J. Physiol. (1969), **204**, *pp.* 159–168
With 3 text-figures
Printed in Great Britain

COMPOSITION OF ALVEOLAR LIQUID IN THE FOETAL LAMB

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(*Received* 24 *February* 1969)

SUMMARY

1. Experiments were performed on foetal lambs at gestations between 125 days and term. The foetus was exteriorized at Caesarean section with the umbilical cord and placental attachment maintained intact. Samples of liquid from the alveolar parts of the lung were withdrawn through a tracheal cannula and samples of lung lymph, plasma and amniotic liquid were also obtained. Measurements were made of total osmolality, concentrations of electrolytes and urea, pH and P_{CO_2}. Titrations were carried out with N/10 HCl and N/10 NaOH. The water content of the liquids was estimated and concentrations expressed per kg H_2O.

2. In alveolar liquid $[H^+]$, $[K^+]$ and $[Cl^-]$ were higher and $[Ca^{2+}]$, [phosphates] and $[HCO_3^-]$ were lower than in plasma or lymph. In amniotic liquid osmolality $[Na^+]$, $[Cl^-]$ and $[Ca^{2+}]$ were lower and [phosphates] higher than in plasma or lymph. Alveolar liquid/plasma ratios of $[HCO_3^-]$, $[Ca^{2+}]$, $[Cl^-]$ and $[K^+]$ differed from ultra filtrate/plasma ratios of these ions.

3. Titration curves demonstrated a very small amount of buffering in alveolar liquid at its *in vivo* pH of 6·27 mostly due to HCO_3^- at an average concentration of 2·8 mM/kg H_2O.

4. It is concluded that foetal alveolar liquid is not an ultrafiltrate of plasma nor a mixture of amniotic liquid and plasma ultrafiltrate, but a special material elaborated by the foetal lung.

INTRODUCTION

The lungs of foetal mammals including humans are filled with liquid (Addison & Howe, 1913; Potter & Bohlender, 1941; Adams, Moss & Fagan, 1963). In the lamb at term the volume which can be aspirated through the trachea is about 25 ml./kg body wt. (Humphreys, Normand, Reynolds & Strang, 1967). Since ligation of the trachea in the rabbit (Jost & Policard,

1948; Carmel, Friedman & Adams, 1965) or congenital bronchial atresia in humans (Potter & Bohlender, 1941) leads to overdistension of the foetal lungs, it is very likely that the liquid is formed *in situ*.

Alveolar liquid has a low concentration of protein (0·03 g/100 ml.; Boston, Humphreys, Normand, Reynolds & Strang, 1968) which suggests that it could be formed by ultrafiltration from lung capillaries. But Adams *et al.* (1963) reported that the liquid differs strikingly from plasma in its low total CO_2 content and pH. The measurements reported in this present paper were made to establish how much the concentrations of substances, and the buffering in alveolar liquid differ from plasma and from lung lymph, which we have taken to represent interstitial fluid. We have also compared alveolar and amniotic liquids. These measurements suggest that alveolar liquid is a special material elaborated by the foetal lung, and neither a simple ultrafiltrate nor a mixture of amniotic liquid and ultrafiltrate.

METHODS

Experimental procedure

Experiments were performed on forty exteriorized foetal lambs with gestational ages between 115 days and term (mean weight 3·9 kg, range 1·87–5·7 kg), many of which were used also for other experiments. In thirty-one experiments anaesthesia in the ewes was induced with thiopentone and maintained with pentobarbitone as previously reported (Humphreys *et al.* 1967); but in nine experiments halothane was given to the ewe by inhalation instead of pentobarbitone. A Caesarean section was performed, a sample of amniotic liquid taken anaerobically into a syringe, and the foetus delivered onto a heated table with the umbilical cord and placental attachment intact. The foetuses were shown to be in good condition by monitoring $P_{a.O_2}$ $P_{a.CO_2}$ and pH_a in carotid artery blood, with results similar to values previously published (Humphreys *et al.* 1967). The thoracic duct component of lung lymph was collected as described by Humphreys *et al.* (1967); and samples of heparinized carotid artery blood were collected anaerobically; alveolar liquid was sampled by cannulating the trachea, clearing the dead space by withdrawing and discarding between 10 and 30 ml. liquid, and gently aspirating the remainder anaerobically into a syringe.

