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GESTATIONAL VARIATIONS IN THE COMPOSITION OF THE FOETAL FLUIDS AND FOETAL URINE IN THE SHEEP

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Early investigations into the composition of the foetal fluids have been reviewed by Needham (1931). More recently other workers, notably Malan, Malan & Curson (1937), Cloete (1939) and McDougall (1949) have examined the composition of the fluids in the sheep, but the relationship of these to the plasmas on the one hand and the urines on the other have been largely neglected in the past. The purpose of the present investigation was to re-examine the composition of the foetal fluids and to make comparisons of the concentration of the constituents present with those in the maternal and foetal plasmas and the maternal and foetal urines.

METHODS

The animals were Welsh Mountain ewes of known conceptual age. The range of foetal age examined was from 45 to 142 days (the gestation period in this breed being about 145 days). Anaesthesia was induced by spinal administration of procaine. The animal was placed in the supine position in a bath of saline at 38° C, the head and thorax being elevated to an angle of 45°, and the foetus was delivered into the bath by Caesarean section (Huggett, 1927). Intravenous supplements of sodium thiopentone (Pentothal, Abbott Laboratories) were given to the ewe if and when required in order to maintain a light plane of general anaesthesia.

Maternal blood was obtained from the dorsalis pedis artery, foetal blood samples from one of the four umbilical vessels. The blood samples were withdrawn into centrifuge tubes containing dry heparin and the plasma obtained by centrifuging for 30 min at 3000 r.p.m. Amniotic and allantoic fluids and maternal urine samples were removed by puncturing the respective sacs and bladder and withdrawing the samples by syringe. Foetal urine samples were obtained from the foetal bladder after the abdomen had been opened under local anaesthesia.

Determinations of the osmotic pressure were carried out with a cryoscope having a Stantel Thermistor as the temperature-sensitive element.

Total nitrogen and non-protein nitrogen (after protein precipitation by trichloroacetic acid) were determined by the Markham micro-Kjeldahl method; amino acid nitrogen by the method of Pope & Stevens (1939) after heat coagulation of the protein; urea by the micro-diffusion

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method (Conway, 1947); sulphate by the barium sulphate turbidimetric method of Sperber (1948); total and inorganic phosphate, creatinine and uric acid by the methods of King (1946). The Na^+ and K^+ concentrations were determined by flame photometer, Cl^- by the method of Sanderson (1952); total reducing substance was estimated by the method of Somogyi (1952) and fructose as described by Bacon & Bell (1948), after deproteinization with NaOH and $ZnSO_4$. Electrometric determination of pH (using a Cambridge pH meter) was carried out on samples which were collected under liquid paraffin.

The accuracy of the estimations of osmolarity of sodium, potassium, chloride, urea and fructose concentrations was of the order of 3%, and the remaining estimations (some of which relied on difference methods) were of the order of 5–10%.

RESULTS

The results of osmotic pressure determinations are shown in Table 1. The osmotic pressure of the foetal plasma is very similar to that of the maternal plasma.

The foetal bladder urine is considerably hypotonic with respect to the plasma and is typically in the range 50–80% of the tonicity of foetal plasma, while the osmotic pressure of the maternal urine varied up to five times that of the plasma.

TABLE 1. Average osmotic pressure values (m-osmole/l.) in the plasmas, foetal fluids and foetal urine

Foetal age (days)	Maternal plasma	Foetal plasma	Amniotic fluid	Allantoic fluid	Foetal urine
45– 69	335 (6)	335 (4)	333 (6)	248 (6)	—
81– 93	332 (4)	344 (5)	327 (5)	286 (5)	239 (4)
104–117	339 (5)	353 (5)	307 (5)	288 (5)	207 (2)
130–142	339 (4)	353 (4)	303 (4)	322 (4)	166 (1)

Figures in parentheses indicate number of observations.

