference between these series must in some way be related to the environment, the greater number being found at Armidale in the New England district of N.S.W., as compared with Deniliquin in the Riverina district.

There was a wide variation in number of caruncles within each homogeneous group of ewes; the overall range was from sixty-four to 145. Caruncles were generally evenly distributed between the two uterine horns but differences of ten were common and there were occasional larger differences of up to thirty-six.

Some ewes were weighed just before slaughter in Series 2, 4 and 5. The coefficient of correlation between ewe weight and number of caruncles was \( -0.11 \) \( (n = 8) \) in Series 2, and was also low in each strain of Series 4 and 5, reaching significance in only one strain in one series \( (r = 0.60, n = 10, 0.02 < P < 0.05) \). The combined coefficients within Series 4 and 5 were both \(+0.26\) and were not significant \( (n = 49 \text{ and } 32) \). Thus within the range of weights encountered, ewe weight appears to be of minor importance in determining the number of caruncles presented to the blastocyst.

NUMBER OF COTYLEDONS

Strain variations

The mean number of cotyledons associated with single foetuses was about seventy-three. As with the number of caruncles there were significant differences between series, for example, sixty-seven in Series 1 and seventy-seven in Series 3, and there was a wide range within homogeneous groups, from thirteen in a uterus containing ninety-four caruncles to 103 in a uterus of 123 caruncles. There was a significant strain difference within series where means ranged from sixty-eight to eighty-one, and there was also a strong suggestion that there are more cotyledons in the placentae of British breeds than in those of Merinos. For example, one of the four Southdown, one of the three English Leicester and one of the six Southdown × Merino ewes in Series 16 had more cotyledons (110, 127 and 100) than any of the Merinos examined, and the means of the English Leicester and English Leicester × Merino were higher than the means of any of the other groups in all series.

There were usually more cotyledons in the gravid than in the non-gravid horn, but the distribution of cotyledons between the two horns was extremely variable; the number in the non-gravid horn ranged from zero to two more than in the gravid horn. Means were approximately forty in the gravid horn and thirty in the non-gravid horn.
Effect of twinning

There were more cotyledons in ditocous than in monotocous ewes in each of the seven series in which more than one ewe bore twins, and the differences from three series were significant; e.g. in Series 3 the numbers were eighty-eight and seventy-seven ($P<0.01$). The difference in Merinos was approximately ten cotyledons but in the Corriedales of Series 12 and the crossbreds of Series 15, the respective differences were eighteen and sixteen; all these differences were significant ($P<0.01$). Each twin foetus was attached to considerably fewer cotyledons than most single foetuses (forty-two compared with seventy-three in Merinos) and this difference was highly significant in the seven relevant series.

Effect of foetal sex

Male lambs were associated with a greater mean number of cotyledons than female lambs in each of the sixteen series, so that the difference is highly
significant; the overall means were seventy-six in males and seventy in females. The difference was in the same direction when the placentae of pairs of male twins were compared with those of pairs of female twins in Series 3, 12 and 15. There were insufficient placentae identified with sex of lambs, for useful comparison to be possible between twins of unlike sex.

**Effect of age and nutrition of ewe**

The number of cotyledons tended to be lower in the older age group (age confounded with parity) in Series 8 and 10 (Text-fig. 2), and when the two series are considered together, the regression of cotyledon number on age is significant (regression coefficient = \(-1.71 \pm 0.70\) cotyledons per year, \(P<0.05\)). Differences were in the same direction in Series 6 and 11.

Variation in the level of nutrition of the ewes during the last 6 weeks of pregnancy produced no consistent effects.

![Text-fig. 3](image-url)

**Text-fig. 3.** Relation between the number of caruncles and the total number of functional cotyledons in Series 3. The regression lines were calculated with the number of caruncles as the independent variate. ○—○, One foetus per uterus; regression coefficient, \(b = 0.39 \pm 0.10\). ⋄—⋄, Two foetuses per uterus; regression coefficient, \(b = 0.58 \pm 0.16\). ▲, Three foetuses per uterus.

