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Ionic control of the rupture of fetal membranes

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Preterm premature rupture of the chorioamniotic membrane frequently leads to preterm birth and perinatal mortality. To ascertain whether the rupture of these membranes is influenced by variations in environmental pH and sodium concentration, we punctured 180 specimens from 9 membranes that were equilibrated in solutions of different pH, and 196 specimens from 10 membranes that were equilibrated in solutions with different sodium concentrations. Whole-membrane mechanical characteristics—strength, stiffness, toughness and ductility—were measured. These characteristics were defined based on a variant of the weakest-link theory: from a pool of three to five specimens, the specimen that had the lowest values of strength and concomitant values of stiffness, toughness, and ductility represents the mechanical characteristics of the entire membrane section. Strength-related mechanical characteristics—strength, stiffness and toughness—correlated negatively with pH ($p < 0.001$, $p < 0.001$, and $p < 0.02$, respectively), while ductility did not correlate significantly with pH. Membrane hydration and thickness correlated positively with pH ($p < 0.001$). The greatest increase in hydration accompanied by the greatest drop in strength, stiffness and toughness was observed between pH values of 3.68 and 5.58, suggesting that insufficient quantities of vaginal H^+ could cause—as well as mark—premature rupture of the chorioamniotic membrane. No whole—membrane mechanical characteristics correlated significantly with changes in sodium concentration. Membrane hydration and thickness had slight positive correlations with sodium concentration ($p < 0.05$). Changes solely in the sodium concentration of the amniotic fluid during normal pregnancy are not sufficient to facilitate rupture at term.

1. Introduction

The discovery of a marker and a cause of the premature rupture of the chorioamniotic membrane is vital to the development and implementation of a treatment for this threat to the fetus. In 1989, Gleeson *et al.* [1] and Ernest *et al.* [2] established independently that vaginal pH can serve as a marker of an increased risk of premature rupture. Whether pH could actually affect the strength of the chorioamniotic membrane was not investigated. In an effort to describe biochemical changes with the progression of pregnancy, several investigators have researched and described variations in amniotic concentrations of sodium, chloride, potassium, and calcium among other components of the amniotic fluid [3–9]. Their findings were primarily used to predict gestational age on the basis of biochemical changes; but the possibility that the strength of the chorioamniotic membrane could be affected by these changes was not considered.

Recently, we proposed that the mechanical characteristics of the fetal membranes are influenced by hydration, which is affected by the thickness of the membrane [10]. Hydration would reduce the mechanical characteristics of the membrane by separating structural fibres and allowing them to move past one another easily, as textile fibres do in loose but not in tight weaves. The degree of separation depends on the strength of the electrostatic repulsion between polyanionic mucopolysaccharides that surround collagen fibres [11, 12]. Consequently, the ionic environment should control the mechanical characteristics of the membrane, by affecting the strength of the electrostatic repulsion, and thus, hydration.

In this experiment, we investigated the effects of environmental pH and sodium concentrations on the mechanical characteristics of the chorioamniotic membrane by puncturing specimens that had been equilibrated in solutions containing different concentrations of H^+ or Na^+. Although the natural environment of the chorioamniotic membrane was not duplicated, we included as many factors as were required to investigate the effect of the ionic environment on the mechanical characteristics of the membrane. Mean values of five mechanical characteristics—strength, secant stiffness, tangent stiffness, toughness and ductility—were measured from force versus deflection traces that were recorded during puncture [13]. Then, the mean value of each whole-membrane mechanical characteristic was plotted versus pH or sodium concentration. The validity of the hypothesis that the ionic environment controls the mechanical characteristics was assessed on the basis of regression lines that fit these data best. These analyses suggest that thc absence of normal quantities of H^+ in the vagina could induce premature rupture of the fetal membranes and that the reduction in the sodium concentration of the amniotic fluid with the progression of gestation is not, alone, sufficient to weaken the chorioamniotic membrane as term approaches.

2. Materials and methods

2.1. Solution compositions

To investigate the effect of changes in environmental pH, five solutions were made that spanned the entire range from above the pH of blood to below that of the vagina. The pH of each solution was buffered with citric acid and dibasic sodium phosphate to values of nominally 7.5, 6.5, 5.5, 4.5, and 3.5. The citric acid/sodium phosphate combination was used, because it allowed the control of pH across a very broad range (from pH 8.0 to pH 2.2) without introducing additional buffers into the solution [14]. The actual pH measurements that were used in regression analyses were taken after sections of tissue were equilibrated in respective solutions (Table I). To avoid the exaggeration of the effect of pH, all other ions were maintained at average levels found in the amniotic fluid: $[Na^+]$, $[Cl^-]$, $[K^+]$, $[Ca^+]$, and $[Mg^+]$ were constant at 132, 102, 4.0, 4.0 and 1.3 mmol/l, respectively [3–9].

The effect of changes in the sodium concentration of the amniotic fluid was studied with five solutions in which both $[Na^+]$ and $[Cl^-]$ were varied between physiological values that were within one standard deviation of the means reported by Anderson and Weber [9] (Table II). Chloride concentration was varied *as well as* sodium concentration, because *both* concentrations decrease during gestation, though the decrease in sodium concentration is considerably greater. The decrease in cation concentration could be modelled with a smaller decrease in $[Na^+]$ and constant $[Cl^-]$, but the latter model would not correspond to physiological conditions as accurately as the former. Nominal $[Na^+]/[Cl^-]$ values of 145/114, 135/107, 125/100, 115/93, and 105/86 mmol/l were chosen. The $[Na^+]$ of the stock solutions was checked with an IL943 Automatic Flame Photometer (Allied Instrumentation Laboratory, Inc., Lexington, MA). While $[K^+]$, $[Ca^+]$, and $[Mg^+]$ were constant at 4.0, 4.0, and 1.3 mmol/l, respectively, the pH of each solution was set to 7.4 but was reduced to 7.2 through the addition of tissue sections.

