

## Studies in Sex Physiology No. 19.

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### The Influence of Age on (a) Amount and (b) Nature and Composition of the Allantoic and Amniotic Fluids of the Merino Ewe.

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#### (a) INTRODUCTION.

IN Study 18 (Malan and Curson, 1936) a few general remarks appeared on the nature of the increase in the amount of allantoic and amniotic fluids in the case of 10 Merino sheep, two having been slaughtered and examined at the end of each month of pregnancy. Whereas it seemed from Table I that the bulk of the increase of the *allantoic* fluid occurred during the second half of the gestation period, this does not appear to be the case when the data in Table I (below) are considered. In point of fact Table I contains additional details about nine sheep not included in Study 18. The variation is striking.

In the case of the *amniotic* fluid the amounts are not so extremely variable and there is apparently little increase before the end of the first month and after the end of the third month. The fluid therefore shows its increase during the second and third months.

#### OUR OBSERVATIONS.

The relevant information is tabulated in Table I.

TABLE I.

Foetus of ewe.	Foetus weight gm.	Allantoic fluid ccm.	Amniotic fluid ccm.	Age of foetus days.
35712	1	55	2.5	31
25924	0.9	48	2.5	31
35984	0.827	46	4	32
44803	1.25	56	6	33
44849	44.5	73	240	60
45082	54.2	95	210	60
35659	70.6	500	200	61
30169	59	195	250	61
35592	82	360	200	64
15337	530	76	520	90
21665	470	76	500	90
33131	617	300	200	92
39904	680	222	365	94
44679	2,730	690	930	121
38521	2,170	555	760	121
35976	2,230	350	450	122
44397	3,300	670	520	145
30514	3,540	1,140	300	146
45023	3,750	3,700	350	150

The data are represented graphically in Chart A where the amounts of the allantoic and amniotic fluids are shown by circles and black dots respectively.

A comparison with the chart (Fig. 493) figured by Needham (1931) shows more or less an agreement, but as there is so much variability it is not considered desirable to draw the curves. As is nevertheless evident, at the end of the first month the amniotic fluid is considerably less than the allantoic fluid, but then increases rapidly and at the end of the third month is apparently more than the allantoic fluid. Towards the end of pregnancy there is no obvious difference between the amounts of the two fluids.

When the amounts of the two fluids are compared with the foetus, as was done by Döderlein for the cow (Needham, Fig. 496) it is seen (Chart B), as pointed out by Needham, that both are much more abundant relative to the foetus weight during the early than in the late stages of pregnancy. This is so pronounced for the allantoic fluid that it has not been possible to include in the chart the values for the age of one month. The fall in the amount of the allantoic fluid per 100 gm. foetus weight with advancing age is much more rapid during the first three months than is the case with the amniotic, as may be expected in view of the less amount of the latter in the beginning, and its more rapid increase during the second and third months. This is demonstrated in Chart B, but it is to be noted that the curves shown are only rough illustrations of the situation and have no other meaning. Chart B is also in fair agreement with that drawn by Needham (Fig. 496).

CHART A.