**Relation to number of caruncles**

The number of cotyledons was positively and significantly \((P<0.01)\) correlated with the total number of caruncles present in Series 1 to 5 (e.g. Text-fig. 3); correlation coefficients ranged from \(+0.56\) to \(+0.81\). Straight-line regression analyses suggested that the number of cotyledons represented a constant proportion of the number of caruncles (70% in monotocous ewes and about 80% in ditocous ewes). Only in the monotocous ewes of Series 3 (Text-fig. 3) did the analyses suggest that changes in the number of cotyledons were proportionately less than corresponding changes in the number of caruncles.

**THE WEIGHT OF INDIVIDUAL COTYLEDONS**

The weight of individual intact cotyledons was extremely variable, ranging from 0.1 to 45 g; foetal cotyledons on the afterbirth were also variable and
ranged from 0·1 to 12 g in the only foetal placenta in which cotyledons were individually weighed. Very heavy cotyledons frequently appeared to consist of two fused single cotyledons. The smaller ones were near the tubal end of the horns, and the large ones near the junction of the two horns, particularly on the mesometrial aspect.

Strain variations
Comparison of cotyledon weight between series in which the material was fixed may not be valid, but within Series 4 the variation between strains (from 4·8 to 8·0 g) was significant (\( P = 0·05 \)). There were no outstanding strain or breed differences in the mean weight of fresh foetal cotyledons of the single lambs.

Effect of twinning
Intact cotyledons in ditocous ewes were about 30% heavier than those in monotoocous ewes in Series 1 to 5 inclusive and the difference was significant in Series 3, 4 and 5. Foetal cotyledons were also heavier in ditocous than in monotoocous ewes (averages of the four pairs of means were 1·74 and 1·31 g; \( P < 0·01 \)).

Effect of age and nutrition of ewe and foetal sex
The weight of the foetal cotyledons was greater in the older age groups (Text-fig. 2) as shown by straight-line regression analysis in Series 8 and 10; the composite regression coefficient was \( +0·082 \pm 0·026 \) g per year \( (P < 0·01) \). Sex differences in the size of cotyledons were neither consistent nor significant. There were no variations associated with differences in the nutrition of the ewes.

Relation to number of cotyledons
The mean weight of intact cotyledons in a monotoocous ewe was inversely related to their number (Text-fig. 4) in all series; correlation coefficients ranged from \(-0·07 \) to \(-0·83 \) and were significant in three series. The low correlation of \(-0·07 \) in Series 2 can be attributed to the restricted range in number (fifty-two to eighty-six in this series). The mean weight of foetal cotyledons was likewise inversely related to the number of cotyledons in twenty of the twenty-one homogeneous groups in all eleven series; correlation coefficients averaged \(-0·33 \).

Although the points are widely scattered, their distribution is consistent with a hyperbolic relationship such that the product of the number of cotyledons per foetus with the mean weight of cotyledons tends to be constant. The same relationship appears to hold in the placentae of both single and twin lambs.

These results show that there is usually a compensation in placental weight when implantation occurs at a restricted number of cotyledonary sites, but this compensation is incomplete (see below). Both maternal and foetal cotyledons are involved, but the data of Series 2 are too scanty to show the relative contribution of each tissue to this compensation. On the average, the foetal contribution to total cotyledon weight was about 40%.
TOTAL WEIGHT OF COTYLEDONS

As with other variates examined, the total weight of cotyledons varied greatly between ewes: e.g. in Series 3 the weight of intact cotyledons ranged from 181 to 810 g, and in the monotocous ewes of Series 7 the foetal cotyledons ranged from 45 to 214 g.