Note that the overlap of pH and $[Na^+]$ and $[Cl^-]$ (cf. Tables I and II) allows one to verify the representativity of both samples, via the similarity of hydration, thickness, and mechanical characteristic values.

TABLE I Components of solutions in which pH was varied[a]

pH	$[Na^+]$[b] mmol/l	$[Cl^-]$[b] mmol/l
7.27	132	102
6.45	132	102
5.58	132	102
4.62	132	102
3.68	132	102

[a] Nominal values of $[K^+]$, $[Ca^+]$, and $[Mg^+]$ were 4.0, 4.0, and 1.3 mmol/L, respectively.
[b] Nominal values based on the weights of salts added.

TABLE II Components of solutions in which $[Na^+]$ and $[Cl^-]$ were varied[a,b]

pH	$[Na^+]$ mmol/l	$[Cl^-]$[c] mmol/l
7.2	141.9	114
7.2	132.8	107
7.2	128.6	100
7.2	113.8	93
7.2	104.4	86

[a] Nominal values of $[K^+]$, $[Ca^+]$, and $[Mg^+]$ were 4.0, 4.0, and 1.3 mmol/l, respectively.
[b] Although the effect of varying sodium concentration was investigated, the chloride concentration was varied as well to model physiological conditions as closely as possible. That is, the chloride concentration also decreases during pregnancy, but not as much as sodium concentration. Thus, results would be exaggerated, if $[Na^+]$ were varied alone.
[c] Nominal values based on the weights of salts added.

2.2. Tissue preparation

For the pH and sodium experiments, nine and ten refrigerated afterbirths of healthy patients who had undergone labour at term. respectively, were taken from Labour and Delivery at North Carolina Memorial Hospital within 36 h after delivery. Since previous experiments showed that the mechanical characteristics of the chorioamniotic membrane do not change significantly when refrigerated for 72 h [10], a maximum refrigeration time of 36 h was deemed unlikely to affect the results of this experiment.

The chorioamniotic membrane was separated from the placenta and cut into five sections that included material ranging from the placental margin to the edge of the rupture site. Each section was placed into a petri dish that contained one of the solutions, covered to prevent evaporation of the solution, and allowed to equilibrate at 25°C for at least 2 h.

After equilibration, the pH of each solution was measured with a Digital pH Meter 107 connected to a gel-filled combination electrode (Fisher Scientific, Philadelphia, PA). The sections were removed from the dishes, and excess liquid was dabbed off of each section. Three to five Tygon tubing rings (ID = 15.88 mm, OD = 20.64 mm, wall thickness = 2.38 mm and height = 7 mm) (Norton Co., Franklin Park, MA, USA) were glued to each section with Loctite 447 cement (Loctite Corp., Newington, CT, USA) as described in a previous experiment [13]. The quantity of tissue available dictated the number of rings that would fit on each section. Membrane specimens that were now affixed to rings were cut apart, and each specimen's thickness was measured. The remaining tissue was placed over a loose aluminum mesh in a weighing boat, weighed, dehydrated in a vacuum for 2 days, and weighed again to determine percentage hydration. The weight of tissue specimens reached a steady state after 2 days in vacuum, as was verified by the lack of further change in specimens that were allowed to dry for 4 days.

2.3. Thickness measurements

Thickness was measured three times on each specimen with a Sony μ-Mate digital micrometer (Sony, Tokyo, Japan) that was connected to an LCD Digital Multimeter (Micronta, Fort Worth, TX) [10, 13]. A sudden drop in electrical resistance, as shown by the multimeter, indicated that both the anvil and the spindle of the micrometer were in contact with the membrane specimen and that thickness could now be measured.

2.4. Mechanical characteristic measurements

As in previous work [13], membrane specimens were punctured by forcing a bullet-shaped probe through specimens that were positioned so that the amnion would contact the probe. The probe diameter (3.18 mm) was equal to one-fifth the specimen diameter (15.88 mm). A model TTCM Instron testing machine (Instron, Canton, MA, USA) was used to record values of force and deflection during puncture. Force versus deflection traces were then used to measure the strength, secant and tangent stiffness, toughness, and ductility of the membrane specimens.* These *whole-membrane mechanical characteristics* of each biaxially stressed specimen represent the force to fracture (N), force per unit deflection (N/m), work to fracture (J), and deflection to fracture (m), respectively. Because the chorion and amnion frequently fail separately [13], the force versus deflection traces can include two peaks (Fig. 1). The first peak is produced by both membranes acting together and is represented in measurements of first strength, secant and tangent stiffnesses, minimum toughness and minimum ductility. The amnion usually breaks first, at a greater force and smaller deflection than the chorion. The chorion is generally represented by second strength, maximum toughness and maximum ductility. Toughness and ductility are cumulative because only one layer of the chorioamniotic membrane has failed, and energy and deflection are still required to complete puncture. Specimens in which the amnion and chorion fail simultaneously have equal values for first and second strengths, minimum and maximum toughnesses, and minimum and maximum ductilities.

