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Effect of thickness on the fracture characteristics of fetal membranes

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The mechanical characteristics of the chorioamniotic membrane were evaluated with a new burst test apparatus by rupturing 35 specimens that were taken from a sample of seven afterbirths. 'Strength', 'stiffness', 'toughness', and 'ductility' were measured. Mechanical characteristics did not change significantly with variation in thickness. While ductility should not correlate with thickness, the lack of a significant increase in strength, stiffness and toughness with an increase in thickness is most unusual and requires an explanation. Subsequently, an additional experiment, which was designed to ascertain the mechanical stability of membrane specimens with prolonged exposure to air, showed a dramatic increase in stiffness as membranes were allowed to dry. The increase in stiffness indicates that strength and toughness also increase with drying, provided that ductility remains constant. Thus, the degree of hydration of the membrane, which is reflected in thickness, regulates mechanical characteristics. The increase in the water content of certain amniotic layers has a lubricating effect on the amnion–chorion interface. This lubrication increases as term approaches. Thus, hydration must increase as gestation progresses, and strength, stiffness, and toughness must decrease until the membrane is weak enough to rupture at the end of gestation. Thus, hydration must increase as gestation progresses, and strength, stiffness, and toughness must decrease until the membrane is weak enough to rupture at the end of gestation.

1. Introduction

In the last four decades, intense interest has developed regarding the characteristics of the chorioamniotic membrane, primarily to understand why this membrane fractures prematurely [1–12]. Initially, several investigators studied the insufficiency of intrauterine forces to fracture the chorioamniotic membrane [1, 3, 5]. Later, the viscoelastic nature of the membrane was determined [7] as well as the effects of fatigue [4], collagen content [9], bacterial growth and inflammatory response [10, 12], and surface energy [11] on the mechanical characteristics of the fetal membranes. By the mid-1970s, membrane characteristics were described in terms of modulus of elasticity, work, and strain [6]. Due to the heterogeneity of the chorioamniotic membranes, measurements of thickness and fracture characteristics were so widely dispersed that they did not appear to correlate with likelihood of rupture *in vivo*.

In this experiment, the chorioamniotic membrane is characterized by the same mechanical property evaluations that are used to study synthetic membranes [13] and hard tissues [14] – namely, 'strength', 'stiffness', 'toughness', and 'ductility'. Five specimens from each chorioamniotic membrane were studied using sophisticated equipment to reduce the imprecision that is intrinsic in the testing of biological tissues. Because the disparity between membrane characteristics of different mothers is so large, each specimen was evaluated independently. The variation *within* membranes de-emphasizes the large differences *among* membranes (i.e. mothers). In this paper, we determine that characteristics of the chorioamniotic membrane *at parturition* are relatively homogeneous and, at best, correlate weakly with membrane thickness. Ironically, this lack of correlation is of great conceptual significance: if the force required to fracture a thick membrane equals the force required to fracture a thin membrane, then the stress the material can withstand decreases as thickness increases. As such behaviour is unlikely, we propose that thickness is indicative of the degree of hydration of the membrane, and that hydration determines the membrane's mechanical characteristics.

2. Materials and methods

2.1. Procurement

The afterbirths of seven patients were recovered from a refrigerator in Labour and Delivery of the North Carolina Memorial Hospital, where they had been stored immediately after delivery in an air-tight plastic tub for 0.5 to 13 h. Time spent in refrigeration has no

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effect on the strength of the chorioamniotic membranes [2] (also see Section 2.6). After the chorioamniotic membrane was cut from the placenta, five specimens per membrane were taken from the vicinity of the site of fracture and affixed to poly(methyl methacrylate) (PMMA) washers (ID 33.25 mm, OD 43.00 mm, width 4.70 mm) using Loctite 447 cement (Loctite, Newington, CT, USA).