The relationship of the cryoscopic values in the two foetal fluids with foetal age are seen in Fig. 1. The allantoic fluid is initially hypotonic but the tonicity rises towards term, whereas the amniotic fluid is approximately isotonic in the early stages of gestation with hypotonic values being found after about 90 days. The foetal bladder urine was found to be hypotonic with respect to the foetal fluids (except in one case).

The total nitrogen concentration in the foetal plasma and in the amniotic fluid shows a mutual correlation as well as a positive correlation with foetal age (Fig. 2). That in the maternal plasma varied from 880 to 1370 mg/100 ml., mean 1117 mg/100 ml.

The total nitrogen of the allantoic fluid remains considerably higher than that of the amniotic over the whole age range examined and varies from 73 to 468 mg/100 ml., with an average value of 273 mg/100 ml. The values show no definite relationship to foetal age except perhaps in foetuses younger than 57 days. A positive correlation of total nitrogen in the foetal urine with foetal age (81–142 days) was found (correlation coefficient, r, $=0{\cdot}875$, $P<0{\cdot}001$), the total nitrogen increasing from 55 to 193 mg/100 ml.

The non-protein nitrogen (N.P.N.) of the foetal plasma was found to be in all cases greater (mean value 45, range 37–50 mg/100 ml.) than that present in the maternal plasma. The amniotic N.P.N. was usually less, while the allantoic N.P.N. was considerably higher, than that of the foetal plasma. Amino acid nitrogen concentration was consistently highest in the allantoic fluid. One sample of foetal plasma estimated for amino acid nitrogen gave a value of 26·6 mg/100 ml. compared with a mean maternal plasma concentration of 14·8 mg/100 ml. (Table 2).

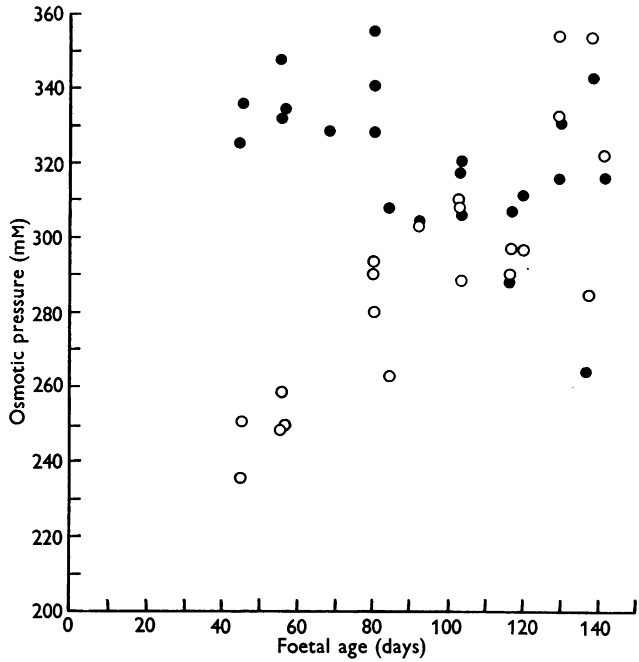

Fig. 1. Correlation of osmotic pressure in the foetal fluids with foetal age. ●, Amniotic fluid; ○, allantoic fluid. From 45 to 142 days the osmotic pressure in the allantoic fluid rises and gives a significant positive correlation with foetal age ($r = 0{\cdot}846$, $P < 0{\cdot}001$). The osmotic pressure in the amniotic fluid shows a significant negative correlation ($r = 0{\cdot}563$, $P < 0{\cdot}01$) over the same age range.

The urea concentration in bladder urine increases with foetal age (Fig. 3). This rise in urea concentration is reflected in the urea concentration of the foetal fluids (Fig. 4).

While the allantoic urea concentration is variable (mean 64, range 12–140 mg/100 ml.), that of the amniotic fluid shows a progressive increase in concentration with gestation age. The permeability of the placenta to urea is reflected in the correlation of the concentrations of maternal plasma urea with foetal plasma urea (Fig. 5).