In order to establish how each membrane would behave at each ion concentration, the whole-membrane mechanical characteristics were determined for each membrane section based on a variant of the *weakest-link theory* [17, 18]. The ramifications of this theory are that mechanical characteristics of the whole membrane section are defined by the specimen with the lowest values of strength and concomitant values of stiffness, toughness, and ductility, because this specimen's failure would compromise the entire membrane section. A uniaxial analogue is a length of chain that is only as strong as its weakest link.

Figure 1 Identification of mechanical characteristics on a schematic force versus deflection curve. First and second strengths are the force to fracture values measured at number (1) and (2), respectively. Secant stiffness is represented by the dotted line, while the dashed line represents tangent stiffness. Minimum toughness (///) corresponds to the area under the curve of the first peak, and maximum toughness is the sum of both the area under the first peak (///) and the area under the second peak (XXX). Minimum and maximum ductilities are the deflection values measured at (3) and (4).

2.5. Data analysis

Values of hydration, thickness, and each whole-membrane mechanical characteristic were averaged and plotted versus the pH or the sodium concentration of the specimens. Correlation coefficients (r) were calculated for the regression lines that fit best through the values of the weakest specimens of each membrane section. These lines were then superimposed over the mean of the minimum values to facilitate visual interpretation (without the clutter of 45–200 data points). The statistical significance of the correlation between ion concentration and hydration, thickness, and whole-membrane mechanical characteristics was ascertained via the probabilities (p-values), which were obtained by using the r-value and the sample size (n) [19].

3. Results

3.1. Appearance of whole-membrane traces

The shapes of force versus deflection traces generally corresponded to that of the trace in Fig. 1, except when the amnion and chorion fractured simultaneously and the trace included only one peak. Differences in mean values of strength and ductility are indicative of differences in the magnitude of traces. The shapes of curves were similar regardless of ion concentration. Changes in stiffness reflect differences in the quotient of [strength/ductility], whereas changes in toughness reflect differences in the product [strength × ductility].

3.2. Effect of pH

Hydration and thickness increased with an increase in pH (Fig. 2), as is shown by mean values and standard

* With regard to *whole membrane mechanical characteristics* (cf. Fig. 1 and [15, 16]), "strength" is the force that resists penetration, while "ductility" is the corresponding deflection of the membrane. "Stiffness" is the quotient of strength and ductility, or (for purely elastic materials) the slope of the first portion of the force versus deflection trace. The *secant* "stiffness" refers to the chord between the first contact and the initial fracture, and the *tangent* "stiffness" refers to the collinear line that can be drawn just below the point of initial fracture. "Toughness" is the product of the strength and ductility, or equivalently, the area under the force versus deflection trace.

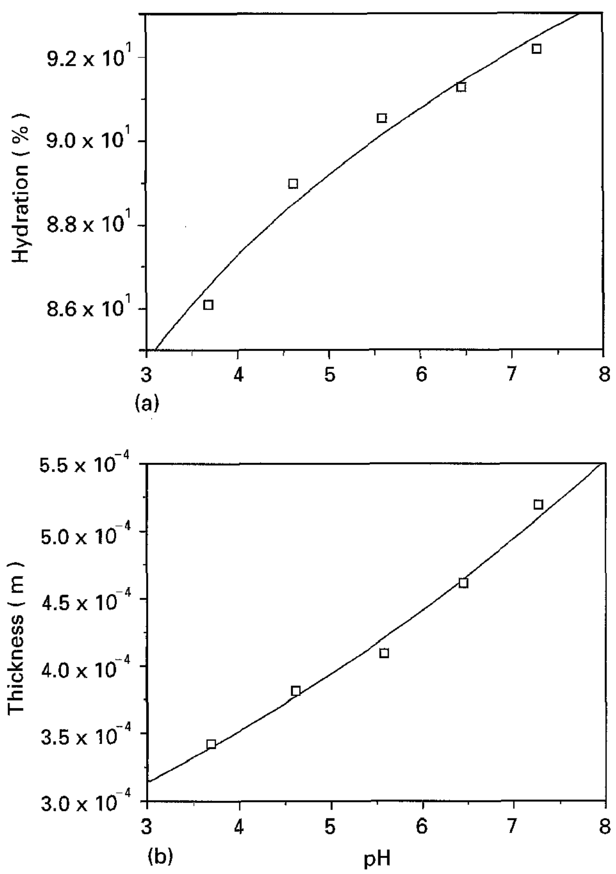

Figure 2 Behaviour of hydration (a) and thickness (b) with change in pH (cf. Table IV for slopes and statistics).

deviations, the latter expressed both in absolute terms (SD) and as a percentage of the mean (%) (Table III). The equation describing the increase of hydration with an increase in pH was logarithmic, while the increase in thickness was best described by an exponential equation (Table IV). Mean values of all whole-membrane mechanical characteristics other than ductility decreased with an increase in pH (Fig. 3). The decreases of first and second strengths, secant and tangent stiffnesses, and minimum and maximum toughnesses with an increase in pH were described best by logarithmic equations (Table IV). The increases in minimum and maximum ductility values with an increase in pH were also described best by logarithmic equations.