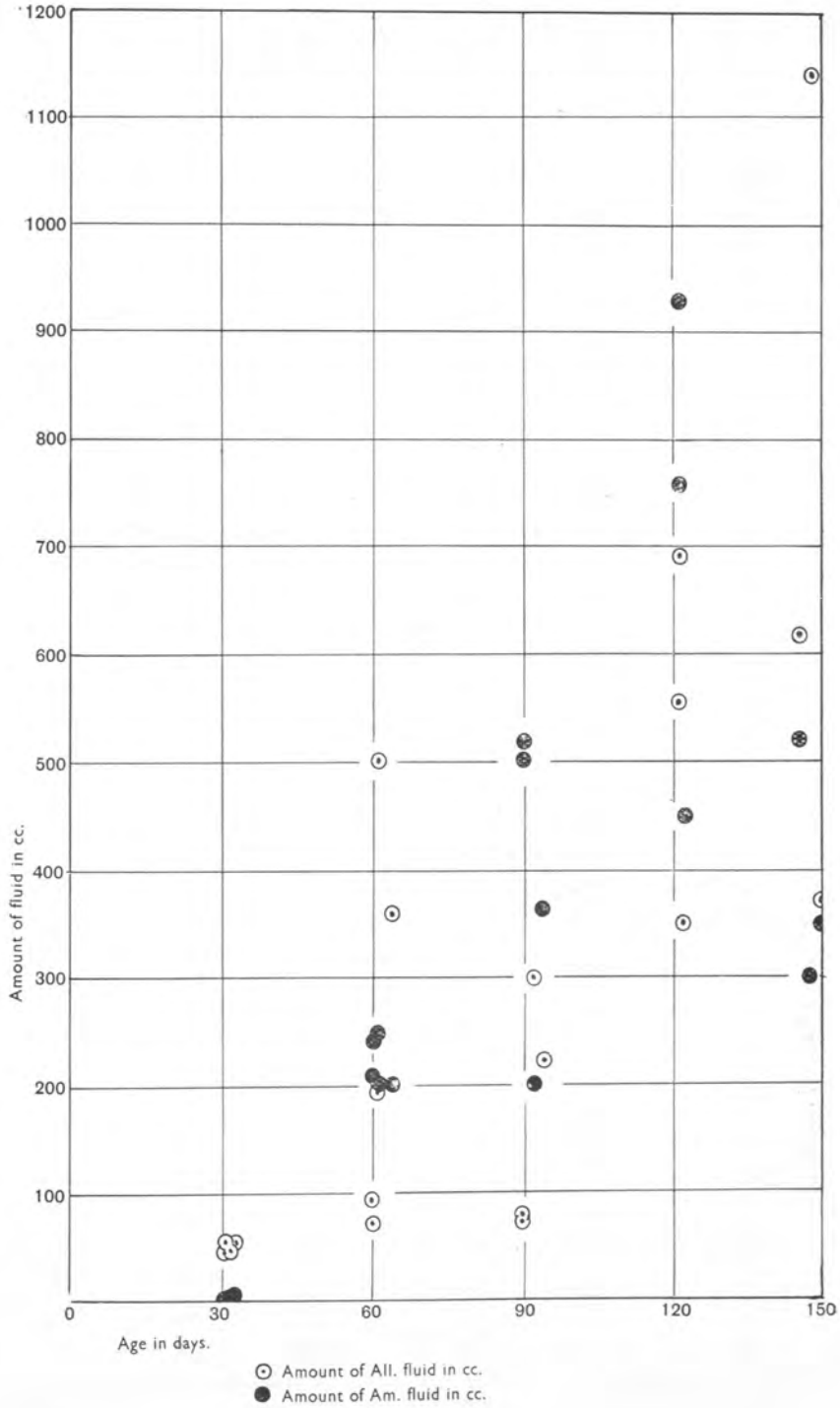
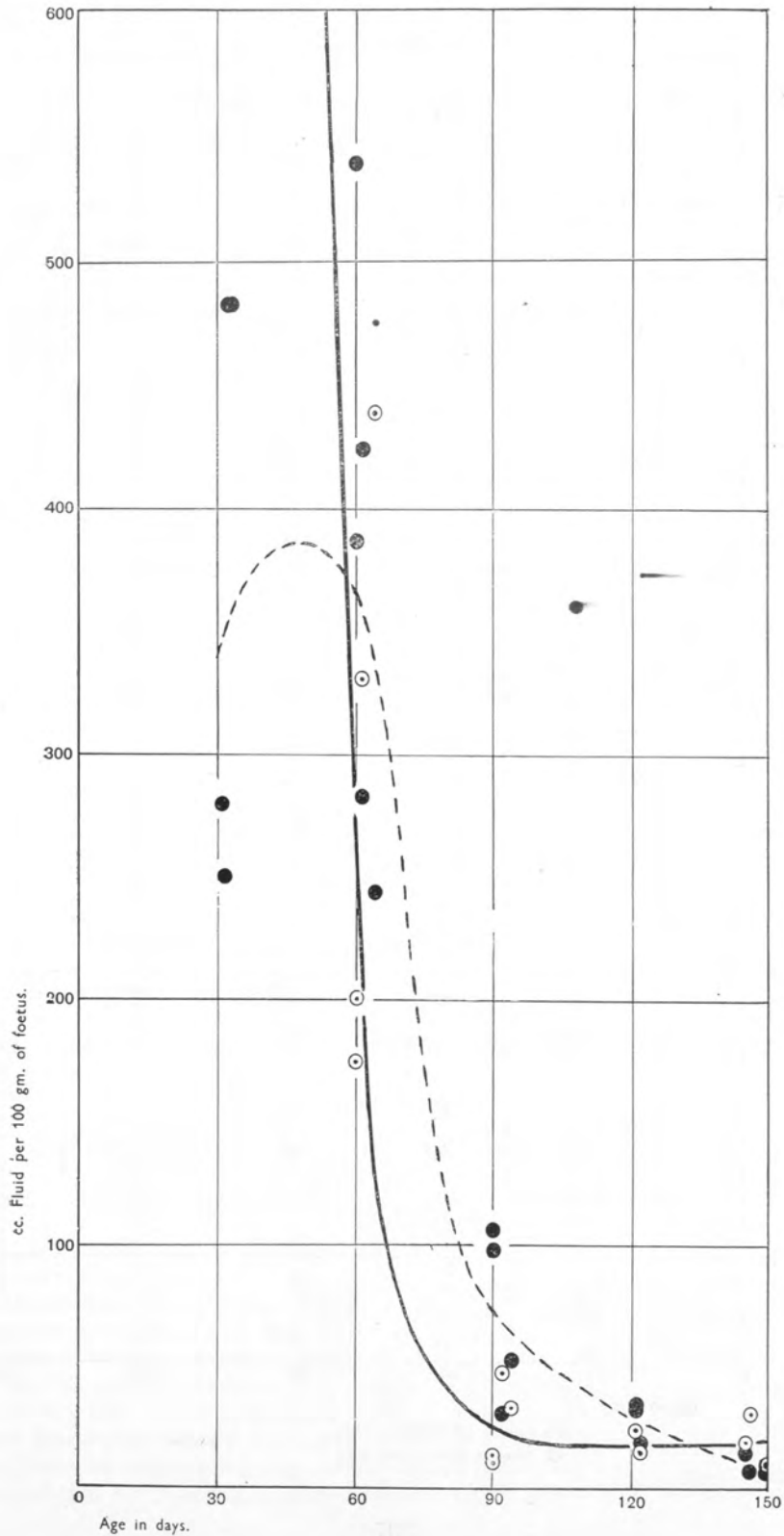