The creatinine values are shown in Table 3. It will be seen that the creatinine concentration in the amniotic fluid exceeds that found in the foetal plasma in the older foetuses but is less than that of the foetal urine. Creatinine values of the allantoic fluid are higher than those of the urine and show a positive correlation with foetal age.

The uric acid concentration of the allantoic fluid in five animals examined exceeded that of the amniotic fluid, the ranges being 1·4–57·4 mg/100 ml. and 0·8–5·5 mg/100 ml. respectively.

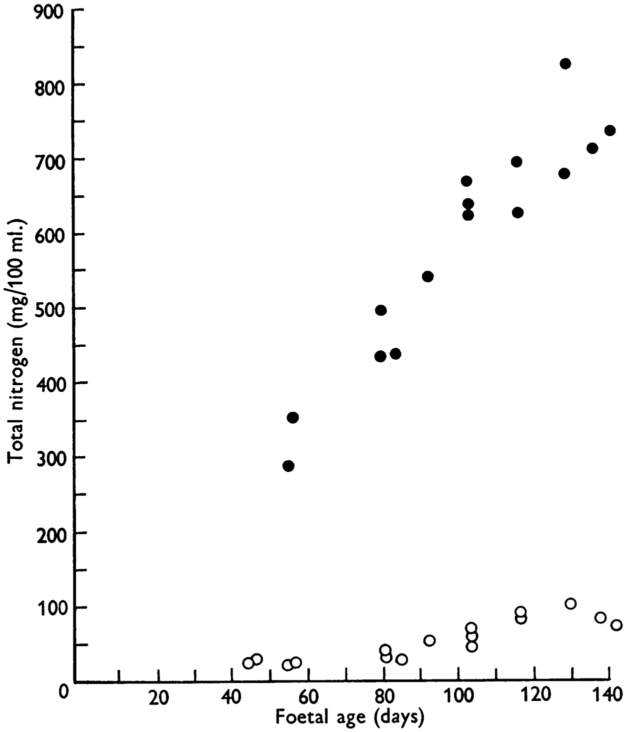

Fig. 2. Correlation of the total nitrogen in the foetal plasma and amniotic fluid with foetal age. ●, Foetal plasma; ○, amniotic fluid. Over the age range examined a positive correlation of the total nitrogen in the foetal plasma to foetal age is present ($r = 0{\cdot}942$, $P < 0{\cdot}001$). A similar correlation is present in the amniotic fluid ($r = 0{\cdot}892$, $P < 0{\cdot}001$).

TABLE 2. Non-protein and amino acid nitrogen in the maternal plasma and foetal fluids (mg/100 ml.)*

	Maternal plasma	Amniotic fluid	Allantoic fluid
Non-protein nitrogen	30 (17–37) *17*	26 (20–40) *7*	210 (136–268) *5*
Amino acid nitrogen	14·8 (7·3–20·0) *5*	3·9 (3·0–4·4) *5*	53 (21·8–75·6) *4*

* Mean (range); no. of observations in italics.