3.3. Effect of sodium concentration

Mean values of hydration and thickness decreased with an increase in sodium concentration (Fig. 4), although the standard deviations remained fairly constant (Table V). The decreases of hydration and thickness were linear (Table VI). The mean values of all whole-membrane mechanical characteristics did not change significantly (Fig. 5), and all regressions were linear (Table VI).

3.4. Interpreting the effects of pH and sodium concentration

Note that because $[H^+]$ is *inversely proportional* to pH, the increase in the concentration of *either* cation (H^+ or Na^+) correlates negatively with hydration and thickness (Figs 2 and 4) and, in the case of H^+, correlates positively with mechanical characteristics other than ductility (Fig. 3).

4. Discussion

4.1. Thickness, hydration and mechanical characteristics

Figs 2 and 4 show that chorioamniotic membrane thickness is a good indicator of its hydration, as was proposed in previous work [10]. Changes in hydration not only mirror but probably cause changes in mechanical characteristics other than ductility. Previously, we hypothesized that the *strength-related mechanical characteristics*—that is, strength, stiffness, and toughness—would be affected by hydration, because the degree of interaction between structural fibres would be limited by the quantity of water between these fibres [10]. Ductility should not change as dramatically as the strength-related characteristics, because ductility is primarily an indicator of fibre orientation as opposed to fibre interaction. Hydration is decreased by increasing the concentrations either of the cations H^+ or Na^+ in the environment of the chorioamniotic membrane (cf. Tables III–VI and the top of Figs 2 and 4). The most likely mechanism by which the ionic environment controls the hydration of the chorioamniotic membrane is by affecting the degree of electrostatic repulsion between polyanionic mucopolysaccharides. This mechanism appears especially probable because two different cations produced similar effects.

4.2. pH and premature rupture

The differences in the values of vaginal pH, between mothers whose membranes ruptured prematurely and those whose membranes did not, allowed investigators to establish that a vaginal pH above approximately 4.5 indicates an increased risk of premature rupture [1, 2]. The pH of the membrane's environment clearly affects the strength-related mechanical characteristics of the *weakest links* in the membrane (cf. Fig. 3 and Tables III and IV).* These characteristics show the greatest decrease between pH values of 3.68 and 5.58. Thus, an increase in pH is probably a cause, as well as a marker, of the premature rupture of the chorioamniotic membrane.

4.3. Sodium concentration and timely rupture

The effects of variations in sodium concentration were not as significant as variations in pH; but, due to the logarithmic nature of pH, the $[H^+]$ concentration was tested over a far greater range than the sodium

* When *all* membrane specimens are analysed, trends between pH and strength-related mechanical characteristics are still discernable, but not statistically significant due to the inherent scatter in these very large pools of data.

TABLE III Percentage hydration, thickness, and "whole-membrane" mechanical characteristics of chorioamniotic membrane specimens in solutions with different pH[a]

	Percentage hydration (%)	Mean thickness (m)	First strength (N)	Second strength (N)	Secant stiffness (N/m)	Tangent stiffness (N/m)	Minimum toughness (J)	Maximum toughness (J)	Minimum ductility (m)	Maximum ductility (m)
For pH = 7.27[b]										
AVE	92.33	5.18×10^{-4}	4.64	3.99	5.07×10^{2}	1.56×10^{3}	1.11×10^{-2}	1.59×10^{-2}	9.46×10^{-3}	1.12×10^{-2}
SD	2.558	1.48×10^{-4}	1.56	1.24	1.92×10^{2}	5.59×10^{2}	4.13×10^{-3}	7.66×10^{-3}	1.54×10^{-3}	2.84×10^{-3}
%	2.770	28.5	33.7	31.1	37.9	35.8	37.3	48.2	16.3	25.4
n^c	9	36	9	9	9	9	9	9	9	9
For pH = 6.45:										
AVE	91.44	4.61×10^{-4}	5.70	4.58	5.53×10^{2}	2.12×10^{3}	1.27×10^{-2}	1.60×10^{-2}	1.03×10^{-2}	1.17×10^{-2}
SD	1.603	1.35×10^{-4}	2.52	2.17	2.54×10^{2}	1.26×10^{3}	5.09×10^{-3}	7.90×10^{-3}	1.60×10^{-3}	2.52×10^{-3}
%	1.753	29.3	44.2	47.4	45.9	59.7	40.2	49.5	15.6	21.5
n	9	37	9	9	9	9	9	9	9	9
For pH = 5.58:										
AVE	90.56	4.09×10^{-4}	5.77	4.43	5.70×10^{2}	2.24×10^{3}	1.22×10^{-2}	1.93×10^{-2}	1.00×10^{-2}	1.21×10^{-2}
SD	1.789	1.18×10^{-4}	1.93	2.11	1.66×10^{2}	1.19×10^{3}	5.08×10^{-3}	1.15×10^{-2}	9.32×10^{-4}	1.95×10^{-3}
%	1.975	28.9	33.5	47.6	29.2	52.9	41.7	59.7	9.3	16.1
n	9	36	9	9	9	9	9	9	9	9
For pH = 4.62:										
AVE	88.77	3.81×10^{-4}	8.18	6.71	8.70×10^{2}	3.22×10^{3}	1.64×10^{-2}	2.07×10^{-2}	9.69×10^{-3}	1.06×10^{-2}
SD	0.956	8.65×10^{-5}	2.52	2.79	3.25×10^{2}	1.47×10^{3}	5.40×10^{-3}	7.87×10^{-3}	1.52×10^{-3}	1.55×10^{-3}
%	1.077	22.7	30.8	41.6	37.4	45.7	32.8	38.0	15.7	14.6
n	9	35	9	9	9	9	9	9	9	9
For pH = 3.68:										
AVE	86.45	3.42×10^{-4}	8.85	7.40	9.33×10^{2}	3.55×10^{3}	1.69×10^{-2}	2.57×10^{-2}	9.49×10^{-3}	1.11×10^{-2}
SD	3.242	1.36×10^{-4}	3.08	1.87	2.90×10^{2}	1.08×10^{3}	9.21×10^{-3}	1.05×10^{-2}	1.50×10^{-3}	2.36×10^{-3}
%	3.751	39.9	34.8	25.3	31.1	30.4	54.4	40.8	15.8	21.3
n	9	36	9	9	9	9	9	9	9	9