Analysis of liquids

Acid-base measurements. Total CO_2 was measured in uncentrifuged anaerobically collected samples of alveolar liquid by the Natelson modification (1951) of the Van Slyke method (1917). This had an S.D. of $\pm 0{\cdot}23$ mM/l. for five repeated measurements on the same sample. The much higher concentrations of total CO_2 in lymph, plasma and amniotic liquid were measured, after equilibration with 5% CO_2, in an Autoanalyser (Technicon) by the method given in the Technicon Handbook (file N-21a). (S.D. of repeated measurements < 1 mM/l.). $[HCO_3^-]$ was calculated by subtracting dissolved CO_2 plus H_2CO_3, estimated as $0{\cdot}03 \times P_{CO_2}$ (Van Slyke, Sendroy, Hastings & Neill, 1928). Measurements of P_{CO_2} by the method of Severinghaus & Bradley (1958) and of pH using a capillary glass electrode were made in samples of alveolar liquid, amniotic liquid and whole blood collected anaerobically by syringe; the pH of lymph was measured in samples collected under oil. Titration curves were obtained by titration in room air with N/10 HCl and N/10 NaOH.

Measurement of electrolyte and urea concentrations. The measurements were made on samples of alveolar and amniotic liquid, centrifuged at 30,000 *g* and 0° C for 10 min. On centrifugation amniotic liquid, pale yellow and faintly opalescent on collection, retained its colour and some opalescence. Alveolar liquid, colourless but more opalescent on collection, became crystal clear on centrifugation. Measurements were also made on six samples of uncentrifuged alveolar liquid and showed no important difference from results after centrifugation. Concentrations of Na^+, K^+ Cl^-, urea and phosphates (as P) were made using an Autoanalyser (Technicon) by methods given in the Technicon Handbook (files N-21a, N-4b and N-1c) and Ca^{2+} by the method of Halse (1968). The coefficients of variation of repeated measurements were 1% or less. Osmolality was measured by freezing point depression (Advanced Instruments osmometer).

Concentrations are expressed per kg H_2O. For this purpose average water concentrations of 956 g/l. for plasma and 960 g/l. for lymph were obtained from the equation of Peters (1953) relating serum protein and H_2O concentrations. These estimates agreed to less than 0·2% with measurements made by evaporating to dryness. For alveolar liquid and amniotic liquid, water concentrations of 990 and 986 g/l. respectively were obtained by evaporating to dryness; these compare with Davson's (1956) value of 990 g/l. for c.s.f. which has a similar protein concentration to alveolar liquid.

RESULTS

Concentration of substances

Table 1 gives a mean values for osmolality, electrolyte and urea concentrations and for pH and P_{CO_2} in alveolar liquid, amniotic liquid, lung lymph and plasma. No differences related to gestational age were observed. No differences in concentration were observed depending on how much liquid was aspirated from the trachea before taking a sample; and in one particular experiment in a pair of samples, one taken after withdrawing 3 ml. and the other after 30 ml., $[Na^+]$, $[K^+]$, $[Cl^-]$ and $[HCO_3^-]$ differed by less than 1 mM/kg H_2O and pH by 0·02. The Table includes values for protein concentration from Boston *et al.* (1968) for alveolar liquid, and from Humphreys *et al.* (1967) for lung lymph and plasma.