CHART B.



## (b) INTRODUCTION.

The nature and composition of the mammalian foetal liquids have been the subject of investigation for centuries and it is therefore surprising that so little precise information is available regarding them. Concerning the human, Tankard, Bagnall and Morris (1934) make the significant comment that "Although there are some analytical figures in the text books they are rarely of recent date and are also incomplete". Although Needham (1931, p. 1534) indicates that the chemical study of foetal fluids offers "quick returns for work done" associated with "the economy of thought involved in trying any handy estimation method", it is disappointing that so little data is available concerning the sheep. Needham in referring to this species mentions (a) Jacqué's work which indicates that "the allantoic fluid is at first hypotonic to the amniotic fluid and afterwards hypertonic to it". Needham adds, "But as usual in this subject such a statement by no means applies to the pig or the cow, in which the allantoic liquid remains hypotonic to the amniotic liquid throughout development, the two approaching somewhat and reaching an almost equivalent osmotic concentration shortly before birth". Again he concludes "one fact alone is universally true, namely that the 'foetal liquids' are always a good deal hypotonic to the blood whether foetal or maternal". (b) Needham, in Table 247, giving the analysis of the amniotic liquid *at term*, includes Jacqué's (1902) figures (in gm%) as follows:—total ash 0·84, soluble ash 0·82, insoluble ash 0·017, NaCl 0·64, and coagulable protein 0·102. (c) In regard to the carbohydrate content of the fluids, Paton, Watson and Kerr (1907) "reported the presence of fructose in" both the fluids of the sheep and cow. They "calculated the carbohydrate nitrogen ratio . . . and found the interesting, though rather enigmatic, result that C/N ratio diminishes in the allantoic as much as it is increased in the amniotic" (p. 1546); and (d) in discussing maternal transudation in foetal secretions Needham, in Table 249, gives an instructive comparison of an analysis of the fluids in the sheep and cow as follows:—

	Allantoic fluid.		Amniotic fluid.	
	Sheep.	Cow.	Sheep.	Cow.
gm. per cent.—				
Protein.....	0·54	0·08	—	—
Total ash.....	0·924	0·275	0·84	—
Insoluble ash.....	0·074	0·025	0·017	—
Soluble ash.....	0·85	0·25	0·82	—
NaCl.....	0·16	0·19	0·64	—
NaCl Soluble ash × 100.....	18·5	76·0	78·0	—

The precise age of the foetuses is not given.

Below are tabulated the more important facts relating to the nature of the foetal fluids of the ruminant and equine, very little precise data being recorded for the sheep.

TABLE II.  
*General Description of Ruminant and Equine Foetal Fluids*(<sup>1</sup>).

	De Bruin. (1901.)	Smith. (1921.)	Craig. (1930.)	Williams. (1931.)
ALLANTOIC FLUID.				
Origin.....	Foetal urine.	Foetal urine.	—	Chiefly from foetal kidneys.
Colour.....	Whitish, colourless at first and later yellow or brownish.	First colourless but later brown.	First colourless but later becomes yellow.	—
Appearance.....	Foamy.	First turbid.	At first slightly turbid.	Clear.
Reaction.....	Neutral.	—	—	—
Amount.....	Increases towards the end of gestation.	—	Greatest at early period.	Varies widely in health.
Specific gravity.....	Thin liquid.	—	—	—
Chemical composition.	Grape sugar, oxalates, albumen, mucus and allantoidin all absent.	Urea, allantoin (chemically related to uric acid), protein levulose, lactic acid and certain salts.	H <sub>2</sub> O, albumen, osazone, urate of urea, lactic acid, phosphates of Na, Ca, Mg and traces of sugar. Later due to renal excretion are erythrin and hippuric acid. (Hippomanes contain oxalate of Ca.)	Albumen, sugar, and urea.
Function.....	—	A second water jacket for foetus and at birth dilates vaginal passage.	—	—
AMNIOTIC FLUID.				
Origin.....	Secreted from internal face of amnion ( <sup>2</sup> ).	Transudate from both mother and foetus.	—	Not fully determined from amnion itself and or from foetal urine.
Colour.....	Amber.	First yellowish-red but reddish towards end, probably owing to meconium.	—	Colourless but in foetal disease may be reddish or other colour.