Text-fig. 4. Relation between the number of cotyledons per foetus and the mean weight (above) and the total weight (below) of (a) intact cotyledons in Series 3 and (b) foetal cotyledons in Series 12. The hyperbolic lines connect points of equal total weight of cotyledons, arbitrarily chosen. The straight lines are drawn to connect the mean points for singles with those for twins.

(a) Intact cotyledons. Singles: ○, 95; ●, 115; □, 135 days. Twins: □, 95; ■, 115; ■, 135 days. (b) Foetal cotyledons. ○, Singles; ●, twins.

Strain variations

Variation between strains in total weight followed the same pattern as the variation in weight of individual cotyledons. There was significance only in the male foetuses of Series 4 (0.05 > P > 0.01), where the means varied between 355 and 500 g, and there were no obvious strain or breed differences in the material examined fresh in Series 15 and 16.

Effect of foetal sex

Differences in the weight of intact cotyledons between male and female lambs were small and not consistent; total cotyledon weight was greater for male than female foetuses in only two of the six comparisons made. On the
other hand, in the foetal placentae, cotyledons of male lambs were consistently heavier than those of females in each of the eleven series (e.g. see Text-fig. 2) so that the difference between the overall means (100 and 80 g) was highly significant.

Effect of twinning

The total weight per ewe of both intact and foetal cotyledons in each relevant series was higher in ditocous ewes than in monotocous ewes, e.g. means of 663 and 480 g at 114 days in Series 3 ($P < 0.05$) and means of 144 and 99 g ($P < 0.01$) in Series 7, 12, 15 and 16. When expressed as total weight per foetus, the difference was in the reverse direction, but was still significant. Compensation in cotyledon weight for reduced number of cotyledons is therefore not complete (Text-fig. 4).

Effect of age and nutrition of ewe

The total weight of foetal cotyledons was significantly higher in older age groups in Series 8 and 10 (Text-fig. 2); the composite coefficient of linear regression was $+4.25 \pm 2.05$ g per year ($0.05 > P > 0.01$). There was however no apparent relation between level of nutrition and total weight of foetal cotyledons.

Changes during pregnancy

Information on the changes in weight of intact cotyledons between 95 and 135 days of pregnancy is provided by Series 3. In monotocous ewes the weight declined significantly, but in ditocous ewes there was a non-significant increase in cotyledon weight with advancing pregnancy (Text-fig. 5).

Accompanying this reduction in cotyledon weight of monotocous ewes, there was a marked tendency towards cotyledonary eversion; in nine out of ten placentae at both the 95th and 114th day the maternal tissue almost completely enveloped the foetal cotyledon, but at 135 days, the foetal tissue tended to surround the maternal in eight out of fourteen placentae, much as
in the normal bovine cotyledon illustrated by Andersen (1926). This change suggests that the ratio of foetal to maternal tissue increases as pregnancy advances.

INCIDENCE OF ABNORMALLY SMALL LAMBS
Among the 416 monotocious ewes in which pregnancy was allowed to proceed to the 135th day or beyond, only four produced outstandingly small lambs of between 1·3 and 1·9 kg. The placentae of the smallest three were also extremely small, the intact cotyledons weighing 79 and 101 g in two of them; the foetal cotyledons weighed a mere 12 g in the third.

DISCUSSION
In the material described here the appearance and distribution of the cotyledons within the uterus were in general agreement with descriptions by other writers (Cloete, 1939; Wallace, 1948; see also review by Amoroso, 1952). However, the average number of seventy-three cotyledons in the Merino in the present study was lower than the average of seventy-eight in the Merino derivations (Rambouillet, Targhee and Columbia) of Hoversland, Watling & Van Horn (1954) and in the Welsh Mountain sheep of Barcroft (1946). It was also substantially lower than the mean of eighty-four reported for Merinos by Cloete (1939) and the mean of eighty-three for Suffolk and Border Leicester × Romney ewes reported by Wallace (1948). The range of cotyledon numbers (14 to 103) found in the present study was much wider than the range given by Amoroso (88 to 96). Likewise the average of 100 caruncles per ewe in the Australian Merinos discussed above, was lower than the average of 150 in Wallace’s sheep. The Australian Merino may thus be at a disadvantage compared with other breeds.