Alveolar liquid and plasma. In alveolar liquid, [protein], $[HCO_3^-]$, $[Ca^{2+}]$ and [phosphate] are significantly lower than in plasma while $[Cl^-]$, $[K^+]$ and $[H^+]$ are significantly higher (P of the above differences $< 0{\cdot}001$ except for [K] where $P < 0{\cdot}05$). In plasma the sum of measured anions (excluding phosphates) is 25 m-equiv/kg H_2O less than the sum of cations; the difference is presumably made up by anionic protein, organic acids and phosphates (Gamble, 1954). In alveolar liquid the sums of anions and cations are similar (anions 2·6 m-equiv/kg H_2O greater than cations). Thus Table 1 probably includes all the quantitatively important anions in alveolar liquid.

Figure 1 compares ultrafiltrate of plasma/plasma ratios ($R_{uf/p}$) given by Davson (1956) with alveolar liquid/plasma ratios ($R_{al/p}$) from measurements on simultaneously collected pairs of samples from seven animals.

TABLE 1. Mean values (± S.E.) for concentration of electrolytes, urea, protein, osmolality and pH and P_{CO_2} in alveolar liquid, plasma, lymph and amniotic liquid. Values are in mM/kg H_2O except where stated

Sample		Na^+	K^+	Ca^{2+}	Cl^-	Phosphates as P	HCO_3^-	P_{CO_2} (mm Hg)	pH (units)	Urea	Osmolality	Protein (g/100 ml.)
Alveolar liquid	$\bar{x}$	150	6·3	0·8	157	< 0·02	2·8	40	6·27	7·9	294	0·027*
	S.E.	1·3	0·7	0·09	4·1	—	0·3	3	0·05	2·7	2	0·002
	n	8	8	7	7	7	9	9	7	8	13	6
Plasma	$\bar{x}$	150	4·8	3·3	107	2·3	24	43	7·34	8·2	291	4·09†
	S.E.	0·7	0·2	0·08	1·3	0·17	1·2	4	0·04	1·4	2	0·26
	n	11	11	7	11	7	10	9	6	7	16	6
Lymph	$\bar{x}$	147	4·8	—	107	—	25	—	7·31	—	—	3·27†
	S.E.	0·6	0·5	—	0·9	—	0·8	—	0·02	—	—	0·41
	n	3	3	—	3	—	4	—	5	—	—	6
Amniotic liquid	$\bar{x}$	113	7·6	1·6	87	3·2	19	54	7·02	10·5	265	0·10
	S.E.	6·5	0·8	0·05	5·0	—	3	7	0·09	2·4	2	0·01
	n	4	3	3	4	2	3	7	5	3	17	3

* Values from Boston *et al.* 1968. † Values from Humphreys *et al.* 1967.

The $R_{al/P}$ values for $[HCO_3^-]$ and $[Ca^{2+}]$ are substantially lower, and for $[Cl^-]$ and $[K^+]$ are higher than the corresponding $R_{uf/P}$ values, and from the S.E.S given by Davson (1956) the differences appear to be significant.

Lymph. There is a lower protein concentration in lymph than in plasma. There were no significant differences between lymph and plasma in the concentrations of the other substances measured in paired samples.

