J. Physiol. (1958) 140, 14–22

RENAL FUNCTION IN THE SHEEP FOETUS

BY D. PAULINE ALEXANDER, D. A. NIXON, W. F. WIDDAS*
AND F. X. WOHLZOGEN†

From the Physiology Department, St Mary's Hospital Medical School, London, W.2

(*Received* 12 *June* 1957)

Leonardo da Vinci, at the commencement of the sixteenth century, recorded his observation that the human foetus produces urine *in utero* (quoted by Needham, 1931). More recently, analyses of foetal bladder samples in the sheep (Jacqué, 1902) and the human (Makepeace, Smith, Dailey & Carrol, 1931) have indicated that the foetus produces a hypotonic urine. The foetal rabbit, cat, opossum, chick and pig show glomerular excretion of ferrocyanide and tubular excretion of phenol red by both mesonephros and metanephros (Gersh, 1937), while human foetal concentration of uric acid has been shown by Kinnumen & Paalanen (1952). Wells (1946) and Daly, Wells & Evans (1947) have shown that within 24 hr of term the foetal rat's kidney is capable of responding to a subcutaneous injection of 0·15 ml. of 50% urea by diuresis. They also observed the blood: urine concentration ratios of urea and creatinine. Some quantitative observations on phenol sulphophthalein excretion by the foetal rabbit kidney during the last week of gestation have been made by Williamson & Hiatt (1947).

In none of the above experiments was the rate of urine production measured, and there would appear to be some difficulty in achieving such measurements in acute experiments on the smaller species. In experiments on sheep, however, it was found practicable to measure rates of foetal urine production in acute experiments during the latter half of pregnancy. This has enabled changes in the pattern of urine composition and flow to be investigated and urine analyses have been compared with similar tests carried out concurrently on blood and foetal fluids.

The relation of urine production to the changing composition in the foetal fluids is described in the preceding paper (Alexander, Nixon, Widdas & Wohlzogen, 1958). The present paper is concerned with the evidence which these experiments give regarding the development of function in the foetal sheep kidney between 61 and 142 days gestation age.

* Present address: Physiology Department, King's College, London, W.C. 2.
† Present address: Physiology Department, University of Vienna.

Preliminary reports were given to the Physiological Society (Alexander, Nixon, Widdas & Wohlzogen, 1955) and the XXth International Physiological Congress (Alexander, Nixon, Widdas & Wohlzogen, 1956).

METHODS

The animals used were Welsh mountain ewes of known conceptual age. The age range investigated was from 61 to 142 days gestation age (full term in this breed being about 145 days). Procaine spinal anaesthesia was used, supplemented when necessary by intravenous thiopentone. The ewe was placed in a bath of saline at 38° C. The head and back were at an angle of 45° above the horizontal. The foetus was delivered by Caesarean section (Huggett, 1927), and, with the umbilical circulation maintained intact, retained close to the mother by means of a sling of lint strapped across her body. The foetal bladder was exposed under local anaesthesia by means of a mid-line incision below the umbilicus. The bladder was gently freed from the umbilical vessels running alongside it. After making a small incision in its wall the bladder was cannulated by means of a piece of glass tubing 2 in. long, with a constriction near one end, ligated in position. This glass tubing was attached to polythene tubing which lay along the body of the foetus, only the open end being maintained above the level of the saline. The urine was withdrawn at intervals from the tubing by means of a fine-drawn teat pipette which could pass to the level of the bladder. The volumes of all samples were recorded, together with the time of sampling.

In the initial experiments foetal blood samples were collected by repeated puncture of the umbilical veins. Later a fine polythene catheter was inserted via a tributary into the lumen of one of the two main umbilical veins, through which all the blood samples were obtained.

The methods used in all chemical analyses and determinations have been described in the preceding paper (p. 1).

