The Composition of Foetal Fluids of Sheep at Different Stages of Gestation

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One of the interesting problems of foetal physiology is the origin, function and interrelation of the allantoic and amniotic fluids. Some information can be obtained by a study of the composition of the two fluids. Most of our knowledge on the subject, in the case of ruminants, is provided by the papers of Jacque (1902), Paton, Watson & Kerr (1907) and Malan, Malan & Curson (1937) on sheep, and of Doderlein (1890) on cows. The older work refers to unspecified material, usually obtained from a slaughterhouse and arranged only according to foetal length or weight. The results of Malan *et al.* (1937) refer to Merino ewes at given stages in gestation and are the most complete. The present work reports the composition of a series of foetal fluids which had been obtained from cross-bred ewes of defined stages in gestation and management during pregnancy. The properties and constituents studied are the specific gravity, dry matter, ash, total and non-protein nitrogen, pH and principal anions and cations of physiological interest.

EXPERIMENTAL

Material. Twelve samples of allantoic and thirteen of amniotic fluid were obtained from some of the ewes in the experiments of Wallace (1948) on the growth of lambs before and after birth. The animals were his 'age series' of ewes. These were of Border-Leicester × Cheviot breed and had been mated with a Suffolk ram. They were fed a standard ration of sanfoin hay and concentrates, which was sufficient to meet requirements for maintenance and reproduction, without fattening the ewe appreciably by the end of pregnancy. They were killed in groups at 28-day intervals during the gestation period.

Treatment of samples. Immediately after slaughter of the ewes, the uterus and contents were removed, the fluids put into beakers and kept in a cold store. Samples for analysis were usually taken within a few days, and stored in waxed tubes with a crystal of thymol at 4° until they could receive attention. The last samples of allantoic fluid contained suspended solid matter, presumably meconium. This was allowed to settle and the supernatant fluid only taken for analysis.

Chemical methods. Specific gravity was determined as the ratio of the weights (to the nearest mg.) of fluid and water in a 10 ml. density bottle at room temperature (17–20°). Dry matter, ash and organic matter were determined by drying 2 ml. of the fluid to constant weight at 110° in a porcelain crucible, ashing as in the Stolte process, described by Peters & Van Slyke (1932), and weighing again.

N was estimated by micro-Kjeldahl, total N on 0·5 ml. allantoic fluid and 1·0 ml. amniotic fluid, and non-protein N on 5·0 ml. filtrate from a mixture of 2·5 ml. fluid and 10 ml. of 10% (w/v) trichloroacetic acid solution; protein N was calculated by difference. In some samples, notably the later ones of amniotic fluid, a clear trichloroacetic acid filtrate could not be obtained, in which case the results are incomplete.

Inorganic ions were estimated by standard methods used in serum analysis as follows: Na (Kramer & Gittleman, 1924); K (Kramer & Tisdall, 1921) precipitation followed by solution in excess ceric sulphate and back titration with 0·02 N-$Na_2S_2O_3$ after adding KI; Ca (Clark & Collip, 1925); Mg (Denis, 1922); chloride (Sendroy, 1937); inorganic P (Fiske & Subbarow, 1925); pH was determined with a quinhydrone electrode and the CO_2-combining capacity in a volumetric Van Slyke apparatus, after equilibrating with alveolar air.

These methods were applied as follows: Ca, Mg, chloride and P were estimated directly on both fluids, and Na directly on the amniotic fluid. Na in the allantoic fluid and K in both fluids were estimated on the ash obtained by the Stolte process (see Peters & Van Slyke, 1932), as direct estimation gave erratic results; when insufficient sample remained to repeat the analysis on the ash, the results are incomplete. Suitable dilutions of the sample were made for the determination of K, Ca, and Mg in the allantoic fluid. In estimating P in the allantoic fluid, difficulty was encountered with samples obtained after the first 2 months of pregnancy, as the values were low and a turbidity developed on the addition of the molybdic sulphuric reagent; however, an approximate value was obtained by extracting the molybdenum blue with *iso*amyl alcohol and comparing with a similarly treated standard.