2.2. Thickness measurements

The thickness of each chorioamniotic membrane specimen was measured with an apparatus similar to that used by Polishuk *et al.* [2]. A Sony μ-Mate digital micrometer (Sony, Tokyo, Japan) was connected to a Fluke 77 Multimeter (John Fluke Mfg. Co., Everett, WA, USA) to ascertain the exact moment of contact. Contacts were fabricated by epoxying the long sides of wires to the flat surfaces of insulating caps that had been slipped over the anvil and spindle of the micrometer. The wires were oriented orthogonally to touch at a centre point only. A water-absorbing nylon filter (MSI, Westboro, MA, USA) of relatively uniform thickness was measured to verify the accuracy of this method of thickness measurement. These thickness measurements were corroborated using the optics of a Kentron Hardness Tester (Kent Cliff Labs., Peekskill, NY, USA). Thickness measurements of the nylon filter with the Sony μ-Mate/Fluke 77 Multimeter and the Kentron Hardness Tester were 0.137 ± 0.008 mm and 0.129 ± 0.009 mm, respectively. This difference was not significant according to a Student's *t*-test. Point-thicknesses of each chorioamniotic membrane specimen were measured at three locations, which were located approximately in the centre of the specimen, since this region would experience the most deformation. To keep membrane specimens moist during thickness measurements, an atomizer was used to blanket them with a thin coat of deionized water, which avoided the addition of cations that may affect thickness or mechanical characteristics [15, 16]. Each specimen was returned to the broth, which was composed of blood and minute quantities of deionized water, immediately after thickness measurements were made. Mean thickness was calculated for each specimen.

2.3. Burst apparatus

An apparatus (Fig. 1) was designed to investigate fracture characteristics of chorioamniotic membranes in conjunction with future tests, such as hydroxyproline content, bacterial infection, and hydration, that will also require sizeable tissue samples. Because fracture characteristics were studied in five specimens per membrane, the fracture surface of the apparatus (15.7 mm) was smaller than most in the literature, except for the device designed by MacLachlan [3]. The apparatus was machined from AISI type 304 stainless steel and consisted of two parts that were bolted together once the membrane was set in place. The bottom half had a gas line and a pressure transducer (0.21 MPa, Series PX236; Omega Engineering, Inc., Stamford, CT, USA) attached. The top half was

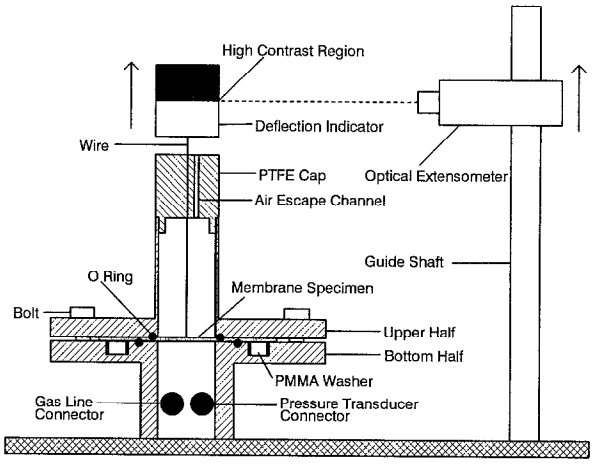

Figure 1 Schematic drawing of the burst test apparatus. Note the location of the PMMA washer and the o-rings that hold the membrane specimen firmly in place. The optical extensometer measures the deflection of the membrane by tracking the indicator, which contacts the membrane specimen via the wire.

capped by a poly(tetrafluoroethylene) (PTFE) cylinder that centred the deflection indicator while minimizing frictional resistance. The deflection indicator was made of an AISI type 304 stainless steel wire that passed through the centre of a hollow white paper cylinder, the top half of which was spray-painted black to provide a high contrast region. The gravitational force exerted on the membrane specimen by the indicator was minimal, 0.86 g. The magnitude of deflection was measured with an Instron Optical Extensometer (Instron, Canton, MA, USA), which followed the high contrast region with a precision of ± 0.01 mm.

2.4. Membrane testing

The PMMA washer with the wet membrane specimen affixed was placed into the groove in the bottom half of the burst apparatus (Fig. 1). The top half was bolted into place, trapping the chorioamniotic membrane between the o-rings to make slipping impossible. To simulate intrauterine conditions, the amnion was inserted facedown (the amnion formed the gas–membrane interface). The displacement indicator was inserted through the PTFE cap and lowered until it contacted the specimen. Nitrogen gas was admitted through a motor-driven valve, which exposed the membrane to a steadily increasing pressure, as opposed to the less precisely controlled pressure increases described previously [1–3, 7, 8].