TABLE II—(continued).

	De Bruin. (1901.)	Smith, (1921.)	Craig, (1930.)	Williams, (1931.)
AMNIOTIC FLUID (contd.)				
Appearance.....	—	—	—	Clear but in foetal disease may be opaque.
Reaction.....	Alkaline.	Alkaline.	Alkaline. Abundant and limpid during first half of gestation and later scantier, viscid and citron or red.	Sheep 100-500 gm. (quotes St. Cyr and Violet).
Amount.....	—	—	—	—
Specific gravity.....	Thin and watery but at about 5 months gestation changes into a mucoid stringy mass.	—	—	Slightly heavier than water.
Chemical composition.	H <sub>2</sub> O 975-991 parts; chlorides, carbonates, calcium, potassium and sodium sulphates 2.6-7.8; urea 2-3.5; kreatin, kreatinin sugar and fat traces; albumen and mucin .8-10.7.	Protein, urea, sugar, lactic acid, kreatin, and some salts.	99 per cent. H <sub>2</sub> O, albumen, glucose, urea, kreatin and other elements of urine and meconium.	Albumen, sugar and urea (and contains bacteria). Foetal diarrhoea is observed in sheep.
Function.....	Protection of foetus and lubrication of vaginal passage at birth.	Water bag for foetus and dilates and lubricates vaginal passage.	Equable temperature, protection and lubrication.	—

(<sup>1</sup>) As far back as 1814, J. F. John (in his *Chemische Tabellen* Needham, p. 222) shows that observations had been made on the foetal fluids of the cow. "In 1821 Lassaigne described the amniotic and allantoic liquids of the cow as containing 'albumen, osazone, a mucin containing nitrogen, amniotic acid, lactic acid, phosphate of calcium, sulphate of soda and potash and the muriates of soda and of ammonium.'" (Needham).

(<sup>2</sup>) Zietschmann (p. 117) shares this opinion.

TABLE III.

*An Analysis of the Foetal Liquids of the Merino Sheep at the end of the 1st, 2nd, 3rd, 4th and 5th Months.*

D.O.B.	Days.	Fluid.	Total (c.c.).	pH.	S-G.	Gm. Solids per 100 c.c.	Gm. Organic matter per 100 c.c.	Gm. Ash per 100 c.c.	Gm. Nitrogen per 100 c.c.	Mgm. K per 100 c.c.	Mgm. Ca per 100 c.c.	Mgm. Na per 100 c.c.	Mgm. P per 100 c.c.
35712	31	Allantoic..... Amniotic.....	55 2.5	—	1.003	0.82	.02	0.8	.087	20	8.24	223	5.95
25924	31	Allantoic..... Amniotic.....	45 2.5	—	1.005	1.06	.285	0.775	.063	30	6.54	254	5.95
35984	32	Allantoic..... Amniotic.....	46 4	8.4 8.6	1.004	1.15	.505	0.645	.061	12	11.8	204	3.45
44803	33	Allantoic..... Amniotic.....	56 6	8.2 8.4	1.003	1.10	.56	0.54	.061	19	11.5	182	3.81
44849	60	Allantoic..... Amniotic.....	73 240	— 7.5	— 1.002	— 1.065	— .2	— 0.865	— .107	— 55.8	— 5.7	— 274.0	— 1.64
45082	60	Allantoic..... Amniotic.....	95 210	7.4 7.6	1.009 1.003	2.485 1.05	2.225 .195	0.260 0.855	.239 .051	9.8 62.0	9.5 5.8	58.8 250.0	2.07 1.51
35659	61	Allantoic..... Amniotic.....	500 200	— 7.3	1.008 1.006	1.60 1.05	.85 .435	0.75 0.615	.207 .019	20.0 69.0	13.7 3.45	288.0 339.0	8.68 1.31
30169	61	Allantoic..... Amniotic.....	195 250	— 7.6	— 1.008	— 1.07	— .195	— 0.875	— .034	— 39.3	— 5.9	— 294.0	— 1.26
35592	64	Allantoic { Pregnant..... Non-pregnant..... Amniotic.....	360 — 200	7.3 7.3 7.3	1.009 1.007 1.008	1.49 1.47 1.09	.87 .85 —	0.620 0.620 1.200	.138 .140 .039	6.0 6.0 36.0	15.7 16.5 5.2	239.0 224.0 347.0	7.94 8.2 1.84
15337	90	Allantoic { Pregnant..... Non-pregnant..... Amniotic.....	39 37 520	6.6 6.8 8	1.018 1.011 1.007	4.23 4.19 1.27	3.37 3.32 .44	0.86 0.87 0.83	.445 .426 .109	65.2 30.8 45.3	6.4 4.8 5.9	111.0 117.6 242.5	8.2 11.3 1.49



TABLE III—(continued).