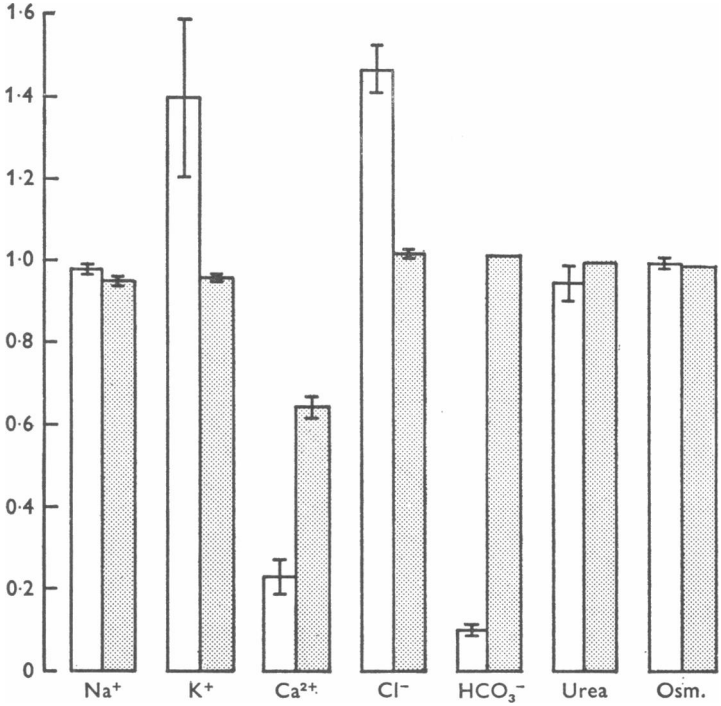

Fig. 1. Mean ultrafiltrate/plasma ($R_{uf/P}$, filled columns) and alveolar liquid/plasma ($R_{al/P}$, open columns) ratios of concentration (mM/kg H_2O) with ± S.E. shown where available as vertical bar. $R_{uf/P}$ ratios from Davson (1956); $R_{al/P}$ ratios from simultaneously collected pairs of samples in seven foetal lambs.

Amniotic liquid. In amniotic liquid $[Na^+]$, $[Cl^-]$ and osmolality are lower ($P < 0{\cdot}001$) and $[HCO_3^-]$, $[Ca^{2+}]$, [phosphate] and pH higher ($P < 0{\cdot}001$) than in alveolar liquid.

Titration curves

Figure 2 shows typical titration curves between pH 4 and pH 10 for alveolar liquid, amniotic liquid, lymph, water and a solution of $NaHCO_3$ (4 mM/l.). The slope of the titration curve for alveolar liquid is much less steep than for lymph or amniotic liquid and indicates a small amount of

buffering in this liquid. The actual position of the titration curve for lung liquid on the ordinate depended on the P_{CO_2} at the start of the titration, which was not controlled, but differences in P_{CO_2} do not affect the slopes of the curve. Figure 3 gives buffer values (mM[H^+] per litre and unit pH change) obtained from the slopes of the curves in Fig. 2 (Van Slyke, 1922).