These differences, and the differences between series in the present study, vitiate comparisons of cotyledon numbers between sheep treated in different ways if the treatments are confounded by breed or strain, e.g. in the comparison made by Metcalf, Meschia, Hellegers, Prystowsky, Huckabee & Barron (1962) between Merinos at 14,000 ft altitude and Welsh Mountain sheep at sea level.

The absence of a strong correlation between number of cotyledons and near-term foetal weight is apparently due to the great, though by no means complete, compensation in size of individual cotyledons which occurs when cotyledon numbers are low, e.g. as in twins, and which has also been recorded by Barcroft & Kennedy (1939). On this account it would not be expected that ewes with a large number of caruncles would produce very much larger lambs than ewes with fewer caruncles, despite the relation between cotyledon number and number of caruncles (Text-fig. 3).

It might be expected that a large number of caruncles would be an advantage where more than one foetus is carried, but in Merinos it appears that few of the caruncles unoccupied by a single foetus are potentially available, because only about ten of them are used by twin foetus (Text-fig. 3). Similarly in the Suffolk and Border Leicester × Cheviot ewes of Wallace (1948), in which there were about 150 caruncles, forty-five were unoccupied in ditocous ewes. This
raises the question whether all structures identified macroscopically as caruncles, are potential cotyledons; it may be that some, especially among those towards the tubal end of the uterine horn, can never become functional. Development of a caruncle into a cotyledon presumably depends firstly upon whether it has the physiological capacity to develop, secondly upon whether, and when, it comes into contact with the chorion, and thirdly on whether the region of the chorion provides the appropriate stimulus. At present there is no evidence to show which of these factors is responsible for the non-involvement of the caruncles at the tubal end of the uterus.

Reasons for involvement of more caruncles in placentae associated with male foetuses, and in those in young ewes, are likewise obscure. The sex differences must arise from embryonic factors, perhaps pressure of fluid within the blastocyst, which Hammond (1927, 1935) and Wallace (1948) suggest to be important in implantation. The age (or parity) difference must originate from maternal factors, perhaps uterine distensibility and size.

Although cotyledon number is poorly related to birth weight, the results show conclusively that there is a statistical relation between the weight of the mature foetus and that of both intact and foetal cotyledons. The great individual variation is sufficient to account for the failure of Barcroft (1946) and Wallace (1948) to detect a relationship in their small samples, although they agree that foetal growth would be reduced if the placenta was very small.

The variability in the present trend material was too great to allow demonstration of more than a straight-line trend between birth weight and cotyledon weight within the range encountered. However, on extrapolation, the line intercepts the body weight axis above zero, so that the true relationship must be curvilinear, as is that in man (McKeown & Record, 1953a, b) and guinea-pig (Eckstein et al., 1955).

The results are also similar to those in man and guinea-pig in that at constant placental weight, male lambs tend to be heavier than female lambs, later born lambs heavier than first born lambs, and single lambs heavier than twin lambs. Yet it has been accepted by others, e.g. McKeown & Record (1953a), that at constant placental weight in the sheep, twin foetuses are heavier than single foetuses. This misconception arises from the nature of the relationship between foetal weight and cotyledon weight; at low placental weights the ratio of foetal weight to cotyledon weight is high and decreases as cotyledon weight increases, i.e. the ratios in two populations should be compared at constant placental weight.

As in man (Warkany et al., 1961) the sheep shows an incidence of very small offspring associated with small placentae. However, there is at present little evidence to show whether the relationship between placental weight and birth weight is one of cause and effect or whether, as Barcroft (1946) pointed out, placental size is simply a reflection of foetal size since the foetal membranes are of foetal origin. The demonstration by Walton & Hammond (1938) of a powerful maternal effect on foetal placental weight in the horse certainly suggests that foetal genotype is of minor importance in the control of placental size.