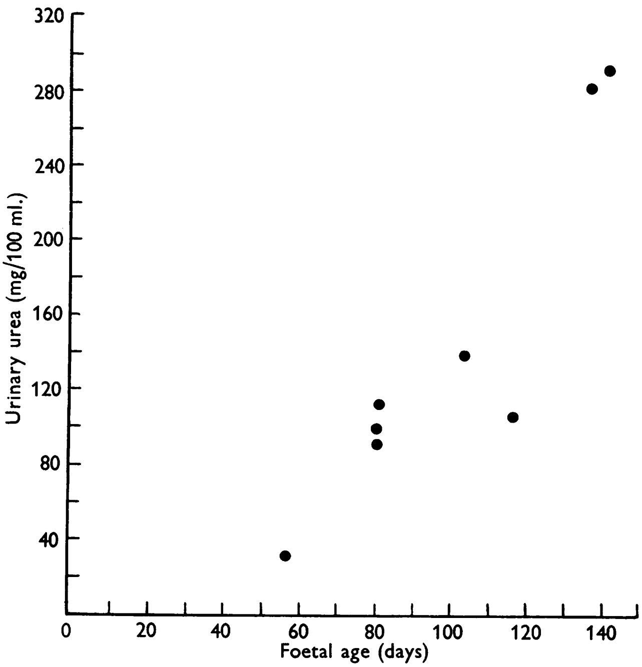

Fig. 3. Correlation of bladder urine urea with foetal age. The concentration of urea present in initial bladder samples of foetal urine shows a significant positive correlation ($r = 0{\cdot}910$, $P < 0{\cdot}01$) with foetal age between 57–142 days.

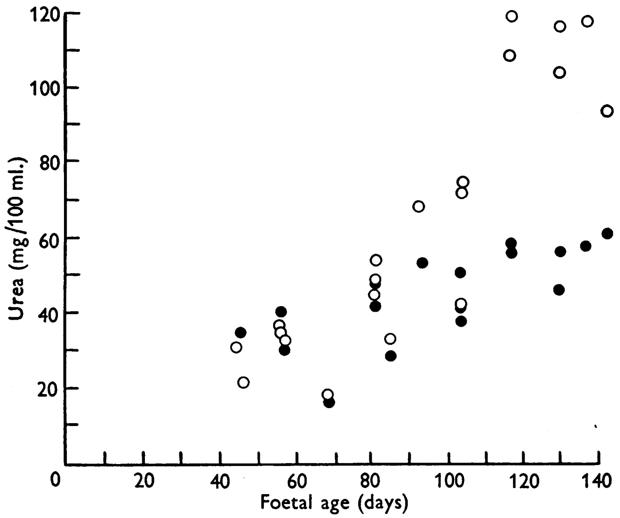

Fig. 4. Correlation of urea in the foetal plasma and amniotic fluid with foetal age. ●, Foetal plasma; ○, amniotic fluid. A significant positive correlation of urea in foetal plasma ($r = 0{\cdot}718$, $P < 0{\cdot}001$) and amniotic fluid ($r = 0{\cdot}893$, $P < 0{\cdot}001$) exists over the age range 45–142 days.

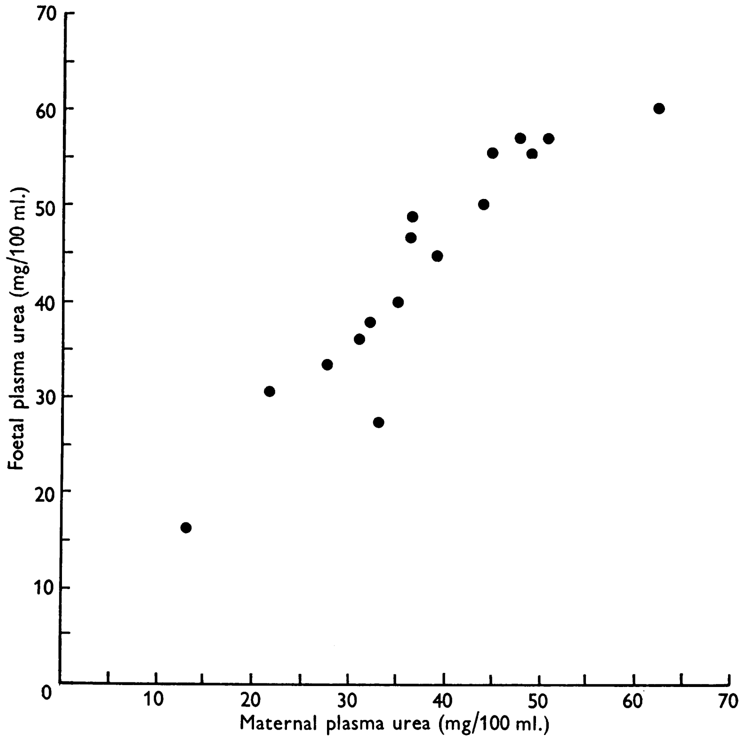

Fig. 5. Relationship of the foetal plasma urea with the concentration encountered in the maternal plasma.