RESULTS

Rate of urine flow

The average minute volume for each foetus was calculated from the volume of urine collected over timed collection periods within the first hour of the experiment. The flow (0·14 ml./min at 61 days) increased to a maximum (0·64 ml./min) at approximately 117 days, then fell again to 0·14 ml./min at 142 days (Table 1). When considered in relation to foetal weight, the minute

TABLE 1

Sheep no.	Sex	Foetal age (days)	Urine flow (ml./min)
926	F.	61	0·14 (0·1 –0·26)
942	F.	65	0·24 (0·08–0·37)
944	F.	71	0·18 (0·15–0·29)
807	F.	81	0·22 (0·08–0·35)
838	M.	85	0·24 (0·08–0·34)
821	F.	93	0·20 (0·05–0·6)
804	M.	104 A	0·43 (0·2 –0·67)
830	M.	104 B	0·54 (0·42–0·64)
820	M.	117 A	0·64 (0·4 –0·94)
814	M.	117 B	0·58 (0·2 –1·2)
934	F.	123	0·47 (0·17–0·57)
817	F.	130	0·46 (0·1 –0·77)
935	F.	137	0·12 (0·07–1·74)
911	M.	141	0·23 (0·16–0·51)
840	M.	142	0·14 (0·09–0·83)

Figures in parentheses indicate range of urine flow encountered during experiment. Except for sheep of 104 days and 117 days, which are subtitled A and B, all the sheep in this table will be referred to by their ages only in subsequent tables.

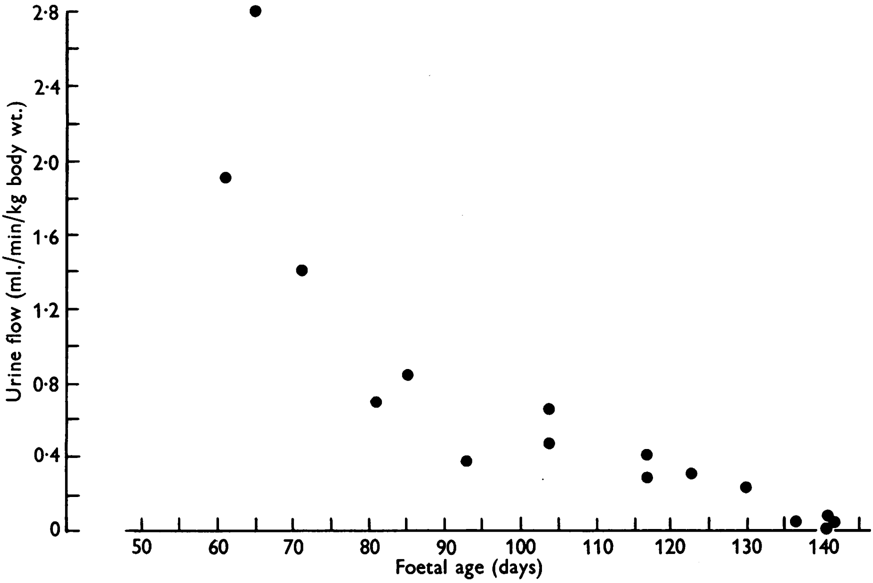

Fig. 1. Variation of urine flow per kg body weight with foetal age.