D.O.B.	Days.	Fluid.	Total (c.c.).	pH.	S-G.	Gm. Solids per 100 c.c.	Gm. Organic matter per 100 c.c.	Gm. Ash per 100 c.c.	Gm. Nitrogen per 100 c.c.	Mgm. K per 100 c.c.	Mgm. Ca per 100 c.c.	Mgm. Na per 100 c.c.	Mgm. P per 100 c.c.
21665	90	Allantoic { Amniotic { Pregnant.... Non-pregnant.....	48 28 500	6.8 6.8 7.4	1.02 1.015 1.008	5.30 5.13 1.31	4.49 4.36 .48	0.81 0.77 0.83	.461 .432 .074	54.6 24.4 30.8	68.2 33.9 9.2	64.5 64.5 247.0	5.50 5.0 0.68
39904	94	Allantoic { Amniotic { Pregnant.... Non-pregnant.....	140 82 365	6.0 6.0 7	1.013 1.014 1.003	3.950 3.815 1.405	2.990 2.825 .595	0.960 0.990 0.810	— .323 .041	123.0 127.0 22.0	14.6 14.8 6.4	141.0 148.0 317.0	18.73 18.73 2.0
38521	121	Allantoic { Amniotic { Pregnant.... Non-pregnant.....	555 760	6.9 6.9	1.015 1.006	3.12 1.38	2.12 1.70	1.00 0.68	.277 .161	225.0 25.0	9.1 6.8	131.0 244.0	3.45 1.057
44397	145	Allantoic { Amniotic { Pregnant.... Non-pregnant.....	670 520	7.1 7.2	1.013 1.005	3.11 1.51	2.04 .77	1.07 0.74	.245 .091	407.0 134.0	5.9 5.7	14.0 178.5	4.64 2.85
45023	150	Allantoic { Amniotic { Pregnant.... Non-pregnant.....	220 150 350	6.0 6.3 6.9	1.015 1.014 1.001	4.82 4.35 1.005	3.75 3.3 .785	1.07 0.82	.416 .381 .112	246.0 222.0 30.0	16.4 15.2 13.4	66.7 66.7 296.0	12.5 12.5 2.86

## DISCUSSION.

Here we will deal not only with the analysis of the amniotic fluid at full term (as was done by Needham) but also with the analysis of *both* fluids at the end of *each* month of pregnancy. As there appeared to be a difference macroscopically between the allantoic fluid of the pregnant and non-pregnant horns, samples of the respective liquids were examined from the second month onwards (2nd month, ewe 35592; 3rd month, all ewes; 4th month, 44679; and 5th month, 45023).

The specific gravity was estimated by the direct weight method and for the *entire* test, including chemical analysis, a minimum quantity of 30 c.cm. was essential. The gaps that appear in the accompanying Table III are in several cases due to insufficient fluid being available, e.g. the amniotic fluid at 1 month. Unfortunately no determinations were made regarding sugar, Mg or Al salts.

Although no hippomane was encountered in the allantoic fluid, a note has been added (see Appendix) regarding its nature since such structures are not uncommon in the sheep.

The results given in the above table (Table III) are unfortunately too incomplete to justify a detailed consideration of the chemical composition of the amniotic and allantoic liquids. Nevertheless, as the study reported in this publication was obviously of a preliminary nature it may be worth while to emphasize some of the indications suggested by the chemical data, especially in the light of the findings reported and discussed by Needham.

The values for hydrogen ion concentration, determined by the B.D.H. Capillator method, may not be indicative of the true values in every case as it was not always possible to carry out the determinations immediately after taking the samples. It would seem, however, that the values indicate greater alkalinity during the early part of gestation than at later stages, a fact which agrees with the observations made on hens' eggs by Gueylard and Portier and quoted by Needham. The pH of the amniotic fluid shows a tendency to be higher than that of the allantoic fluid of the same period as the values of Aggazzotti also indicate in the case of chickens. Determinations of the pH of foetal blood was omitted and is strongly indicated whenever possible in future work.

According to Needham hardly any investigations have been made of the specific gravity of the constituents of the hen's egg and apparently none of those of other terrestrial eggs. Groebbs has shown that the specific gravity decreases during the course of development of a number of wild birds' eggs. The values given in the table remain extraordinarily constant for both amniotic and allantoic fluids during gestation.