2.5. Membrane characteristics

Two plots were obtained for each chorioamniotic sample: the Instron Optical Extensometer generated a signal corresponding to membrane deflection, and the pressure transducer generated a signal corresponding to the pressure that deflected the membrane. By synchronizing the signals and multiplying the pressure by the cross-sectional area, force traces could be plotted on a Fisher Recordall Series 5000 (Houston Instrument, Austin, TX, USA), and 'strength', 'stiffness' (secant and tangent), 'toughness', and 'ductility' could be

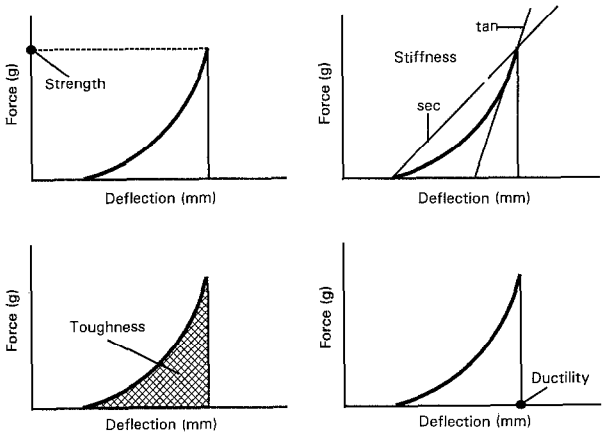

Figure 2 Determination of chorioamniotic membrane characteristics [strength, stiffness (secant and tangent), toughness, and ductility] on an idealized J-curve of force versus deflection.

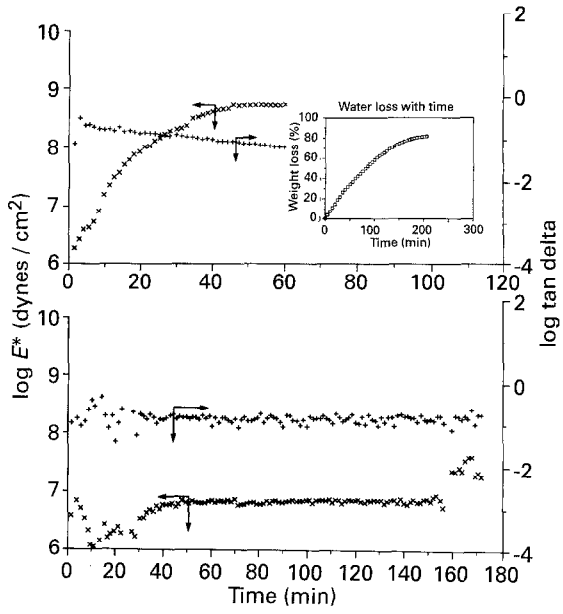

Figure 3 Dynamic mechanical properties of chorioamniotic membranes as a function of time: top, an unsupported membrane specimen that was allowed to dehydrate as shown in the inset; and bottom, a supported membrane specimen that was kept moist. From the left-hand and right-hand coordinates, the storage modulus (E^*, which is proportional to stiffness) and the damping ratio of the real and imaginary moduli (tan delta) are obtained [21].

evaluated for each specimen (Fig. 2). The quotation marks indicate that the definitions of the terms used to describe these characteristics are different from definitions commonly used in engineering disciplines. Strength (g) is the force required to fracture the membrane specimen. Stiffness ($g\,mm^{-1}$) is the amount of force required to deflect the specimen at unit distance. Toughness (g mm) is the amount of work required to deflect the specimen until it fractures. Ductility (mm) is the distance the membrane specimen was deflected at fracture.

2.6. Membrane stability

To ascertain whether prolonged storage in a refrigerator affects the mechanical characteristics of chorioamniotic membranes, four membranes were tested over a period of 72 h starting immediately after delivery. Specimens were removed from different regions of the membrane as rapidly as possible, and the remaining tissue was returned to its broth in the refrigerator. Five specimens from each membrane were tested at the end of five time-intervals. The first time-interval was as short as membrane retrieval and apparatus setup allowed—1–3 h after delivery. No significant change in the membrane characteristics or thickness was observed with the passage of time: correlation coefficients (r) were 0.121, 2.08×10^{-2}, and 5.43×10^{-2} for thickness, strength, and ductility, respectively.