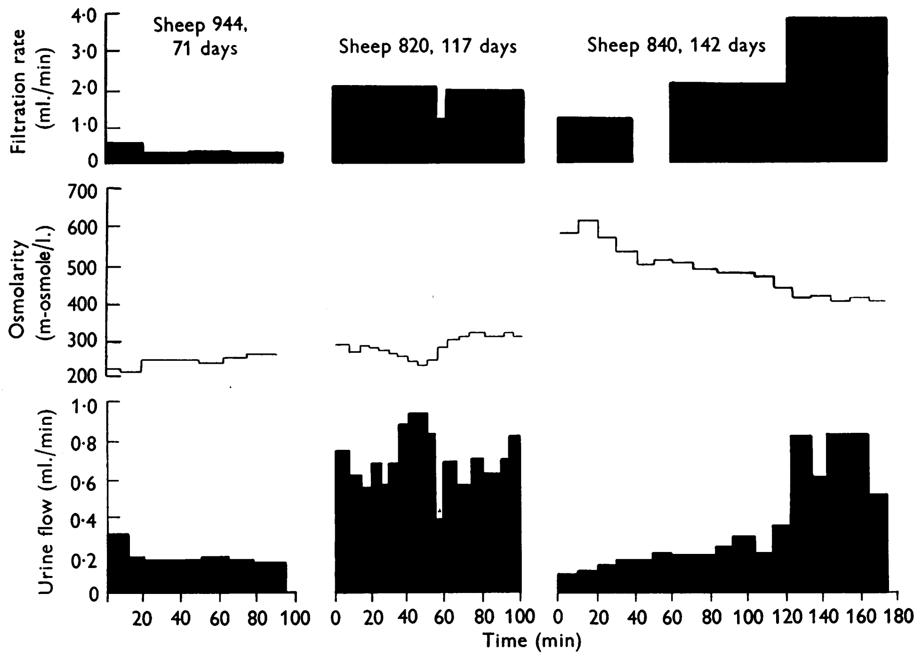

Fig. 2. Protocols of sheep aged 71, 117 and 142 days, showing variation of filtration rate and osmolarity with urine flow. Most values for filtration rate represent pooled samples covering periods of 40 min or more.

volume/kg body weight declined progressively from 1·9 ml./min/kg at 61 days to 0·04 ml./min/kg at 142 days (Fig. 1). The corresponding adult value (calculated from catheter samples on four ewes, three pregnant, one non-pregnant) was 0·02 ml./min/kg body weight.

Range of minute volume in individual foetuses

The urine flows shown in Table 1 represent the average of changing minute volumes: the extreme values encountered in each experiment are shown in brackets. Frequent variations were most marked in the 117-day foetuses, but less in the younger foetuses. The 137- to 142-day foetuses showed the capacity to produce a wide variation in urine flow but also maintained for periods of an hour or more the steadiest urine flows observed. These tendencies are illustrated in the protocols of three experiments in Fig. 2.

Composition of urine in relation to foetal age

The concentration in relation to age of the electrolytes and non-electrolytes mentioned in this paper is given in detail in the preceding paper (Alexander, *et al.* 1958). Three points are relevant to the present paper.

(1) The tonicity of the bladder urine samples described in the preceding paper was always less than that of the foetal plasma. However, during the experiments on the foetuses aged 137–142 days the urine excreted became hypertonic within an hour (about one and a half times the tonicity of the plasma) and maintained this concentration throughout the experiment.

(2) The presence of fructose in sheep foetal plasma and also, in considerable quantity, in the foetal fluids led to an investigation of its excretion by the foetal kidney.

(3) The low plasma concentration of creatinine in all but the near-term foetuses rendered it impracticable to estimate this substance on account of the limitation in volume of the blood samples which could be taken.

In view of the variable rates of urine production at different ages, and in the course of an experiment, it seemed desirable to obtain a measure of renal function by calculating the plasma clearances for various substances.

Foetal plasma clearances

Non-electrolytes. Estimation of the endogenous plasma creatinine, fructose or urea concentrations made it possible to determine the plasma clearance values for these substances by the foetal kidney. In the foetuses aged 137–142 days the plasma clearances of these three substances were very similar. In a number of the younger foetuses the quantity of blood which could be removed was limited and only the urea and fructose determinations were made. In many experiments urine samples had to be pooled to obtain sufficient for analysis. In each experiment two or more clearance values were obtained consecutively. Average values are given in Table 2. These values illustrate two points; first there is similarity of the urea and fructose clearance values in the younger foetuses as well as in the older and secondly the plasma clearance values rise with increase in gestation age.

TABLE 2

Age (days)	Urine flow (ml./min)	Plasma clearance (ml./min)			Mean clearance rate (ml./min/kg)
		Urea	Creatinine	Fructose	
61	0·13	0·15	—	0·21	2·4
65	0·28	0·37	—	0·37	4·3
71	0·15	0·26	—	0·32	2·2
85	0·24	0·64	—	0·66	2·3
104A	0·44	0·97	—	0·8	1·0
117A	0·74	2·0	—	1·9	1·2
117B	0·58	1·1	0·88	0·94	0·5
130	0·36	0·94	1·0	1·2	0·5
137	0·17	1·4	—	1·4	0·5
141	0·28	1·4	—	1·3	0·5
142	0·14	1·3	1·4	1·7	0·4

Shannon (1938) made eight determinations of the inulin clearance and exogenous creatinine clearance in an unanaesthetized adult sheep. His mean value for the inulin clearance was 58·5 ml./min. The creatinine: inulin clearance ratio was 1·03. Plasma inulin clearance estimations performed on a series of ewes (three pregnant and one non-pregnant) under spinal anaesthesia gave values similar to those of Shannon (Alexander, unpublished), and represented a clearance rate of 1·9 ml./min/kg. More recently Parry & Taylor (1955) found that the endogenous creatinine clearance in eight normal adult pregnant sheep was only 80 % of the exogenous creatinine clearance.