In regard to the total solids per 100 ml. fluid, it is evident that the allantoic liquid is significantly higher than the amniotic fluid and it would be interesting to make a complete partition of these solids. From the values of the other constituents given it is apparent that with the exception of sodium higher concentrations of the constituents are invariably present in the allantoic fluid.

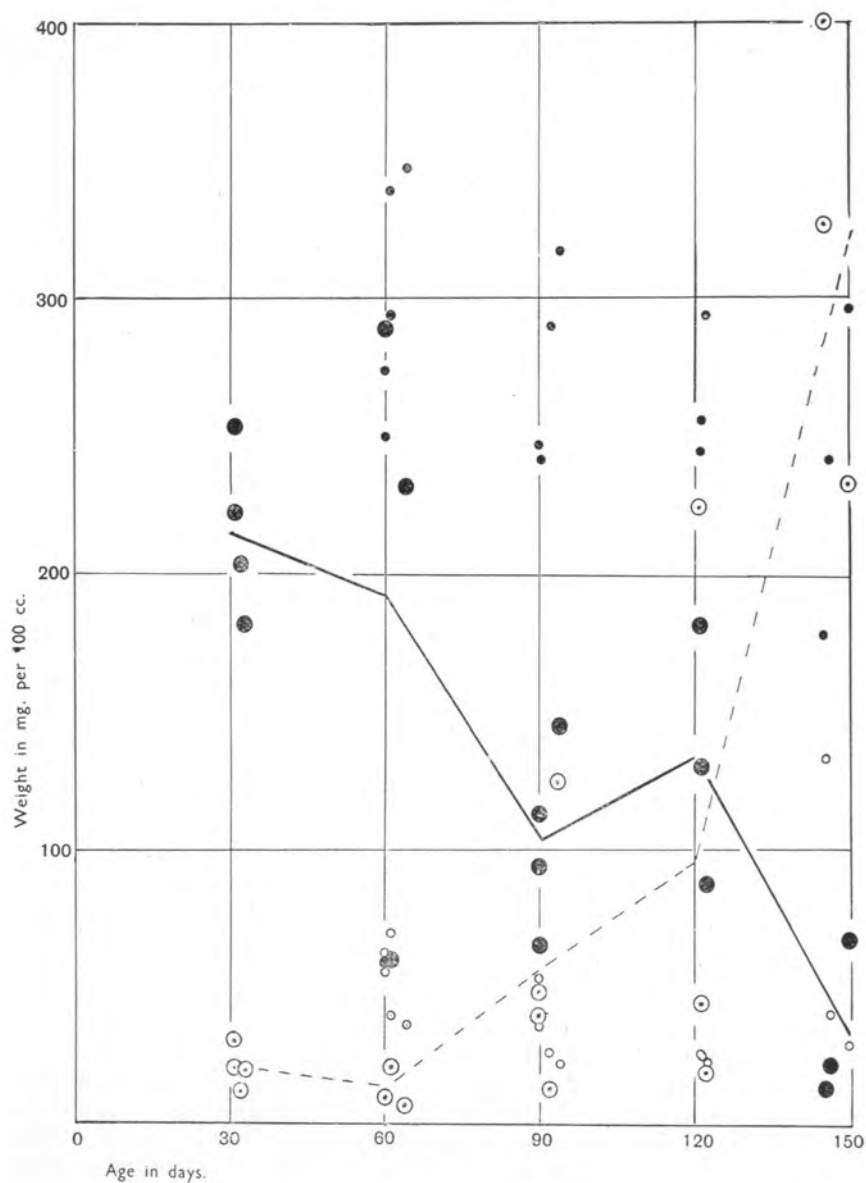
There is also a noticeable increase in the total solid content of the allantoic fluid round about the third month of gestation which is due in part to the formation of excretory products according to Needham. The values do not show a further rise after this period. The amniotic fluid apparently also increases in concentration after approximately three months. In the absence of mammalian amniotic and allantoic fluids given by other workers it may be mentioned that in the case of sheep there is by no means as marked an increase in total solids as in the chick embryo where approximately 29 times and 5 times the values obtained on the 9th day of incubation were obtained on the 14th day for the amniotic and allantoic liquids respectively. In the sheep's foetal fluids also the increase in total solids for both kinds of fluid was primarily due to an increase in the organic fraction of the fluids.

The total ash content of both the amniotic and the allantoic fluids fluctuates but remains fairly constant unless the increases registered in the allantoic fluid just prior to parturition indicate a higher tendency. A comparison with the results of Jacqué which are also indefinite suggests that further data should be collected on the ash contents of these fluids.