RESULTS

The results of analyses are given in Table 1, together with details of the samples. The allantoic fluid showed an increase, as gestation proceeded, in specific gravity, non-protein nitrogen and other organic matter, potassium and magnesium, and a decrease in chloride and inorganic phosphorus; the values for calcium were higher in the middle than at the beginning or end of gestation. The amniotic fluid, on the other hand, was much more constant in composition. It had a lower specific gravity, lower organic matter, non-protein and protein nitrogen and magnesium contents, and higher sodium and chloride contents, than the allantoic fluid. The potassium, calcium and phosphorus contents fell within the range of values of this fluid.

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Table 1. *The composition of foetal fluids of sheep at various stages of gestation*

Stage (days)*	No. of foetus*	Wt. of foetus (g.)*	Sex	Quantity of fluid (ml.)*	Sp.gr.	Dry matter (g./100 ml.)	Ash (g./100 ml.)	Organic matter (g./100 ml.)	Total N (g./100 ml.)	N.P.N. (g./100 ml.)	Protein N (g./100 ml.)	pH	Na^+ (mg./100 ml.)	K^+ (mg./100 ml.)	Ca^{++} (mg./100 ml.)	Mg^{++} (mg./100 ml.)	Cl^- (mg./100 ml.)	P (inorganic) (mg./100 ml.)	CO_2-combining capacity (ml./100 ml.)
									Allantoic fluid										
28	6	0·47	—	32	1·0082	0·85	0·6	0·25	0·118	0·086	0·032	8·00	—	47	9·7	3·7	230	10·4	102
28	8*a*	0·48	—	18·8	—	0·75	0·6	0·15	—	—	—	8·09	177	21	12·0	2·9	240	13·9	60
56	3*b*	47	—	93	1·0106	1·8	0·45	1·35	0·214	0·185	0·029	7·31	163	20	11·9	6·4	240	4·8	31
56	10	46	—	74	1·0156	2·3	0·6	1·7	0·315	0·210	0·105	6·87	139	38	9·3	8·7	249	7·4	9
84	11*a*	525	M.	74	1·0186	—	—	—	0·350	0·287	0·063	6·89	—	—	24·4	22·9	80	0·9	15
84	11*b*	486	M.	59	1·0174	1·1	0·8	0·3	0·343	0·236	0·107	7·36	—	—	20·7	19·3	113	0·4	22
84	9*b*	490	F.	49	—	—	—	—	0·770	0·546	0·224	6·36	—	—	6·3	19·0	51	<1	—
112	14	1624	F.	308	1·0204	4·3	1·25	3·05	0·323	0·280	0·043	4·49	—	40	46·0	34·1	43	<1	—
112	12*a*	1985	F.	248	1·0218	—	—	—	0·404	0·368	0·036	—	—	—	—	—	—	0·4	—
112	12*b*	2248	M.	422	1·0205	4·15	1·05	3·1	0·374	0·330	0·044	5·14	262	119	9·5	41·0	70	0·5	—
140	7	6226	M.	1050	1·0233	3·9	1·15	2·75	0·360	0·350	0·010	6·46	266	141	4·6	79	42	0·5	15
140	15	5925	F.	1167	1·0202	3·7	1·3	2·4	0·304	0·293	0·011	6·64	86	330	2·5	30·5	40	—	10
									Amniotic fluid										
28	6+2	0·47+0·55	—	0·85+0·87	—	—	—	—	0·08	0·08	0·00	—	—	—	—	—	—	—	—
28	8*a*+*b*	0·48+0·55	—	1·19+1·19	—	—	—	—	—	—	—	—	302	—	—	—	378	—	—
56	3*b*	47	—	164	1·0074	—	—	—	0·038	0·031	0·007	7·47	296	67	7·1	1·4	438	2·8	47
56	3*a*	42	—	184	1·0074	1·15	0·9	0·25	0·038	0·033	0·005	7·47	293	57	6·3	1·3	446	2·7	44
56	10	46	—	149	1·0082	1·0	0·8	0·2	0·061	0·038	0·023	7·39	258	75	6·6	2·2	409	6·1	35
84	11*a*	525	M.	630	1·0074	—	—	—	0·034	0·028	0·006	7·67	309	—	6·9	1·5	424	1·5	45
84	11*b*	486	M.	675	1·0066	1·15	0·8	0·35	0·032	0·029	0·003	7·60	312	37	7·0	1·6	432	1·3	48
84	9*b*	490	F.	602	1·0070	—	—	—	0·035	0·025	0·010	7·12	302	—	6·7	1·4	447	2·0	37
112	14	1624	F.	255	1·0079	1·35	0·85	0·55	0·076	—	—	6·68	287	—	10·8	2·8	448	0·7	—
112	12*a*	1985	F.	738	1·0071	1·5	0·9	0·6	0·055	—	—	4·86	293	53	9·2	3·0	430	3·0	—
112	12*b*	2248	M.	1263	1·0077	1·5	1·15	0·35	0·069	—	—	4·53	284	47	8·2	2·8	416	2·5	—
140	7	6226	M.	1684	1·0078	1·2	0·75	0·45	0·072	—	—	8·40	279	43	5·9	3·4	382	1·3	67
140	15	5925	F.	1416	1·0075	1·1	0·65	0·45	0·073	—	—	6·46	233	33	4·3	2·6	348	1·5	18