If foetal weight is, at least in part, determined by placental size then it seems
that a substantial foetal weight of, say, 1.5 kg may be obtained through quite a small placenta (Text-fig. 1). The apparent decline in placental efficiency as cotyledon weight increases may have a three-fold origin. Firstly it may be due to an increased proportion of inactive placental tissue in the heavier placentae, and secondly it may be due to increased difficulty of extraction of nutrients from the maternal blood. This would tend to occur if maternal metabolism did not keep pace with foetal demand, so that the concentration of nutrients in maternal blood declined as foetal weight increased. A tendency for rapid foetal growth to reduce maternal blood glucose concentration in ditocous ewes fed suboptimally has already been described (Reid & Hinks, 1962). The reduction became marked at about 110 days when foetal weight would have been about 1.5 kg (Cloete, 1939) which corresponds roughly to the point at which placental efficiency appears to decline markedly. A third explanation is that there is a significant passage of nutrients across the inter-cotyledonary area of the placenta, and that this passage is relatively more important when the weight of cotyledons is low. This possibility cannot be completely rejected, but it seems to be unlikely since, from general appearance, the blood flow through the inter-cotyledonary membranes is low.

The difference between the foetal weight–cotyledon weight relationships for monotoeuous and ditocous ewes may also be explained, at least in part, by the greater difficulty of the ditocous ewes in meeting the metabolic demand of the growing foetuses (Reid & Hinks, 1962). The apparent age differences in the relationship may have a similar origin, but explanations for the sex differences must lie elsewhere, e.g. in the vascularity of the placenta or placental transfer mechanisms.

The nutritional reduction of foetal weight at constant placental weight is not unexpected since the placenta would have reached its maximum weight by about 90 days gestation (Cloete, 1939; Barcroft & Kennedy, 1939), i.e. before undernutrition began. There is, however, a suggestion that late undernutrition of the ewe reduces placental weight if the ewe is bearing twins but not if a single lamb is carried (Thomson & Thomson, 1949). Earlier undernutrition, which appears to elevate cotyledon weight (Foote et al., 1959), may also have resulted in a reduction of foetal weight at constant placental weight.

Maximum weight of intact cotyledons in monotoeuous ewes is clearly reached soon after mid-pregnancy and there is a subsequent slow decline (Barcroft & Kennedy, 1939; Cloete, 1939; and the present results), but the pattern of change in ditocous ewes is still not clear. Barcroft & Kennedy suggested that the weight of the placenta in these ewes declined faster than in monotoeuous ewes, but the present results show the reverse trend. Relative weight changes of maternal and foetal cotyledonary tissue during pregnancy are completely unknown; but the tendency for cotyledonary eversion in late pregnancy as reported here and by Cloete (1939) suggests an increase in weight of the foetal cotyledon, at a time when foetal demands are increasing. The work of Barcroft & Barron (1946) suggests that a decline in weight of maternal cotyledons is more likely at this time, but it remains to be seen how much the changes described by them are the result of individual variation within the few sheep they examined.
It has been shown in this paper that there is a great increase in weight of individual cotyledons when cotyledon numbers are low, and there appears to be a similar compensation in the cow (Petskoi, 1955) and in the deer, Dama dama (Hamilton, Harrison & Young, 1960), but the mechanisms that control this compensation and hence the total weight of cotyledons, remain in the realms of conjecture.

Cotyledonary weight can however be no more than a crude indication of placental function as indicated by the great variations observed. Indeed, Barcroft & Barron (1946) reported increasing vascularity of the maternal part of the cotyledon while the weight of the whole was declining, and Flexner & Pohl (1941) found that permeability per unit weight of the rat placenta to $^{24}$Na varied widely while placental weight remained almost constant. There is need therefore for a better measure of overall placental function than placental weight, but at the present time none is available.

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