TABLE 3. Concentration of creatinine found in the plasmas, foetal urine and fluids.

	Creatinine (mg/100 ml.)				
Foetal age (days)	Maternal plasma	Foetal plasma	Foetal urine	Amniotic fluid	Allantoic fluid
69	0·4	0·8	—	—	2·0
81*	0·4	0·8	—	0·4	3·7
		0·1	—	0·8	4·0
81	1·2	2·7	2·8	1·8	13·0
85	0·8	1·2	—	1·9	14·3
93	0	—	—	4·3	13·2
104	2·8	2·6	—	4·9	14·0
104	1·3	2·9	—	2·9	10·7
117	0·9	1·5	—	10·2	—
117	1·4	2·7	6·4	6·8	24·9
130	1·2	2·7	—	6·4	27·5
130	2·3	3·1	—	9·4	59·0
137	2·0	3·4	24·4	9·1	44·0
140	—	—	—	—	—
142	2·5	4·2	28·8	6·1	66·0

* Twins.

No consistent trend is discernible in the sodium concentrations in either of the foetal fluids but that in the amniotic fluid (mean 114, range 85–146 m-equiv/l.) is higher than that in the allantoic fluid (mean 70, range 32–131 m-equiv/l.) throughout gestation. In general the potassium concentration of the amniotic fluid (mean 10·6, range 4·7–30 m-equiv/l.) declines as term is approached, while the converse is suggested by the values encountered in the allantoic fluid (mean 10·2, range 1–33 m-equiv/l.). The mean maternal and foetal plasma sodium concentrations were 160 and 152 m-equiv/l. respectively, while the potassium concentrations were 6·0 and 6·7 m-equiv/l. respectively. In foetal urine, the sodium concentration declines (Fig. 6) with foetal age while there is a suggestion of a rise in the potassium concentration.

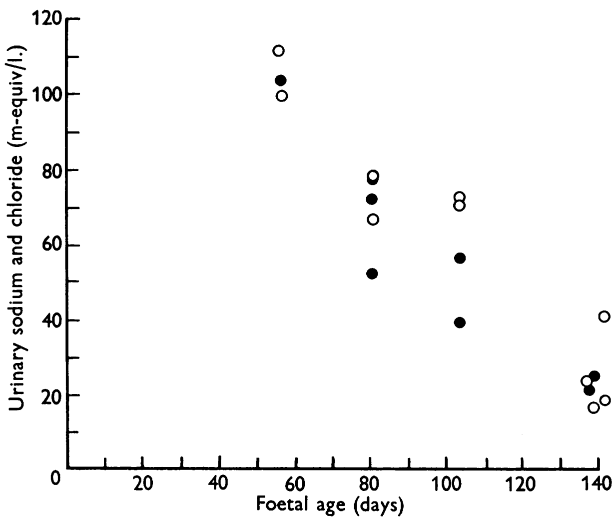

Fig. 6. Correlation of bladder urine sodium and chloride concentrations with foetal age. ○, Sodium; ●, chloride. There is a significant negative correlation of sodium ($r = 0{\cdot}938$, $P < 0{\cdot}001$) and of chloride ($r = 0{\cdot}944$, $P < 0{\cdot}001$) with foetal age between 57 and 142 days.

The chloride concentration present in the foetal bladder urine shows a steep decline as term is approached (Fig. 6).