The total N content of the allantoic fluid is considerably higher than that of the amniotic fluid which supports Needham's statement on the origin of the fluid, viz.: the activity of the foetal kidneys and hence the depository of a proportion of the embryo's waste products. It would also seem that the concentration of N rapidly increases from the first to the second and third months of gestation. The N content of the amniotic fluid fluctuates very appreciably but apparently bears no direct relation to the N content of the allantoic liquid. However, a N partition is essential and is bound to contribute valuable data towards a discussion of the relative importance and significance of the presence of N fractions.

In regard to the remaining constituents which were determined, viz.: calcium, phosphorus, sodium and potassium, a comparison with the two values of each of these given by Needham according to Kamei for avian liquids is interesting. In the latter case it would appear that the Na values of both allantoic and amniotic liquids exceed the K values very considerably at both the periods of incubation given. This observation applies also to the amniotic fluids of the sheep's foetuses for all the months of gestation given in the table but not to allantoic fluids. In this case the Na content decreases as gestation advances while the reverse is true for K. The significance of this difference between the allantoic and amniotic fluids is not clear although the results of Groenewald on calf blood analysis may be quoted in this connection. At birth the K values—like those of the allantoic fluid—were well above normal while the Na values were well below normal for bovines. Both the increase of K and decrease of Na in the allantoic fluid during gestation appear to be too great to be unimportant for although the increase in K may be explained on the basis of the damning effect of excretory products containing K in the allantoic fluid the decrease in Na must clearly be explained on an entirely different basis. (See Chart C.)

CHART C.



● mg. Na per 100 cc. in All. fluid.  
 ○ mg. Na per 100 cc. in Am. fluid.  
 ● mg. K per 100 cc. in All. fluid.  
 ○ mg. K per 100 cc. in Am. fluid.  
 ——— Straight lines joining the averages of mg. Na per 100 cc. in All. fluid.  
 - - - - Straight lines joining the averages of mg. K per 100 cc. in All. fluid.

Phosphorus and calcium occur in greater concentrations in the allantoic than in the amniotic fluid and do not appear to be subject to regular increases or decreases during gestation although the fluctuations in the values are remarkable. Further work in view will probably provide more and therefore better data for discussing the significance of the values.

In conclusion attention should be drawn to the remarkable uniformity on the whole in the chemical composition of the allantoic fluids from the pregnant and non-pregnant horns of the uterus. The few differences in composition, although very considerable in some cases, must await the accumulation of additional data for their elucidation.

## SUMMARY.

*Sheep (Merino).*

	Allantoic liquid.	Amniotic liquid.
Origin.....	"On the whole the evidence is decidedly in favour of both AM/L and AL/L in mammals being of foetal origin." (Needham, p. 1562.)	"Probably first a transudate and is afterwards added to by the foetal urine." (Needham, p. 1547.)
Colour.....	Varies from colourless waterlike fluid at end of first month, through a pale lemon at end of 2nd month, through orange at end of 4th month, to amber at full term.	Varies from colourless waterlike liquid at end of 1st month, through a pale lemon at end of 2nd month, through an orange at end of 3rd month, back to a pale lemon or even colourless liquid at full term.
Appearance....	Varies from transparent or translucent appearance at end of first month to a translucent or opaque appearance from end of 2nd month onwards.	Varies from a transparent appearance up to the end of the 2nd month to a translucent at the end of the 3rd month, to an opaque and viscid fluid at the end of the 4th month and onwards.
Reaction.....	More alkaline than amniotic liquid. See table 3.	—
Amount.....	44 c.cm. (1 month), to 1,140 c.cm. (5 months). (Table I.)	2.5 c.cm. (1 month), to 930 c.cm. (4 months). (Table I.)
Specific gravity..	Values remarkably constant throughout gestation period.	See table 3.
Chemical composition	See table 3.	See table 3.
Function.....	Protection of foetus and dilation of vaginal passage.	Protection of foetus and lubrication of vaginal passage.

## ACKNOWLEDGMENT.

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## APPENDIX.

*A Note on the Hippomane.*

Both Jenkinson (1913) and Zeitschmann (1923) describe the hippomane as a regular disc-like structure of varying size occurring in the equine, bovine and sheep. The origin is said to be due to an accumulation of embryotroph or uterine milk<sup>(3)</sup> which in some way collects in a fold of the allanto-chorion and becomes thrust inward (invaginated) towards the allantoic cavity. As indicated by the above authors the hippomane exists firstly as a vesicular structure, *i.e.* a diverticulum of the allanto-chorion containing embryotroph, secondly as a wart-like body attached by a stalk to the allanto-chorion (see Figs. 1 and 2) and thirdly as a free object in the allantoic cavity after rupture of the stalk. The density naturally varies according to age, the early structure being soft and composed of

<sup>(3)</sup> Embryotroph is "an emulsion of cellular debris and some blood" (Arey, p. 86) added to "the increased glandular secretions (containing glycogen)" (Arey, p. 83) of the uterus, and which is absorbed directly by the trophoblast until the more efficient haemotropic type of nutrition has been developed.

cellular debris, blood and secretion from the surrounding uterine mucosa. The next stage would be represented by a firmer consistence owing to connective tissue proliferation. Finally, organisation would cease and the degenerating mass would become impregnated with salts derived from the allantoic fluid, *e.g.* ammonium magnesium phosphate, oxalic and uric acids, this being the case particularly in the equine. The colour varies from brown to olive green in the mare, a dirty white to orange in the cow, and a brown in the ewe. In the goat the colour is dirty white.