* Data quoted from Wallace (1948).

DISCUSSION

Before discussing the results, it is appropriate to consider possible effects of the treatment of the experimental material in terms of conditions and processes obtaining in the womb of the living ewe. Post-mortem changes may set in before the removal of the fluids from the foetal sacs. These might alter the composition through differences in the permeability of the membranes separating the two fluids, or in the functioning of the foetal kidney. The possibility of such changes was emphasized by Jacque (1902), but not explicitly avoided by others. They are considered to be negligible in the present work.

After taking the samples there may be a loss of dissolved gases. Should the fluid contain any appreciable amount of carbon dioxide, the pH value and carbon dioxide content would then both be affected. In the present work, samples could not be obtained anaerobically and so the carbon dioxide combining capacity, rather than the carbon dioxide content, was determined. The pH values reported may consequently be too high. Reliable figures for pH and carbon dioxide content have, therefore, yet to be reported.

During storage of the samples, enzymic changes might occur. Any due to micro-organisms were avoided in the present work. It is not known, however, whether the fluids themselves contain enzymes derived from the foetus. As they are known to contain fructose the possibility of changes, which might affect the acidity, remains. This might have the additional effect in those samples of allantoic fluid, which contained suspended solid matter, of bringing more ions into solution.

The present results are mostly in general agreement with those already reported in the literature, but the following differences may be noted. Malan *et al.* (1937) reported a number of much lower values for the specific gravity, especially of the amniotic fluid. They also found higher values for the phosphorus in the allantoic fluid in the latter part of gestation, but it is not clear whether their results refer to the inorganic, as in the present work, or to the total phosphorus. Doderlein (1890) gives some significantly higher values for the calcium in the amniotic fluid.

The changes in the non-protein nitrogen, organic matter, potassium and calcium contents of the allantoic fluids suggest an increasing excretion of foetal urine as gestation proceeds. This implies that the composition of the allantoic fluid reflects the developing metabolic activity of the foetus, and the developing functional efficiency of its kidney. The decreased chloride in the 84-day samples, and the increased volume of the 115-day samples may therefore indicate the appearance of tubular activity. Although little is known of the composition of ruminant foetal urine, the limited data of Doderlein (1890) and Jacque (1902) support the view that urinary excretion does take place. Jacque (1902), however, reached somewhat different conclusions as to the routes of excretion on the basis of his extensive cryoscopic investigations. He considered that urine was excreted successively via the urachus alone, the urachus and urethra and finally the urethra alone, as gestation proceeded. The present results, however, may not necessarily be incompatible with this. Urinary excretion may not follow a rigid pattern in a given species, and may possibly be affected by variable factors, such as the management of the ewe during pregnancy.