Alexander, Nixon, Widdas & Wohlzogen (1955, 1958) have shown that the sheep foetus is capable of producing considerable amounts of urine. The introduction of this urine, low in Na and Cl, into the foetal fluids theoretically would result in a progressive reduction in the sodium and chloride concentration of the foetal fluids as gestation proceeds (Fig. 7). It will be seen, however, that the amniotic fluid chloride concentration, initially higher than that in the plasma, falls slowly as term approaches, being below plasma chloride concentration after 100 days, whilst the allantoic chloride concentration falls sharply until it reaches about 6 m-equiv/l. in the region of 90 days and then remains relatively unchanged until term. The sodium concentrations of the foetal fluids do not reflect the changes in the chloride concentrations.

If the amniotic and allantoic fluid chloride concentrations are plotted against urinary chloride concentration, as in Fig. 8, then the values are found to lie on opposite sides of the diagonal line which would represent the expected concentration if either the amniotic or allantoic fluid was merely unchanged urine collected in the respective sacs. The points for amniotic fluid lie above this line and this is consistent with the view that in amniotic fluid urine only represents part of the volume and that there is some other contribution to the formation of this fluid. The fact that the points for allantoic fluid all lie below the line indicates either an addition of water or a removal of chloride. Since

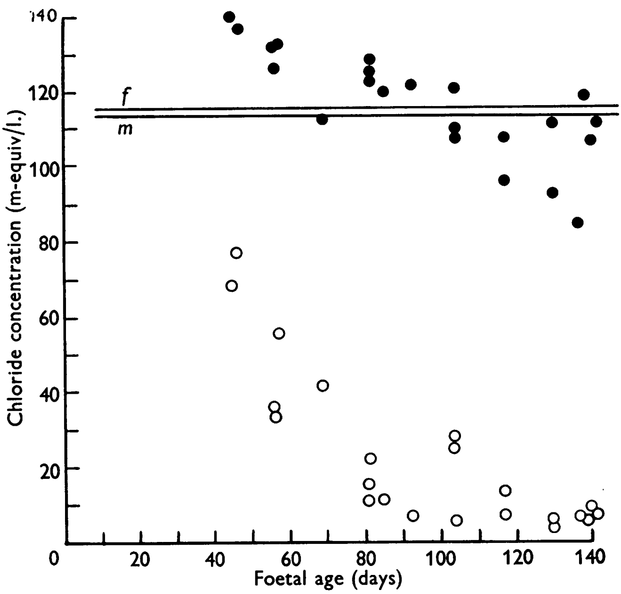

Fig. 7. Decline of chloride concentrations in the foetal fluids with increasing foetal age. ●, Amniotic fluid; ○, allantoic fluid. Chloride concentrations in both fluids show a significant negative correlation with foetal age between 45 and 142 days; amniotic fluid ($r = 0{\cdot}798$, $P < 0{\cdot}001$), allantoic fluid ($r = 0{\cdot}809$, $P < 0{\cdot}001$). *f*, mean foetal plasma chloride; *m*, mean maternal plasma chloride.

the allantoic fluid is hypotonic throughout gestation and osmotic forces would favour a removal of water, the addition of water seems unlikely. The absorption of Cl ions from the allantoic fluid would appear the more likely hypothesis but, as both plasma and amniotic fluids have a higher chloride concentration, any movement of chloride towards those fluids would be against a concentration gradient, and it would be necessary to postulate an active process or a Donnan-type equilibrium.

The foetal plasma phosphate concentrations are slightly above those found in the simultaneous maternal plasma sample. Amniotic fluid phosphorus values are in the main less than those found in the allantoic fluid. When attempting phosphorus determinations in the allantoic fluid, turbidity was encountered, similar to that observed by McDougall (1949).

The sulphate concentration present in the allantoic fluid was found to be considerably higher than that of the amniotic fluid.

The phosphate and sulphate concentrations together with pH values are shown in Table 4.