Because knowledge regarding the stability of the chorioamniotic membrane with prolonged exposure to air was lacking, the reliability of data was assessed with additional tests. Weight losses were measured via thermogravimetric analysis using a 950 Thermogravimetric Analyser (Dupont, Wilmington, DE, USA) and corroborated by conventional gravimetric analysis using a Sartorius Balance, Model 1712MP8 (Brinkman Instruments Co., Westbury, NY, USA). The membrane lost enough water to reduce its weight by at least 83% within 3 h (Fig. 3, top, inset). With an Autovibron, Model DDV-II-C (IMASS Inc., Accord, MA, USA), the effects of drying on the viscoelastic properties of the membrane were measured by evaluating the membrane's stiffness via its elastic modulus as a function of time (Fig. 3). These plots show a profound stiffening (from 10^6 to 10^9 dynes/cm^2) of the chorioamniotic membrane as the membrane dehydrates (Fig. 3, top). When the membrane specimen was kept moist, the elastic modulus remained so low that the specimen had to be supported by a thin base of soft rubber to prevent slumping. The addition of this rubber is responsible for the initial, upward shift in the baseline elastic modulus from 10^6 to 10^7 dynes/cm^2 (Fig. 3, bottom versus top). Because 'burst' testing was completed within 2 min after removal of the specimen from the broth, the present measurements of membrane characteristics fall safely within the time-interval preceding the dehydration-induced stiffening. If physiochemical changes occur during this period, they do not affect the mechanical characteristics.

2.7. Data analysis

Every specimen from each membrane was included as a datum, because the resulting regression represents behaviour within as well as between the membranes. Correlation coefficients (r) were calculated to establish the significance of the relationship between each membrane characteristic and thickness. The probability (p) was ascertained using the r-value and the sample size (n). The mean, standard deviation, and coefficient of variation for the five membrane characteristics and thicknesses were calculated. Between- and within-subject variances were also represented as percentages of the means.

3. Results

The data (Table I) were subjected to a logarithmic transformation and plotted as a function of thickness

TABLE I Experimental data for mechanical characteristics of chorioamniotic membranes

Membrane and specimen number	Thickness mean value (mm)	Strength (g)	Stiffness sec (g/mm)	Stiffness tan (g/mm)	Toughness (g mm)	Ductility (mm)
I 1	0.363	1100	244	903	630	4.50
2	0.577	1490	184	532	2700	8.10
3	0.447	944	113	650	605	8.34
4	0.407	477	52	349	248	9.21
5	0.744	1150	167	812	742	6.90
II 1	0.171	2380	338	1540	4270	7.05
2	0.227	3210	486	2050	9370	6.60
3	0.247	2520	310	1400	7630	8.13
4	0.168	2450	355	1340	4800	6.90
5	0.221	2290	315	5160	6080	7.26
III 1	0.958	1930	276	682	4700	6.99
2	0.980	1950	286	1430	3400	6.81
3	1.516	3910	852	1778	21000	4.59
4	0.640	2010	381	1190	3800	5.28
5[a]	1.216	—	—	1930	—	—
IV 1	0.350	913	128	528	651	7.14
2	0.377	985	101	1420	928	9.75
3	0.433	1130	158	1090	957	7.17
4	0.360	1670	257	843	2780	6.51
5	0.537	1200	164	652	1050	7.32
V 1	0.263	313	44	195	130	7.05
2	0.311	595	59	245	476	10.10
3	0.269	1170	177	764	1300	6.60
4	0.326	1220	152	728	950	8.01
5	0.067	626	85	378	300	7.38
VI 1	0.429	1590	285	1150	2200	5.58
2	0.165	1670	252	636	3000	6.63
3	0.388	1390	213	511	2500	6.54
4	0.478	1560	342	705	2800	4.56
5	0.309	646	86	354	260	7.50
VII 1	0.351	2520	382	865	11700	6.60
2	0.211	1720	239	1250	2910	7.20
3	0.464	1630	201	1070	2480	8.10
4	0.329	1510	244	527	1370	6.18
5	0.193	1240	157	657	1240	7.89

[a] This specimen ruptured just outside the measurable range, so only thickness and stiffness (tan) (see Fig. 2) could be ascertained.