[a] See Table 1.
[b] Average pH of solutions after membrane sections had equilibrated.
[c] Hydration was only measured once for each membrane section that represented the membrane at a given pH. Thickness and mechanical characteristics were measured on each of three to five membrane specimens (depending on section size) taken from each membrane section. "Whole-membrane" mechanical characteristics were identified as such to indicate that the smallest value of strength and concomitant values of stiffness, toughness, and ductility were selected from the three to five specimens taken from each membrane section. These values represent the weakest, and thus the limiting, part of the membrane section.

TABLE IV Equations describing the behaviour of hydration, thickness and 'whole membrane' mechanical characteristics with increasing pH[a]

Dependent variable, y	Equation	n^b	r^c	$p <^d$
Hydration:	$y = 75.43 + 8.618 \ln(\text{pH})$	45	0.711	0.001
Thickness:	$y = 2.20 \times 10^{-4} \exp(0.116\text{pH})$	180	0.439	0.001
Strength:				
First	$y = 17.0 - 6.17 \ln(\text{pH})$	45	0.540	0.001
Second	$y = 14.2 - 5.24 \ln(\text{pH})$	45	0.527	0.001
Stiffness:				
Secant	$y = 1780 - 650 \ln(\text{pH})$	45	0.529	0.001
Tangent	$y = 7430 - 2900 \ln(\text{pH})$	45	0.535	0.001
Toughness:				
Minimum	$y = 2.88 \times 10^{-2} - 8.85 \times 10^{-3} \ln(\text{pH})$	45	0.364	0.02
Maximum	$y = 4.37 \times 10^{-2} - 1.44 \times 10^{-2} \ln(\text{pH})$	45	0.369	0.02
Ductility:				
Minimum	$y = 9.33 \times 10^{-3} + 2.73 \times 10^{-4} \ln(\text{pH})$	45	0.045	N.S.[e]
Maximum	$y = 1.02 \times 10^{-2} + 6.85 \times 10^{-4} \ln(\text{pH})$	45	0.077	N.S.

[a] See Figures 2 and 3.
[b] Sample size. The sample sizes for the mechanical characteristics represent the lowest value of a mechanical characteristic measured among three to five specimens taken from each of five membrane sections per membrane.
[c] r is the correlation coefficient.
[d] The p-value represents the probability.
[e] Any p-value > 0.05 is considered not significant (N.S.).