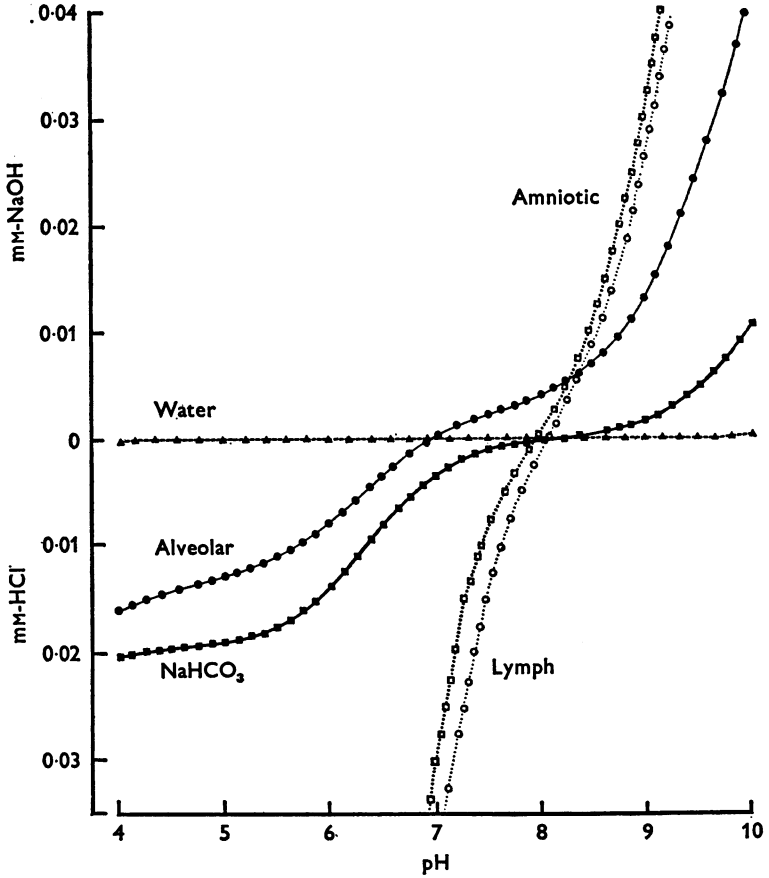

Fig. 2. Titration curves of 5 ml. alveolar liquid (●), amniotic liquid (□), lung lymph (○), water (▲), and $NaHCO_3$ solution, 4 mM/l. (■). Ordinate: mM-HCl and mM-NaOH added. Abscissa: pH.

In a solution of a single buffer, pK values are given by points of maximum slope, and at a pK,

buffer concentration (mM/l.) × 0·575
= buffer value (mM[H^+]$l.^{-1}$ pH^{-1}) (Van Slyke, 1922).

This relationship is illustrated in Fig. 3 where the 4 mM/l. solution of $NaHCO_3$ has a buffer value of 2·3 at its pK. The $NaHCO_3$ solution gave a

standard buffer curve with an expected pK at 6·37. The curve for alveolar liquid is similar to that for $NaHCO_3$ solution but differs slightly in shape from the standard curve, indicating the presence of small amounts of additional buffer in the low pH range and relatively large amounts in the upper part of the range. The buffering additional to bicarbonate in the low pH range is likely to be due to intermediary metabolites (e.g. lactic acid and Krebs cycle acids) with pK values below 6, and in the upper pH range

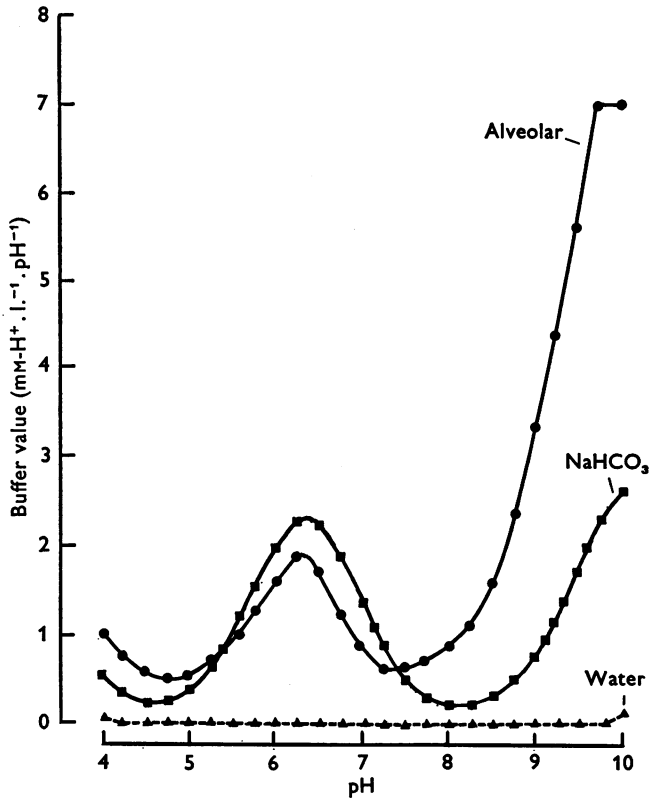

Fig. 3. Buffer values obtained from the slopes of curves shown in Fig. 2 of alveolar liquid (●), water (▲) and $NaHCO_3$ (4 mM/l.) (■). Ordinate: buffer value mM $H^+.l.^{-1}.pH^{-1}$. Abscissa: pH.

to amino acids with pK values mostly between 8 and 12. The closeness of the peak in buffer value of alveolar liquid to the pK of aqueous HCO_3^- solution suggests that most of the buffering in alveolar liquid at this pH is due to its HCO_3^- content. The mean $[HCO_3^-]$ in alveolar liquids where titration curves were performed was 2·7 (± 0·4 S.E.) mM/kg H_2O. At the pK of HCO_3^- this would give a mean buffer value of 1·5 (± 0·2) mM $H^+.l.^{-1}.pH^{-1}$, which compares with a measured mean buffer value of

2·2 ($\pm$ 0·2) mM.H^+.l.$^{-1}$.pH^{-1} at the pK of HCO_3^-. Thus in alveolar liquid at its *in vivo* pH there is a small amount of buffering (average buffer value 0·7) in addition to HCO_3^-.