TABLE II Linear regression analysis of logarithmically transformed chorioamniotic membrane characteristics[a]

Mechanical characteristic, y	Slope, m	Intercept, b	n[b]	r[c]	p value[d]
Log Strength (g)	0.190 (g/mm)	3.22 (g)	34	0.205	NS[e]
Log stiffness					
sec (g/mm)	0.275 (g/mm^2)	2.42 (g/mm)	34	0.251	NS
tan (g/mm)	0.207 (g/mm^2)	3.02 (g/mm)	35[f]	0.184	NS
Log toughness (g mm)	0.45 (g mm/mm)	3.4 (g mm)	34	0.224	NS
Log ductility (mm)	− 0.0890 (mm/mm)	0.802 (mm)	34	0.280	NS

[a] $y = mx + b$, where y is the logarithmically transformed membrane characteristic, m is the slope, x is logarithmically transformed thickness, and b is the intercept
[b] Sample size
[c] r is the correlation coefficient
[d] The p value represents the probability
[e] NS = not significant
[f] See Table I

(Table II and Figs 4–8). The changes in the slopes of the membrane characteristics with increasing thickness are not significant for a sample size of 34 (Table II). Nonetheless, the measures of strength, stiffness, and toughness showed weak positive associations with membrane thickness, whereas ductility was slightly inversely related to membrane thickness. Thickness is the only variable component of the cross-sectional area over which tensile forces applied to the edges of the specimen may be distributed, because the

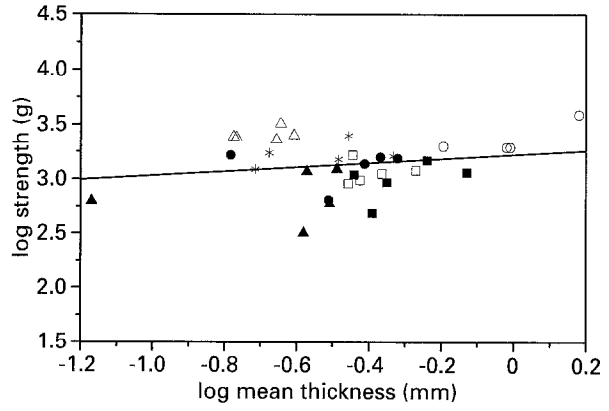

Figure 4 Logarithm of strength versus logarithm of mean thickness for 34 specimens taken from seven chorioamniotic membranes: I ■; II △; III ○; IV □; V ▲; VI ●; VII ⁎; Total ——.

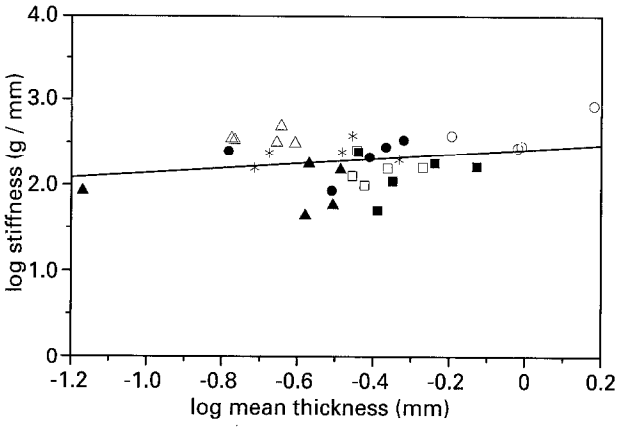

Figure 5 Same as Fig. 4, except the ordinate represents stiffness sec.

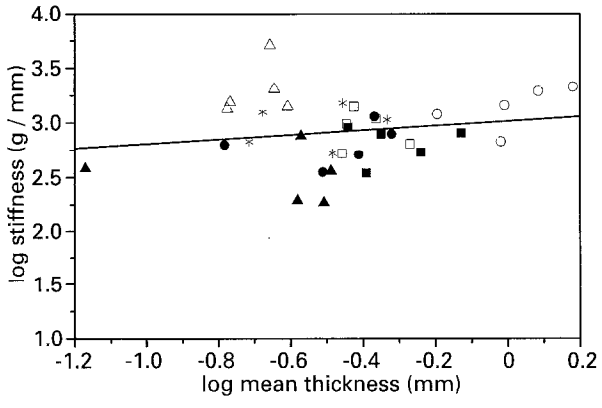

Figure 6 Same as Fig. 4, except the ordinate represents stiffness tan.

diameter of every membrane specimen is equal. Thus, one would expect a linearly proportional relationship between thickness and strength, stiffness, and toughness. The statistics highlight the mean values for the membrane characteristics and the distribution of variability between and within chorioamniotic membranes (Table III). The coefficient of variation was in the range of 50–85% except for toughness (130%) and ductility (18%). Apportioning the total variation into between-membrane and within-membrane components yielded divergent results across the six measures. Only for thickness and strength was between-membrane variation dominant, while ductility was particularly influenced by within-membrane variation. As expected, variance distributions for stiffness and toughness lie between strength and ductility, because the former characteristics are proportional to the latter as the quotient of (strength/ductility) and the product of (strength × ductility), respectively.

