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Elastic Properties and Yield Stress of Fetal Membranes

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Abstract— **This is a presentation of a biomechanics project from almost 30 years ago, that, despite the long delay, is both relevant today and may be interesting from a historical perspective. Premature rupture of the amniotic sac membranes enclosing the fetus is, as yet, a not fully understood process, but may related to the mechanical properties of those membranes. The late biomechanics pioneer Tom McMahon and I developed a method for testing the yield stress of fetal membranes and found it to be homogeneous throughout individual samples. The method was: 1. insensitive to initial stretch, 2. avoided damaging contact with the membrane during stress-testing, and 3. modeled the mechanical properties using a power-law relationship. Because the clinical samples were from normal deliveries, clinical correlation remains as an unfinished aim of this project.**

I. The Project

When I was an undergraduate (1980-83) at Harvard in Engineering and Applied Sciences, the late Tom McMahon was my undergraduate adviser. While I was taking his class, "Systems Analysis with Physiological Applications," in 1982, he mentioned that he was looking for a student to participate in a research project. I volunteered, mainly because I needed research experience and a solid recommendation for medical school, despite the fact that I really wanted to do neurophysiology. The project turned out to be on the physical properties of fetal membranes, far off from my usual interests. But I carried out the project and wrote it up for class credit, and then delayed completing a manuscript for submission to a journal. No one pressured me to complete it, and in the years since I have learned valuable lessons about the temptation to move onto other endeavors after the data is collected. Tom McMahon died on 14 Feb. 1999 at the age of 55 from a complication of back surgery. He was a wonderful teacher, lab leader, writer, and person. I am indebted to him for giving me my start in academic research, for showing how running a lab can be fun (particularly if you take a day here and there to go cycling with the staff,) and how you can be a serious fiction writer and engineer at the same time.

A. Motivation & Innovation

Premature rupture of the amniotic sac membranes is associated with prematurity and amniotic fluid infections. But the nature of the association is unclear; does inflammation of the membranes produce local weakness or is the membrane intrinsically prone to rupture, leading to a post-rupture infection? This was the motivating question. I believe it arose from a conversation between Tom and Shirley G. Driscoll, M.D., a faculty member in Pathology at Harvard Medical School, as they served on a Health Sciences and Technology committee. There was previous work in the area that demonstrated a great variability in physical strength [1] but which did not explain premature rupture [2]. Tom had concerns about the reliability of the methods used previously, particularly as they involved clamping strips of membrane and therefore might cause damage that weakened the samples, and he had an approach in mind that would yield more consistent measures, although it may very well have been closely based on previous work [1].

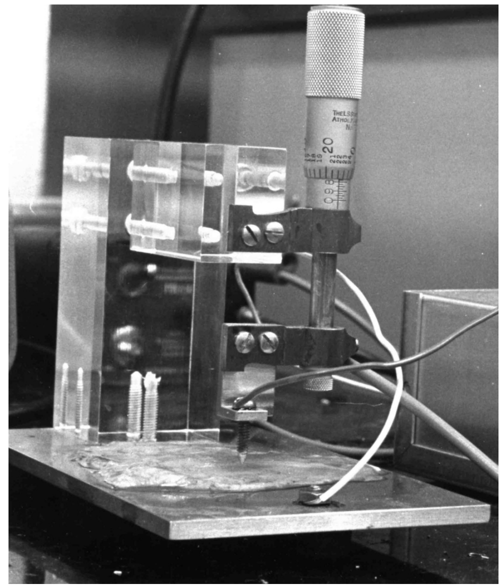

Fig. 1. A micrometer mounted on an insulated brass base, with continuity detector attached to base and micrometer point

The methods: Two devices were constructed for this project. The first was an electronic contact calipers that was constructed by mounting a metal micrometer on perspex blocks so that is was suspended over a brass base (Fig. 1). A brass screw sharpened to a point was attached to the micrometer. A 741 op amp was used as a comparator to detect contact of the screw with any moist material placed on the electrified base. The second device was a membrane clamp (Fig. 2). It consisted of a 3" outside diameter perspex pipe cemented to a flat base. The pipe had two Luer-lock type taps, the upper one a pressure tap and the lower an infusion port. A groove near the top held a 2-220 O-ring in place. After a membrane was draped over the pipe, two 2-222, O-rings were slipped on, one below the inside ring and one above. Two semicircular perspex pieces, containing grooves