Table 2. *Comparison of average compositions of sheep amniotic fluid and serum*

	Amniotic fluid (mg./100 ml.)	Amniotic fluid (m-equiv./l.)	Serum* (m-equiv./l.)
Na^+	287	124·8	158·6
K^+	51·5	13·1	8·9
Ca^{++}	7·2	3·6	5·2
Mg^{++}	2·2	1·8	2·8
Total cations	—	143·3	175·5
Cl^-	416·5	117·3	104·2
P (inorganic)	2·3	about 1·0	1·7
CO_2	46·3	20·7	25·0
Total anions	—	139·0	130·9

* Based on data in Shearer & Stewart (1931) and Dukes (1943).

The results for the amniotic fluid justify the calculation of an average composition (cf. Table 2). When expressed as m-equiv./l. the sums of the anions and cations agree to $\pm 1\cdot5\%$, indicating that the analyses for the main ionic constituents are fairly complete. Inspection of the data suggests comparison with the composition of sheep serum, and an average composition for this fluid, based on the literature, has been included in Table 2. The amniotic fluid is seen to have a slightly lower sodium and higher chloride content, and a calcium content nearer to the ionizable than to the total calcium of serum. This suggests that the amniotic fluid may be a transudate of serum. The possibility may be sufficiently tested for the present, by considering the relations of the predominant ions, sodium and chloride, in the two fluids. The conditions for a Donnan equilibrium are found to hold to $\pm 6\%$. To establish this view more definitely, concurrent analyses of amniotic fluids, and maternal and foetal sera from the same uteri, are required. That sodium can readily pass from the maternal serum to the amniotic fluid has been shown for rats and guinea pigs by the radioactive tracer studies of Flexner & Gellhorn (1942). A similar permeability in the structurally different placenta of the sheep would at least allow the possibility of an ionic equilibrium between the two fluids.

A direct relation between the amniotic and allantoic fluids across the placental membranes seems improbable from the present results. Therefore, if the amniotic fluid is absorbed by the foetus, the foetal digestive tract and kidney would be the main agents determining the quantity and composition of the allantoic fluid. Hence the present 'passive' study of the composition of the two fluids gives us the following picture of their origin and interrelation: the amniotic fluid arises as a transudate of the maternal serum and the allantoic fluid comes from the amniotic fluid by the intervention of the foetus. This view could be tested further by a combination of the more active experimental approach of some of the earlier workers with present-day techniques, and would need to be correlated with both physiological and anatomical evidence.

SUMMARY

1. The composition, mainly ionic, of a series of allantoic and amniotic fluids obtained, at five regular intervals during gestation, from ewes fed during pregnancy to maintain their weight to the end of pregnancy, has been studied.

2. As gestation proceeded the allantoic fluid showed an increase in non-protein nitrogen and other organic matter, potassium and magnesium, and decrease in chloride and inorganic phosphorus. The values for calcium were higher in the middle than at the beginning or end of gestation.

3. The amniotic fluid was more constant in composition; it had a lower specific gravity and organic matter, non-protein and protein nitrogen and magnesium contents, and higher sodium and chloride contents than the allantoic fluid. Potassium, calcium and inorganic phosphorus contents were within the ranges for allantoic fluid.

4. Comparison of these results, with data available for the composition of sheep serum and foetal urine, suggest that the amniotic fluid is in Donnan equilibrium with the maternal serum, and that the chemical differences between the amniotic and allantoic fluids are due to the intervention of the digestive tract and kidney of the foetus.

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A Photoelectric Flame Photometer

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The flame photometer is an instrument for determining the concentrations of certain metals in solution by measuring the intensity of the light emitted by them when the solution is sprayed under controlled conditions into a non-luminous flame. Lundegårdh (1929–34) developed an apparatus in which cations in solution were sprayed into an air-acetylene flame, and the spectra produced photographed and compared with similar spectra from standard solutions. More recently several workers (for literature, see Barnes, Richardson, Berry & Hood, 1945; Boon, 1945) have designed instruments in which the light from the flame is passed through optical filters on to a photosensitive element (selenium cell or phototube) and the current so produced is measured. Such instruments may be bought in the United States (from the Perkin-Elmer Corporation, Glenbrook, Connecticut, or the National