In the present series of experiments glomerular filtration rate in the foetus has been taken as the mean of the plasma clearance values of endogenous urea, creatinine and fructose and the percentage of filtered water reabsorbed by the foetal kidney has been calculated by use of this 'glomerular filtration rate' with the result shown in Table 3. Between 61 and 130 days gestation age the kidney absorbs one-third to two-thirds of the filtered water. After 130 days the proportion increases to 80–90 % of that filtered. This increase in the percentage of water reabsorbed coincides with an increase in the osmolarity of the urine collected. All urine samples collected from foetuses younger than 130 days gestation age showed an osmolarity lower (usually about two-thirds) than that of the foetal plasma. In the foetus aged 130 days the urine produced at the beginning and towards the end of the experiment was hypertonic, while all the other urine samples were hypotonic to varying degrees. The three foetuses aged 137–142 days produced hypertonic urine throughout the experiment (except for the initial bladder samples, which were slightly hypotonic in two foetuses). (See Fig. 2.)

Electrolyte excretion. The Na^+, K^+ and Cl^- content of all urine and plasma samples was determined, except for the plasma samples of foetuses aged 61–71 days and 137–141 days. In calculations requiring these latter values the mean of all other foetal plasma analyses was used.

The electrolyte concentrations in the foetal plasma are given in the preceding paper, as also are those of the initial bladder samples. However, it should be noted that in foetuses of the older age group (137–142 days) the urine produced subsequent to the initial bladder sample showed markedly greater electrolyte concentration. This finding suggests that while the foetus is capable of producing hypertonic urine it is not necessarily doing so *in utero*.

TABLE 3. Variation with foetal age in the percentage of water and electrolytes reabsorbed from the plasma filtrate

Foetal age (days)	Percentage reabsorbed			
	K^+	Na^+	Cl^-	H_2O
61	87	64	64	28
65	77	48	53	24
71	80	65	66	52
85	88	81	82	63
93	76	63	73	49
104A	59	77	76	50
104B	62	48	59	44
117A	72	71	71	62
117B	47	56	57	40
130	52	72	64	66
137	74	95	96	91
141	62	82	80	84
142	77	95	95	92

The amount of Na^+, K^+ and Cl filtered and reabsorbed per minute was determined for each foetus, using the 'glomerular filtration rate' as calculated above. The percentage reabsorption of these electrolytes is given in relation to age in Table 3. On the basis of the small number of observations that have been made it appears that Na^+ and Cl^- reabsorption run closely parallel, ranging from approximately 60% reabsorption at 61 days to 90% reabsorption at 142 days. In contrast the K^+ reabsorption, already 80% or more at 61 days, does not appear to increase with increase in foetal age. Comparison of these figures for electrolyte reabsorption with the corresponding values for water reabsorption (Table 3) shows that in the youngest foetuses studied the electrolyte reabsorption is significantly greater than the water reabsorption. This discrepancy accounts for the hypotonic urine observed in the young foetuses.

The foetal plasma clearance of 'total phosphate' was estimated in three foetuses. At 85 days a clearance of 0·23 ml./min was obtained as compared with a chloride clearance of 0·12 ml./min. In one foetus of 104 days the corresponding values were 0·46 ml./min for total phosphate and 0·44 ml./min for chloride, while in the second foetus of 104 days the values were 0·14 ml./min and 0·26 ml./min.

The plasma protein concentration in a number of the foetuses and ewes was calculated from the nitrogen determinations tabulated in the preceding paper. These values, ranging from 2 g/100 ml. at about 60 days to 4 g/100 ml. at 142 days, are in agreement with those obtained by Meschia (1955) for sheep foetuses of the same age range.