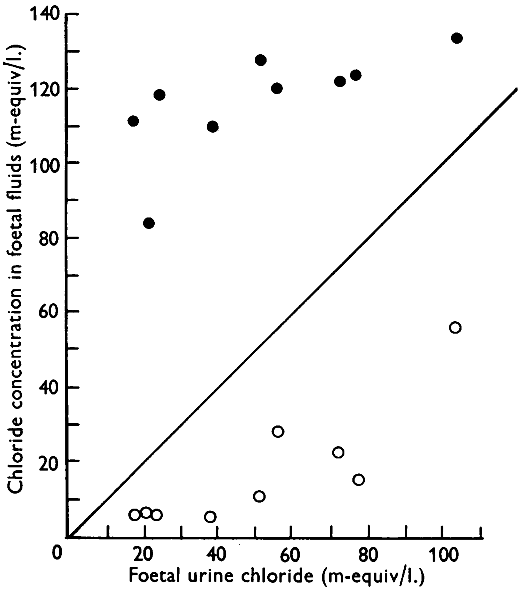

Fig. 8. Relationship of the urinary chloride with that encountered in the foetal fluids. ●, Amniotic fluid; ○, allantoic fluid. The diagonal line illustrates the relationship that would be obtained if the foetal fluids were composed of unaltered foetal urine.

TABLE 4. Total and inorganic phosphorus, sulphate and pH values found in the plasmas, urine and foetal fluids (mg/100 ml., except pH)*

	Maternal plasma	Foetal plasma	Foetal urine	Amniotic fluid	Allantoic fluid
Total P	11·0 (7·9–13·4) *13*	11·7 (9·6–13·9) *14*	4·8 (1·1–11·2) *5*	2·7 (0·6–8·8) *17*	7·3 (1·9–23·8) *16*
Inorganic P	7·8 (4·4–11·3) *8*	7·4 (5·6–8·5) *4*	—	2·3 (1·5–4·1) *9*	—
Inorganic SO_4	—	—	—	3·6 (0·5–8·7) *9*	55·1 (22·9–88·0) *9*
pH	7·36 (7·2–7·57) *4*	7·55 *1*	—	7·37 (6·95–7·52) *6*	6·92 (6·1–7·34) *5*

* Mean (range); no of observations in italics.

The wide range of concentrations of glucose and fructose in the foetal blood and in the foetal fluids was of the same order as that encountered by Barklay, Haas, Huggett, King & Rowley (1949).

An attempt to assess the relative proportions of electrolytes and non-electrolytes in the fluids has been made by considering the contribution of the osmotically more important substances to the total osmolarity. The great variability of composition, particularly in urine samples, makes this assessment subject to considerable error, but the data illustrate the different patterns of plasma and amniotic fluid on the one hand and allantoic fluid and urine on the other.

Thus in results from foetuses in the age range 130–142 days, electrolytes (Na^+, K^+ and Cl^-) contribute on average 77 and 74% to the osmolarity of foetal plasma and amniotic fluid respectively. The corresponding figures for allantoic fluid and urine are 28 and 34%. Calculation shows that in the latter fluids a substantial percentage of the osmolarity must be due to nitrogenous substances other than urea and creatinine.

The sugars may contribute 2–3% of the plasma and amniotic fluid osmolarity, while this contribution may reach 6–10% in the case of allantoic fluid and urine. Substances other than nitrogenous compounds and sugars which have been estimated are only of secondary importance, and about 10–20% of the osmolarity of amniotic and allantoic fluids is not accounted for.

DISCUSSION

Jacqué (1902), in his work on the osmotic pressure of the amniotic and allantoic fluids and a few foetal urine samples, postulated that in the sheep foetus urine passed into the allantoic sac via the urachus up to a gestation age of 90 days. Thereafter the urine passed progressively more and more into the amniotic sac with increasing foetal age, owing to occlusion of the urachus and the patency of the urethra. A further postulate was that because of the longer urethra in the male, the resistance to the passage of urine would be greater in this sex than that in the female at a comparable foetal age, and hence the proportion of urine flowing to the allantoic sac would be greater than that flowing to the amniotic; this, however, is not supported by the present data. The rate of urine formation by the foetal sheep kidney from at least 81 days to term is more than sufficient to account for the volumes of allantoic fluid encountered (Alexander *et al.* 1955, 1958). This ability on the part of the kidney to elaborate a fluid is present at a very early age. Davies (1952) has shown that the mesonephros of the embryo sheep is functionally active in embryos of only 4 mm in length (18 days post-fertilization); this activity is reflected by the appearance of fluid within the allantoic sac having a relatively high fructose concentration, yet at this stage of development little or no amniotic fluid is present. From observations on the pig, McCance & Dickerson (1957) have suggested that secretory processes centred in the allantoic membrane play a part initially in the formation of the allantoic fluid.