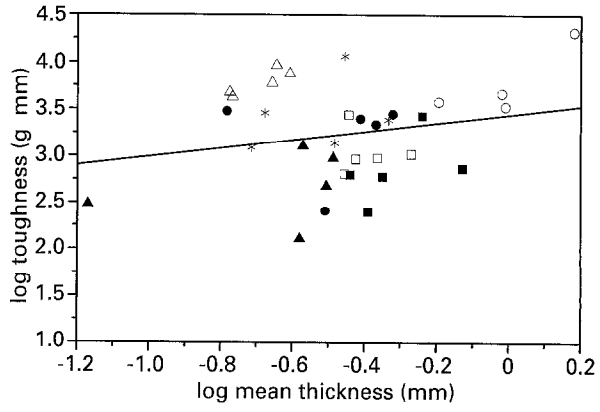

Figure 7 Same as Fig. 4, except the ordinate represents toughness.

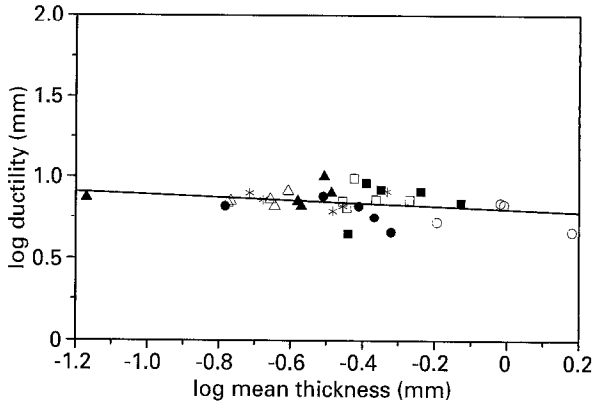

Figure 8 Same as Fig. 4; except the ordinate represents ductility.

4. Discussion

4.1. Morphological and mechanical model

Before forming a hypothesis to explain the behaviour of the chorioamniotic membranes, their structure and how it interacts with membrane characteristics as a function of thickness must be considered.

Because the amnion carries the majority of the load imposed on the chorioamnion [2], this component of the membrane is of primary interest. Bourne [17] reports that the compact layer of the amnion carries the majority of the load placed on this membrane, and does not vary greatly in thickness. Of all layers in the chorioamniotic membrane, the fibroblast layer and, to a far greater extent, the spongy layer show the largest proportional variation in thickness due to fluid and mucin content (also known as mucopolysaccharides—proteoglycans, glycosaminoglycans, glycoproteins, and other materials that, among other functions, act as mechanical cushions, hygroscopic agents, lubricants, and adsorbents [18, 19]). Each of the three aforementioned amniotic layers (compact, fibroblast, and spongy) is composed of a relatively lower density of randomly oriented fibres (the fibres, which are

TABLE III Statistics of the burst test results: mean, variability, and between- and within-subject variance

	Thickness mean values (mm)	Strength (g)	Stiffness		Toughness (g mm)	Ductility (mm)
			sec (g/mm)	tan (g/mm)		
Mean	0.443	1560	240	1070	3200	6.87
Standard deviation	0.309	783	150	878	4100	1.74
Coefficient of variation (%)	70.5	50.1	64	84.5	130	18.0
Between-membrane variation (%)	76.1	63.5	46	37.5	32	10.9
Within-membrane variation (%)	23.9	36.5	54	62.5	68	89.1