DISCUSSION

Davies & Davies (1950) demonstrated histologically that the mesonephros of the sheep has almost completely degenerated by the 100 mm stage. This stage represents 55 days gestation age calculated on the basis of the formula of Huggett & Widdas (1951) relating foetal length to gestation age. Davies (1950) states that his observations of the development of the metanephric arterial blood supply indicate that the metanephros may be functioning by the 40th day of gestation. The data obtained from the present experiments on sheep foetuses between 61 and 142 days of pregnancy are related therefore solely to metanephric function.

The foetal sheep metanephros has been found to produce a higher urine flow per unit body weight than the basal urine flow in the adult ewe. This high urine flow results from the early development of a high filtration rate, coincident with a low capacity for tubular water reabsorption.

In the younger foetuses the filtration rates are greater than the adult resting values when expressed per unit body weight. This high filtration rate in sheep foetuses is the reverse of that expected in the human by Gruenwald & Popper (1940) on the basis of their histological studies. To compare the filtration rates per unit body weight or per unit surface area affords little indication of the efficiency of the individual glomerulus of the foetus for two reasons. In the foetus the percentage of total body weight represented by the kidneys is roughly in inverse proportion to the foetal age. Secondly, the proportion of total kidney tissue represented by the glomeruli is greater in younger than in older foetuses.

As consideration of individual glomeruli is not possible comparison must be made of the over-all efficiency of foetal glomerular filtration with that of the adult. Ideally this should be expressed per unit of body water as suggested by McCance & Widdowson (1952) for the infant. This unit would be especially appropriate in considering development of foetal function where the percentage total body water is altering rapidly with gestation age. However, as total body water is not known in the present series of animals the unit of body weight has been taken as a rough index for comparison.

Calculation of the foetal colloid osmotic pressure on the basis of the plasma protein concentrations shows that the colloid osmotic pressure of the foetal plasma increases with gestation age. At about 60 days the oncotic pressure is estimated at 8 mm Hg. By 141 days this has increased to 18 mm Hg as compared with 25 mm Hg in the adult ewe.

In two of the older foetuses a continuous recording of foetal blood pressure throughout the experiment was made by a cannula passed via a tributary into the lumen of one of the umbilical arteries. The mean blood pressure thus recorded by capacitance manometer was 45 mm Hg. The adult ewe's femoral arterial pressure averaged 123 mm Hg. The difference between the arterial blood pressure and the pressure due to the plasma proteins is smaller in the near-term foetus than in the adult ewe and this may account for the nearly fourfold difference in the corresponding filtration rates.

In the course of each experiment variations in the filtration rate were observed but these were greatest in foetuses near term (see Fig. 2). These variations, which were reflected in the urine flow, indicate some instability in the control of the glomerular filtration pressure which was not reflected in the umbilical blood-pressure recording.

In the present paper foetal renal tubular function has been considered only with respect to the development of water and electrolyte reabsorption. At 65 days, when the filtration rate per unit body weight was the highest measured, the proportion of water reabsorbed is only 25 %. There is a steady increase with foetal age from 65 days to 140 days, when 80 % or more is reabsorbed. During the period 61–130 days sodium and chloride reabsorption is of the order of 60–80 % and the potassium reabsorption slightly higher. From 130 to 140 days sodium and chloride reabsorption rises to approximately 95 % and potassium is of the order 60–80 %. The combined effect of these different rates of water and electrolyte reabsorption results in the formation of hypotonic urine between 61 and 130 days gestation age and subsequently of hypertonic urine between 130 and 142 days gestation age.

Thus, early in development the metanephric tubules are capable of osmotic work in the sense of active electrolyte reabsorption, while it is only late in gestation that they begin to develop the adult capacity for active water reabsorption and production of a hypertonic urine.

SUMMARY

1. The urine production of fourteen sheep foetuses between 61 and 142 days gestation age has been measured.

2. The rate of urine flow was maximal at 117 days. It then declined towards term. Expressed on the basis of flow per unit body weight the urine production of even the most advanced foetus was observed to be in excess of adult resting values.

3. The glomerular filtration rate per unit body weight was greater than the adult value at 61 days, but by 142 days it had decreased to about one quarter of the adult value.