The production of a hypotonic urine as a mode of formation of allantoic fluid has interesting physiological consequences. In the early stages of gestation it may help to maintain the osmotic pressure of the foetal plasma at such a value that fluid is not lost to the maternal circulation. The hypotonic fluid, if stored in a sac of sufficient impermeability, could help in producing the general distension of the uterine cavity. That its contents remain hypotonic and reflect the tonicity of foetal urine strongly suggests a relatively small rate of water transfer, or alternatively that the rate of water transfer is balanced by an active removal of electrolytes. The present studies of concentrations suggest that an active removal of Cl^- may occur but so far there is no direct evidence of such a transfer.

As gestation proceeds the increased rate of production of urine and its elimination into a cul-de-sac of the type envisaged would result in the accumulation of a volume of fluid which could not be accommodated in the uterus and would be many times the volume of allantoic fluid actually found. It is clear that either an increased rate of reabsorption of fluid must occur from the allantoic sac or urine must pass into the amniotic sac. The latter alternative does not in itself offer a sufficiently large volume to accommodate the urine produced but the swallowing of amniotic fluid and its passage along the gastro-intestinal tract offers the most probable explanation of an increased rate of reabsorption.

The present study of the relationship of the concentrations in the various fluids coupled with measurements of urine production (Alexander *et al.* 1958) strongly supports Jacqué's view that from about 90 days' gestation age substantial quantities of urine must enter the amniotic sac via the urethra. In all our experiments it has been possible to demonstrate patency of the urethra but the control *in vivo* of sphincters or other factors affecting the relative distribution of fluid between amniotic and allantoic sacs is still unknown.

It is not asserted that foetal urine is the only source of amniotic fluid—all the evidence from the analysis of its composition is that it is more closely related to plasma than urine, particularly in younger foetuses. An admixture of a fluid of plasma-like composition with foetal urine could, however, best explain the findings for amniotic fluid for older foetuses.

Reynolds (1953) has shown that the secretions of the buccal cavity and respiratory tract can amount to 15 ml./hr in the foetal sheep. Thus, these and other foetal secretions may add appreciably to the volume and composition of the amniotic fluid. A further factor involved in the modification of the amniotic fluid composition is presumably brought about by the swallowing movements previously mentioned, which are observed in late foetuses. The stomachs of foetuses are frequently distended with fluid.

The reabsorption of fluid in the gastro-intestinal tract would make possible a maintenance of fluid balance in the rapidly growing foetus and its associated fluids, in which continued urine production was offset by a gastro-intestinal reabsorption, without postulating any significant change in the absorptive activities of the foetal membranes.

SUMMARY

1. The composition of foetal fluids in the sheep has been compared with that of maternal and foetal plasma and urine during the latter half of pregnancy.

2. Foetal urine is hypotonic, but otherwise its composition has typical urinary characteristics. This composition is closely related to that of allantoic fluid and is the probable source of that fluid.

3. Amniotic fluid has osmotic and other characteristics more closely related to those of foetal or maternal plasma but there is evidence of an admixture with foetal urine in the latter part of pregnancy which confirms the observations of Jacqué (1902).

4. Consideration of the volume of urine produced and evidence of its distribution to the amniotic sac suggest that ingestion and absorption of fluid by the gastro-intestinal tract may play a part in the regulation of the volume of foetal fluids in older foetuses.

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