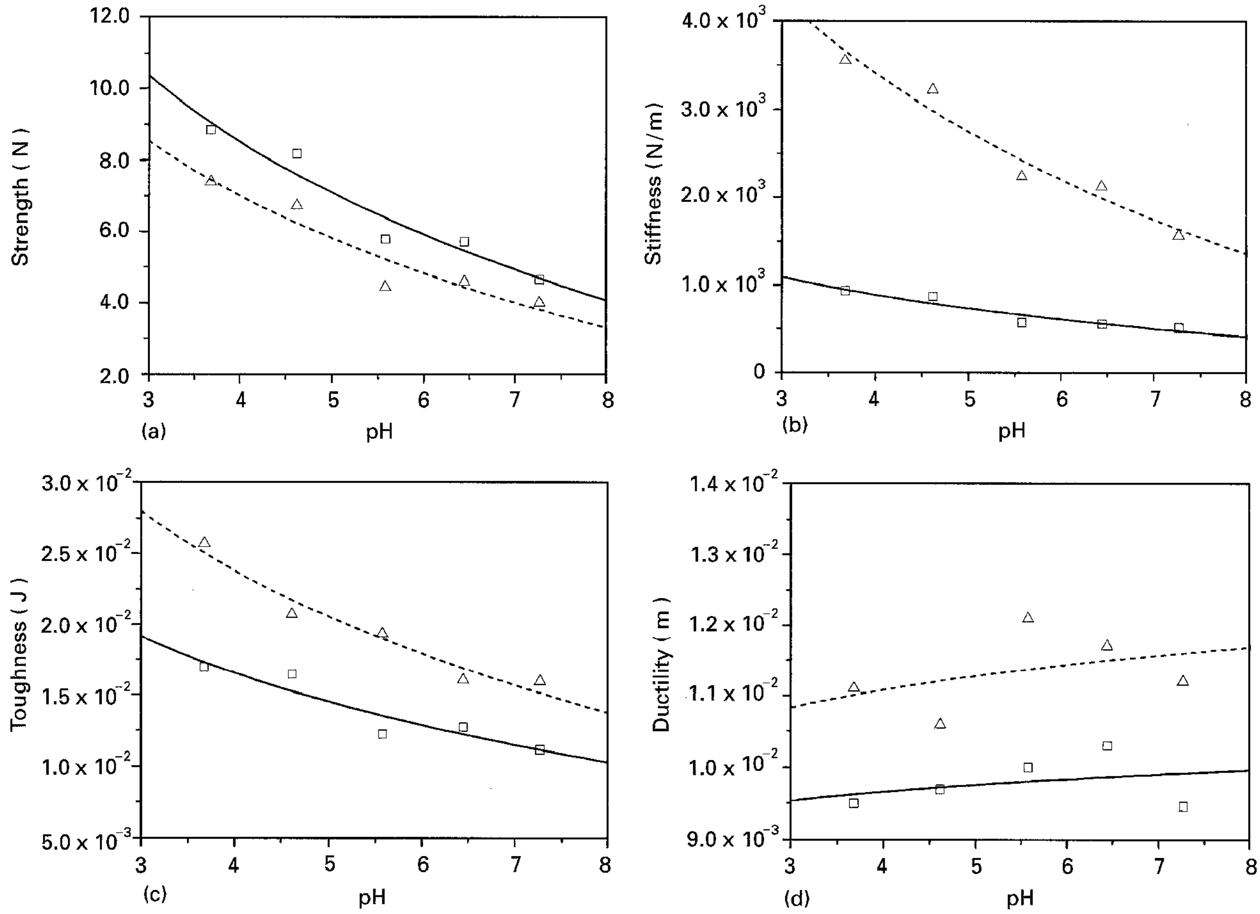

Figure 3 Behaviour of "whole membrane" mechanical characteristics with change in pH (cf. Table IV for slopes and statistics) : (a)–□– first strength, ---△--- second strength; (b)–□– secant stiffness, ---△--- tangent stiffness; (c)–□– minimum toughness, ---△--- maximum toughness; (d) –□– minimum ductility, ---△--- maximum ductility.

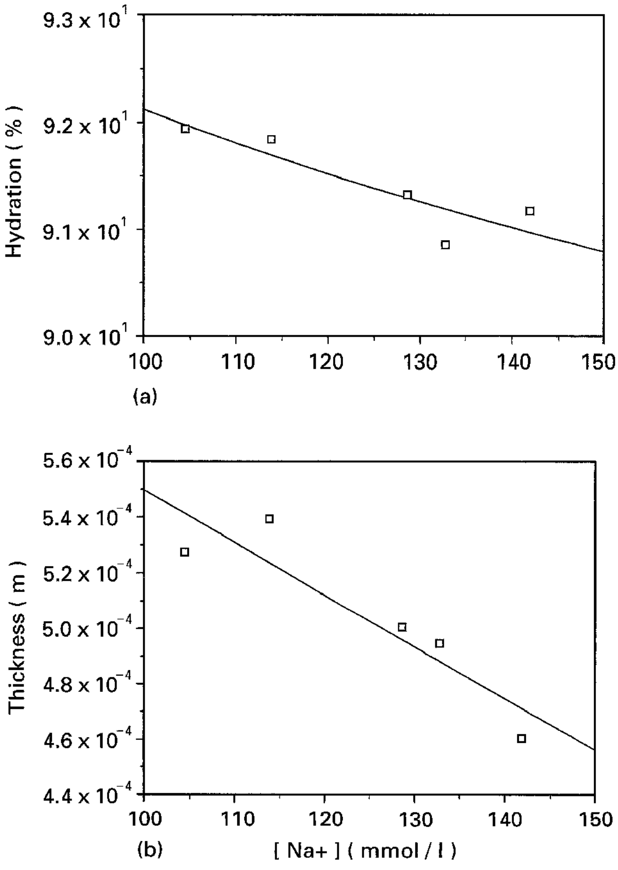

Figure 4 Behaviour of (a) hydration and (b) thickness with change in [Na^+] (cf. Table VI for slopes and statistics).

concentration and was not balanced by a simultaneous increase in anion concentration.

The effect of decreasing sodium and chloride concentrations in the amniotic fluid as gestation progresses [3–9] was modelled by varying the concentrations of these ions *in vitro*. These ionic changes *alone* are not great enough to produce changes in the mechanical characteristics of the chorioamniotic membrane that are of sufficient magnitude to dwarf the variance between chorioamniotic-membrane specimens, though hydration is affected slightly (cf. Figs 4 and 5 and Tables V and VI). *In vivo*, however, the effects of cation depletion are probably compounded by the increase in plasma concentrations of total estrogens and progesterone [20], which have been shown to increase the hydration of connective tissue [21]. Further, electron micrographs taken at different stages of pregnancy show a loosening of the network of fibres that make up the amnion in the third trimester [22]. Altogether the strength, stiffness, and toughness of the chorioamniotic membrane are probably reduced as gestation progresses in order to facilitate rupture at term, but a more sophisticated experiment will be required than the one described in this paper to prove it.