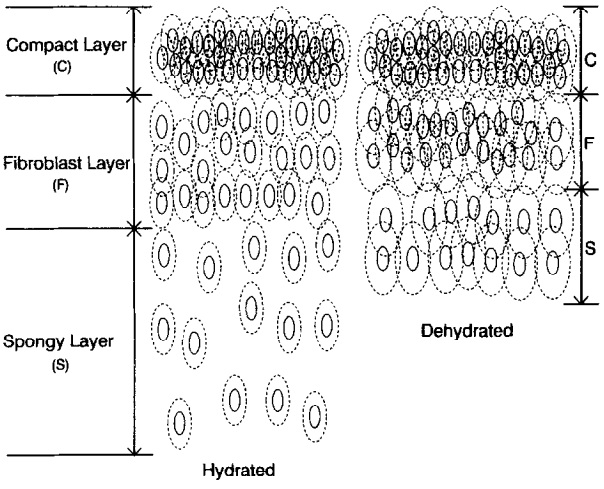

Figure 9 Schematic drawing of longitudinally aligned fibres in cross-section as they would appear in a hydrated amnion (left); and a dehydrated amnion (right). Fibres would only be parallel to each other when the membrane is in tension, otherwise fibre orientation within the layer is random. The solid circles delimit the surfaces of the fibres, while the boundary regions (affected area) are delineated by the dashed circles. Note that the increase in internal friction, as a consequence of dehydration, increases the sizes of these boundary regions.

bundles of collagen fibrils, are oriented in all directions equally in a plane perpendicular to the applied force; fibres are not as likely to traverse layers as they are to remain within them), the ends of which are adjacent—not attached—to each other (Fig. 9, left). Consequently, the response of the fibres to an imposed force depends on internal friction. (Internal friction is the result of viscous drag, which manifests itself by forming a boundary region around the fibre that will affect and be affected by movements of the fibre (Fig. 9). When boundary regions of different fibres overlap, the fibres are joined to an extent determined by the proximity of fibres, and react to the imposed forces as an unit.) The lower the fibre density, or, the greater the hydration of the spongy layer, the greater the 'lubrication' between the membranes [17, 20] or the weaker the internal friction between fibres linking the amnion to the chorion via the spongy layer (Fig. 9). In connection with the importance of fluid content of the amnion, Bourne [17] and Petry [20] record a tendency of the fibroblast and spongy layers to expand when placed in water and contract when placed in an acidic solution; that is, the environment can control the hydration of the amnion. This is consistent with the 'poly-anionic character of glycosaminoglycans' [19] and proteoglycans, which causes repulsion between molecules. Water might act as a weak acid, while stronger acids and cations in solution would neutralize the charges more effectively, decreasing repulsion and voiding excess water. In contrast, the compact layer is virtually unaffected by its environment [17]. Thus, the strength of the compact layer should be quite substantial and stable because internal friction remains constant and strong, as the boundary regions overlap in the absence of a large quantity of fluid between fibres (Fig. 9).

When the drying curve that was used to determine membrane stability is reviewed, a dramatic increase in elastic modulus versus drying time is observed (Fig. 3, top). This amounts to an *increase in stiffness*, because the separation between fibres in the fibroblast and spongy layers has decreased (Fig. 9, right); that is, the resistance of fibres to alignment has increased, due to an increase in internal friction. Similarly, *a rise in strength* with drying suggests that the effective sum of cross-sectional areas of fibres over which stress may be distributed has increased. This rise may also be explained by the fact that previously separated fibres come close enough to act as if crosslinked – as the compact layer must (Fig. 9, right). Fibres are effectively connected when the internal friction resisting longitudinal displacement exceeds their tensile strength. (Tensile strength of fibres is probably also somewhat decreased, due to the hydrophobic nature of interfibrillar bonding. The increased partial pressure of water outside of the fibres causes an increase in water between the fibrils, which will affect hydrophobic bonding [18].) Because fibres share or pass through several boundary regions along their length, longitudinal rigidity is maintained while reorientation is accommodated. Since the fibres are not very extensible [17, 20], ductility mainly reflects the change in orientation of fibres by an applied force. Because fluid removal through diffusion and evaporation has no effect on the initial fibre orientation, *ductility is not affected* by drying. A slight decrease in ductility is possible, because fibre reorientation is more difficult and longer fibres may not have a chance to align completely before rupture. *Toughness increases* with drying, because strength has increased while ductility has effectively remained constant.

To confirm that the lack of correlation found between strength and thickness was not an anomaly, the results of a puncture test, which involved the use of probes to fracture 275 specimens from an additional eleven membranes, were included in the regression (Fig. 10). The inclusion of these data shows