4. Electrolyte (Na^+, K^+, Cl^-) reabsorption occurs in excess of water reabsorption between 61 and 130 days, resulting in the production of hypotonic urine.

5. Tubular water reabsorption does not exceed 66% until 130 days: 80–90% reabsorption occurs between 130 and 142 days, coinciding with the production of hypertonic urine.

We are indebted to Dr D. A. Davey for the blood-pressure determinations by capacitance manometer. This work was aided by A.R.C. and M.R.C. grants to Professor A. St G. Huggett.

REFERENCES

ALEXANDER, D. P., NIXON, D. A., WIDDAS, W. F. & WOHLZOGEN, F. X. (1955). Urine production in the foetal sheep. *J. Physiol.* **130**, 13–14*P*.

ALEXANDER, D. P., NIXON, D. A., WIDDAS, W. F. & WOHLZOGEN, F. X. (1956). Functional development of the foetal kidney in the sheep. *Abstr. XXth int. physiol. Congr., Brussels.*

ALEXANDER, D. P., NIXON, D. A., WIDDAS, W. F. & WOHLZOGEN, F. X. (1958). Gestational variations in the composition of the foetal fluids and foetal urine in the sheep. *J. Physiol.* **140**, 1–13.

DALY, H., WELLS, L. J. & EVANS, G. (1947). Experimental evidence of urine secretion by the foetal kidney. *Proc. Soc. exp. Biol., N.Y.*, **64**, 78–80.

DAVIES, J. (1950). The blood supply of the mesonephros of the sheep. *Proc. zool. Soc. Lond.* **120**, 95–112.

DAVIES, J. & DAVIES, D. V. (1950). The development of the mesonephros of the sheep. *Proc. zool. Soc. Lond.* **120**, 73–93.

GERSH, I. (1937). The correlation of structure and function in the developing mesonephros and metanephros. *Contr. Embryol. Carneg. Instn*, **27**, 33–58.

GRUENWALD, P. & POPPER, H. (1940). The histogenesis and physiology of the renal glomerulus in early post-natal life. *J. Urol.* **43**, 452–458.

HUGGETT, A. ST G. (1927). Foetal blood-gas tensions and gas transfusion through the placenta of the goat. *J. Physiol.* **62**, 373–384.

HUGGETT, A. ST G. & WIDDAS, W. F. (1951). The relationship between mammalian foetal weight and conception age. *J. Physiol.* **114**, 306–317.

JACQUÉ, L. (1902). De la genère des liquides amniotique et allantoidien; cryoscopie et analyses chimiques. *Mém. cour. Acad. R. Belg.* **63**, 3–117.

KINNUMEN, O. & PAALANEN, A. (1952). Fostrets njurfunktion. *Nord. med.* **47**, 19–20.

MCCANCE, R. A. & WIDDOWSON, E. M. (1952). The correct physiological basis on which to compare infant and adult renal function. *Lancet*, **263**, 860–862.

MAKEPEACE, A. W., SMITH, F., DAILEY, M. E. & CARROL, M. P. (1931). The nature of the amniotic fluid. A comparative study of human amniotic fluid and maternal serum. *Surg. Gynec. Obstet.* **53**, 635–644.

MESCHIA, G. (1955). Colloidal osmotic pressures of foetal and maternal plasmas of sheep and goats. *Amer. J. Physiol.* **181**, 1–8.

NEEDHAM, J. (1931). *Chemical Embryology*, vol. **1**, 108. Cambridge University Press.

PARRY, H. B. & TAYLOR, W. H. (1955). Renal clearances of creatinine and *p*-amino-hippurate in normal pregnancy and toxaemia of pregnancy in the sheep. *J. Physiol.* **127**, 54–55*P*.

SHANNON, J. A. (1938). Excretion of inulin, creatinine, xylose and urea in the sheep. *Proc. Scc. exp. Biol., N.Y.*, **37**, 379–380.

WELLS, L. J. (1946). Observations on the secretion of urine by kidneys of foetal rats. *Anat. Rec.* **94**, 504.

WILLIAMSON, R. C. & HIATT, E. P. (1947). Development of renal function in foetal and neonatal rabbits. *Proc. Soc. exp. Biol., N.Y.*, **66**, 554–557.