5. Conclusions

The ionic environment appears to control the whole-membrane mechanical characteristics and thickness of

TABLE V. Percentage hydration, thickness, and "whole-membrane" mechanical characteristics of chorioammiotic membrane specimens in solutions with different sodium and chloride concentrations.[a]

	Percentage hydration (%)	Mean thickness (m)	First strength (N)	Second strength (N)	Secant stiffness (N/m)	Tangent stiffness (N/m)	Minimum toughness (J)	Maximum toughness (J)	Minimum ductility (m)	Maximum ductility (m)
For $[Na^+]$ = 141.9 mmol/l[b]:										
AVE	91.14	4.60×10^{-4}	2.64	2.01	2.70×10^{2}	9.88×10^{2}	5.80×10^{-3}	7.80×10^{-3}	9.76×10^{-3}	1.13×10^{-2}
SD	1.135	1.58×10^{-4}	7.65×10^{-1}	4.64×10^{-1}	9.32×10^{1}	3.47×10^{2}	2.76×10^{-3}	3.84×10^{-3}	2.16×10^{-3}	2.62×10^{-3}
%	1.246	34.4	28.9	23.1	34.5	35.1	47.6	49.2	22.1	23.2
n[c]	10	40	10	10	9	10	9	9	9	9
Fot $[Na^+]$ = 132.8 mmol/l:										
AVE	90.86	4.94×10^{-4}	2.55	2.26	2.81×10^{2}	8.82×10^{2}	5.74×10^{-3}	9.15×10^{-3}	8.97×10^{-3}	1.10×10^{-2}
SD	1.106	1.40×10^{-4}	8.13×10^{-1}	7.54×10^{-1}	5.98×10^{1}	2.18×10^{2}	3.02×10^{-3}	4.67×10^{-3}	1.42×10^{-3}	2.44×10^{-3}
%	1.218	28.4	31.9	33.4	21.3	24.8	52.7	51.1	15.8	22.2
n	10	39	10	10	10	10	10	10	10	10
For $[Na^+]$ = 128.6 mmol/l:										
AVE	91.33	5.00×10^{-4}	3.17	2.46	3.12×10^{2}	9.60×10^{2}	8.18×10^{-3}	1.12×10^{-2}	9.96×10^{-3}	1.17×10^{-2}
SD	1.598	1.68×10^{-4}	1.49	8.40×10^{-1}	1.01×10^{2}	2.21×10^{2}	6.72×10^{-3}	6.86×10^{-3}	2.02×10^{-3}	1.85×10^{-3}
%	1.749	33.6	46.9	34.1	32.3	23.1	76.6	61.4	20.3	15.9
n	10	39	10	10	10	10	10	10	10	10
For $[Na^+]$ = 113.8 mmol/l:										
AVE	91.84	5.39×10^{-4}	2.79	2.22	2.92×10^{2}	1.01×10^{3}	5.68×10^{-3}	1.02×10^{-2}	9.18×10^{-3}	1.23×10^{-2}
SD	1.284	1.49×10^{-4}	8.68×10^{-1}	9.24×10^{-1}	9.53×10^{1}	2.51×10^{2}	2.53×10^{-3}	4.01×10^{-3}	9.53×10^{-4}	1.98×10^{-3}
%	1.398	27.6	31.4	41.7	32.6	24.8	44.6	39.2	10.4	16.1
n	10	39	10	10	9	10	9	9	9	9
For $[Na^+]$ = 104.4 mmol/l:										
AVE	91.94	5.27×10^{-4}	2.60	2.06	2.83×10^{2}	9.37×10^{2}	5.80×10^{-3}	7.78×10^{-3}	9.54×10^{-3}	1.12×10^{-2}
SD	1.136	1.77×10^{-4}	8.26×10^{-1}	7.34×10^{-1}	1.10×10^{2}	2.88×10^{2}	2.32×10^{-3}	3.21×10^{-3}	1.46×10^{-3}	1.72×10^{-3}
%	1.235	33.5	31.7	35.6	39.0	30.8	40.1	41.2	15.3	15.5
n	10	39	10	10	10	10	10	10	10	10

[a] See Table 2.
[b] Sodium concentration of solutions after membrane sections had equilibrated.
[c] Hydration was only measured once for each membrane section that represented the membrane at a given $[Na^+]$ concentration. Thickness and mechanical characteristics were measured on each of three to five membrane specimens (depending on section size) taken from each membrane section. "Whole-membrane" mechanical characteristics were identified as such to indicate that the smallest value of strength and concomitant values of stiffness, toughness, and ductility were selected from the three to five specimens taken from each membrane section. These values represent the weakest, and thus limiting, part of the membrane section. Four types of complications could reduce the sample size (n) of a mechanical characteristic: a specimen could tear from the side of the tube section—no characteristics could be measured; a specimen could slip from the hold of the clamp—only strength could be measured; the force—deflection curve could be irregular (presumably indicating small-scale slipping)—only strength and tangent stiffness could be measured; a specimen could slip from the clamp between first and second peaks—all characteristics other than maximum toughness and maximum ductility could be measured.