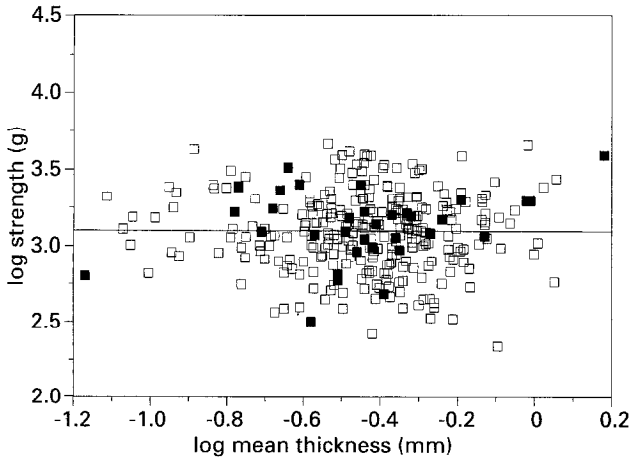

Figure 10 The behaviour of strength versus thickness in 309 specimens that were taken from eighteen chorioamniotic membranes. Seven (34 specimens) of the 18 membranes were fractured in a burst test, while the other 11 (275 specimens) were fractured with probes in a puncture test. The regression shows an even more pronounced lack of correlation between strength and thickness than that of the burst test data only, while the range of dispersion remains similar: correlation coefficient (r) is 5.16×10^{-3}, the slope (m) is -0.006, and the intercept (b) is 3.09 (cf. with Table II and Fig. 4). ■ Burst test; □ Puncture test.

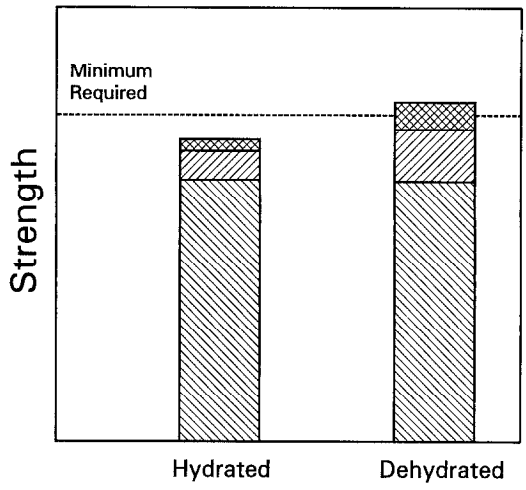

Figure 11 Relative contributions of the major layers of the amnion to its strength in the hydrated state (left); and the dehydrated state (right). "Xs" represent the contribution of the spongy layer, "//s" represent that of the fibroblast layer, and "\\s" represent that of the compact layer.

an even smaller correlation between strength and thickness than that calculated for the 34 specimens tested by the burst test (cf. Fig. 10 and Table II).

4.2. Hypothesis for timely rupture of the membranes

To explain the lack of correlation between strength and thickness that is so unusual but substantiated by the puncture test, we propose that the fibroblast and spongy layers of the amnion are weakened by an increase in hydration—indicated by an increase in thickness—as term approaches. If one assumes that the chorioamniotic membrane requires *all* layers of the amnion to attain some minimum strength (Fig. 11, right), then a decrease in the fibre density of the spongy and fibroblast layers (cf. Fig. 9, right versus left) would dispose the entire membrane to fracture as parturition nears (Fig. 11, left). Consequently, stronger membranes must swell to a greater extent in order to approach a universally equivalent strength at term. In other words. membrane thickness reflects initial strength. The sensitivity of the spongy and fibroblast layers to the water concentration and acidity of their environment provides a likely mechanism for the control of their hydration, and thus, strength and lubrication of the amnion–chorion interface.

If the findings of Bourne [17] and Petry [20] regarding the connection between swelling and lubrication are combined with those of Hills and Cotton [11] relating the surface energy (lubrication) of the epithelial surface of the chorion to gestational age, one deduces the following: the reduction in surface energy of the amnion–chorion interface is concomitant with the swelling of the fibroblast and spongy layers and the increasing probability for rupture of the chorion due to growth of the fetus or labour contractions. Thus, the amnion is isolated mechanically from the more corruptible chorion as the amnion is weakened; possibly the amnion is being sensitized or linked to conditions on the fetal side.

5. Conclusions

Since mechanical characteristics of chorioamniotic membranes are not affected by a variation in thickness, the material responsible for the variation in thickness does not improve the strength of the membrane, and may even weaken it. We suggest that water is this weakening agent, because we found that dehydration effects a dramatic change in elastic modulus and because other authors [17, 20] found that a change in thickness of chorioamniotic membranes primarily reflects a change in hydration. Thus, originally stronger chorioamniotic membranes must absorb more water as term approaches than originally weaker ones so that membranes of all thicknesses will require an equivalent force to fracture at term.

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