DISCUSSION

Electrolyte composition of alveolar liquid. The results given in Table 1 show that alveolar liquid in the foetal lamb has an electrolyte composition which is quite different from either amniotic liquid or carotid artery plasma, which in the foetus is likely to differ only slightly from pulmonary artery plasma due to a contribution which has passed through the lungs. The mixing together of amniotic with a plasma filtrate formed in the lungs could not account for the composition of alveolar liquid, because [Ca^{2+}], [phosphate] and [HCO_3^-] are lower and [Cl^-] and [H^+] higher in alveolar liquid than in either of the other two liquids.

By expressing the measured concentrations in alveolar liquid per kg H_2O and comparing them with Davson's (1956) values for plasma ultrafiltrate, we have shown that the differences in ionic concentrations of alveolar liquid from plasma are not simply imposed by the Donnan equilibrium as a result of the difference in protein concentration. Indeed it seems likely that alveolar liquid is a special material actively elaborated by the foetal lung in contrast to lung lymph, which has an electrolyte composition similar to plasma, and which has been shown by Boyd, Hill, Humphreys, Normand, Reynolds & Strang (1969) to have the macromolecular composition expected from the filtration of plasma through porous capillary walls.

The present experiments provide no evidence as to the part of lung in which the liquid is formed. Since no variations in composition were found depending on how much liquid was aspirated before taking the sample, the whole cellular lining of the lung must be capable of maintaining the observed electrolyte concentration differences from plasma. Plainly the largest area across which the concentration gradients are maintained consists of alveolar walls. Electron micrographs of lung, such as those published by Weibel (1963), show distinct capillary endothelial and alveolar epithelial cell layers. Schneeberger–Keeley & Karnowsky (1968) have shown that, while there are pores or clefts between pulmonary capillary endothelial cells, the junctions between alveolar cells appear to be tight. The low protein concentration and special electrolyte composition of alveolar liquid would appear to depend on the properties of the alveolar cell layer.

Acid base composition of alveolar liquid. The titration curves were performed in air at an effectively zero P_{CO_2}, but since the liquid was not thoroughly equilibrated with air before starting the titration, variable

amounts of dissolved CO_2 would be present in the liquid, which would affect the pH at the time of starting the titration and hence the position of the titration curve on the ordinate. But the slope of the curve at any pH, and hence the estimate of buffering, would be unaffected by these differences in $[CO_2]$, which were therefore considered unimportant.

The titration curves show that most of the effective buffering in alveolar liquid at its *in vivo* pH is provided by its small concentration of HCO_3^-. The pH of 6·27 can be explained by equilibration of this poorly buffered liquid with a P_{CO_2} of 40 mm Hg. The low $[HCO_3^-]$ could be due either to the active exclusion of this substance or to the active addition of a strong acid, which would lower $[HCO_3^-]$ by releasing CO_2 with its diffusion into plasma. If the active addition of acid to alveolar liquid is the reason for its low $[HCO_3^-]$, the acid is probably HCl as any other would be represented as a deficit in measured anions compared with cations.

The foetal lung is derived from foregut (Hamilton, Boyd & Mossman, 1962) and contains carbonic anhydrase (Berfemstam, 1952). It is possible that alveolar liquid is secreted by a process similar to HCl secretion in the stomach, which has been shown to depend on transfer of H^+ from H_2CO_3, and to require carbonic anhydrase (Davies, 1948).

This work was supported by a grant from the Medical Research Council in laboratories constructed with aid of a grant from the Wellcome Trust.

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