Zeitschmann (p. 151) states that in many stock-rearing centres it is believed that hippomanes if fed with salt on bread promote the secretion of milk.

Figs. 2-5 show hippomanes of the cow, donkey, sheep and goat (natural size).

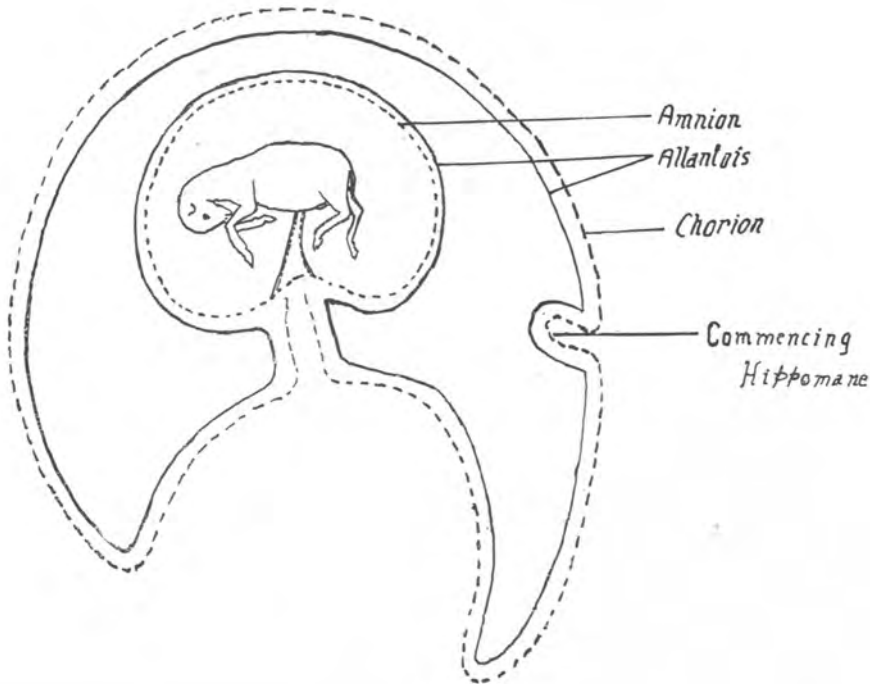


Fig. 1.

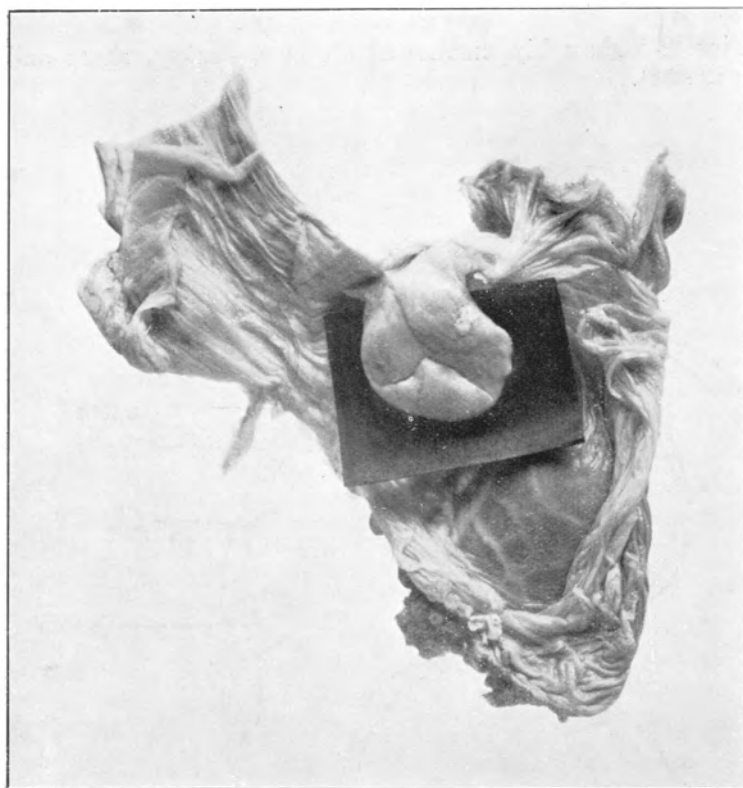


Fig. 2.





Fig. 3.



Figs. 4 and 5.