TABLE VI Equations describing the behaviour of hydration, thickness and "whole membrane" mechanical characteristics with increasing $[Na^+]$[a]

Dependent variable, y	Equation	n[b]	r[c]	$p <$[d]
Hydration:	$y = 0.0274[Na^+] + 94.82$	50	0.290	0.05
Thickness:	$y = -1.88 \times 10^{-6}[Na^+] + 7.38 \times 10^{-4}$	196	0.158	0.05
Strength:				
First	$y = 1.14 \times 10^{-3}[Na^+] + 2.61$	50	0.000	N.S.[e]
Second	$y = 8.46 \times 10^{-4}[Na^+] + 2.10$	50	0.000	N.S.
Stiffness:				
Secant	$y = -0.224[Na^+] + 316$	48	0.032	N.S.
Tangent	$y = -0.278[Na^+] + 990$	50	0.000	N.S.
Toughness:				
Minimum	$y = 1.31 \times 10^{-5}[Na^+] + 4.64 \times 10^{-3}$	48	0.045	N.S.
Maximum	$y = 5.03 \times 10^{-6}[Na^+] + 8.61 \times 10^{-3}$	48	0.000	N.S.
Ductility:				
Minimum	$y = 4.45 \times 10^{-6}[Na^+] + 8.93 \times 10^{-3}$	48	0.032	N.S.
Maximum	$y = -8.88 \times 10^{-6}[Na^+] + 1.26 \times 10^{-2}$	48	0.055	N.S.

[a] See Figures 4 and 5.
[b] Sample size. The sample sizes for the mechanical characteristics represent the lowest value of a mechanical characteristic measured among three to five specimens taken from each of five membrane sections per membrane.
[c] r is the correlation coefficient.
[d] The p-value represents the probability.
[e] Any p-value > 0.05 is considered not significant (N.S.).

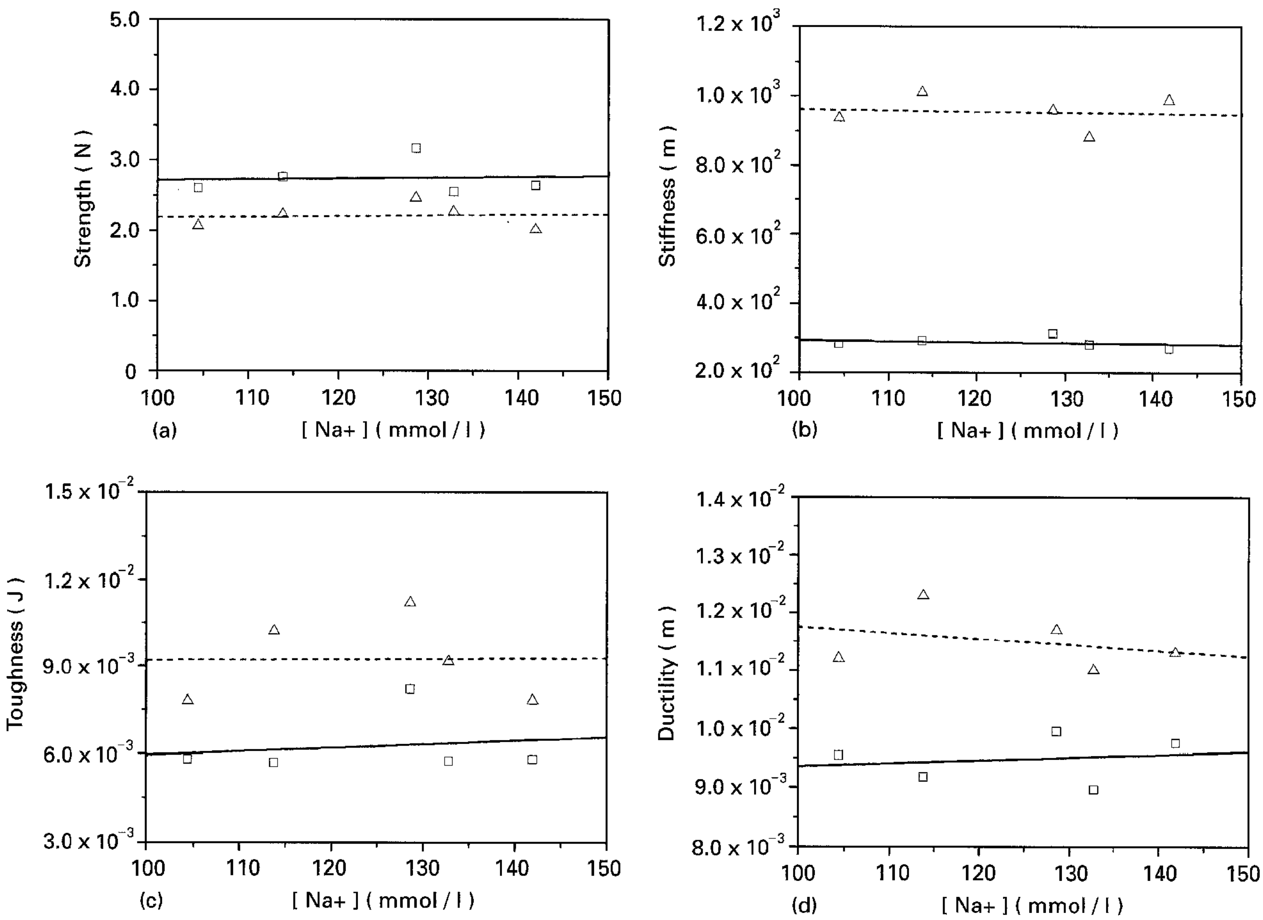

Figure 5 Behaviour of "whole membrane" mechanical characteristics with change in $[Na^+]$ (cf. Table VI for slopes and statistics and Fig. 3 for symbol and line notation).

the chorioamniotic membrane by determining its hydration. Thus, abnormally low concentrations of H^+ in the vagina could *cause* premature rupture of the fetal membranes. Decreases in sodium concentration during gestation do not alter strength-related mechanical characteristics sufficiently to be interpreted as facilitating rupture at term.

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