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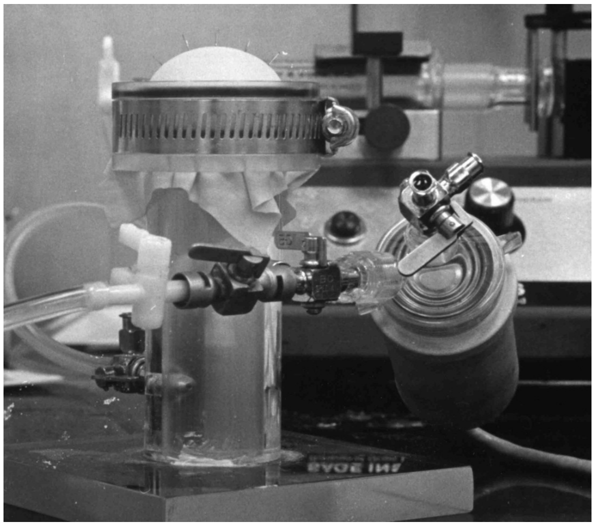

Fig. 2. A Perspex membrane inflator, with three O-rings (two outside, one inside) to securely and gently clamp the edges of the membrane. Two ports allowed for infusion (left) and pressure transduction (right).

in the proper positions were placed around the outside O-rings. A metal hose clamp was fitted into a wide groove on the outside of these pieces and tightened until the O-rings were observed to make about 2 mm of contact with the membrane surface. The pipe was pressurized with a syringe pump filled with mineral oil and the membrane was inflated submerged in 37° mineral oil. Pressure in the pipe was transduced and recorded.

The clever part: Tom sketched out the membrane clamp, I made mechanical drawings, using the skills I learned in Stuyvesant High School in NYC, and it was skillfully constructed by a wonderful machinist, Daniel Spillane, who worked in the Engineering Sciences Bldg. (ESB) at 40 Oxford St. The problem was relating the measured bursting pressure of the membranes to bursting stress. But Tom had worked on a similar problem when calculating the stresses in heart muscle related to ventricular aneurysms [3]. Dan Bogen had written a program in FORTRAN that integrated the differential equations describing the inflation of an elliptical membrane, computing apical extension, other extensions, heights, and curvatures of about 70 points from apex to edge. I was able to simplify his program, given that we were inflating circular membranes that started from a planar configuration, rather than elliptical sections of bulging heart muscle. Experimental data could then be used to calculate the constant k in the uniaxial stress-strain relationship,

$$\sigma = \mu(\lambda^k - 1), \tag{1}$$

where σ = stress, λ = extension (length / unstrained length), and μ and k are constants.

For example, rubber is known to have $k = 2$ [4] while myocardium has $k = 16$. In fact, inflation of a rubber membrane of known thickness in the test apparatus yielded a pressure-volume curve best matched by the $k = 2$ curve produced by the program. Rubber and fetal membranes were photographed during inflation and the apical deflection and

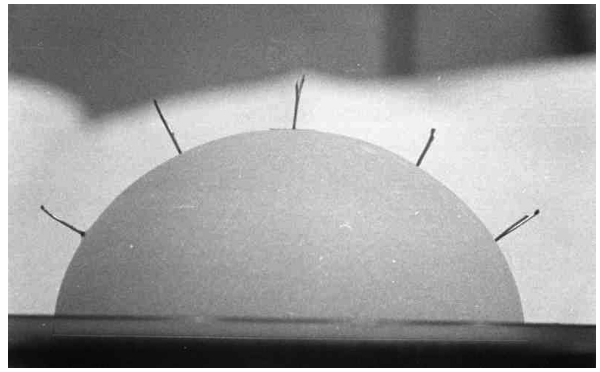

Fig. 3. A latex membrane with glued meter surface markings, inflated with water in the membrane clamp

radius of curvature were derived from digitized photographs (Fig. 3) The inflation-pressure curve of the fetal membranes was best predicted by a $k = 7$, with the greatest error at the greatest inflation (Fig. 4).

The Messy Part

Because fetal membranes emerge attached to placentas, generally in hospitals, I had to initially conduct experiments at Brigham and Women's Hospital, where Dr. Driscoll worked. Being a pathologist, she had space in the morgue, and that's where I set up. I brought the apparatus, artificial amniotic fluid (to store and wet the membranes), and some test placentas to the morgue and got to work. I found that the elastic properties of the membranes were stable for about six hours, after which time the bursting pressure began to decline. So I was able to move my apparatus back to the ESB, as long as I tested the membranes within six hours of delivery. The way this would work was: 1. a call would be made to the lab. 2. I would hop on my bike and ride to the hospital, 3. I would get the placenta with attached membranes, and 4. I would put them in my saddle bag and ride back to the ESB. Then I would trim off pieces of the membranes large enough to be securely clamped, keeping them moist while I measured their thickness in a relaxed state. My labmates were disgusted by the operation, but one of them was making artificial mucus for his coughing machine [5], so he couldn't really talk.

Thickness: A key measure in determining the value of was the thickness measurement, which turned out to vary significantly (up to threefold). But the computed yield stress per unit width of membrane was much more consistent (240-300 N/m.) This suggested a uniformity in strength that made sense from a mechanistic standpoint, as a balloon is only as strong as its weakest portion. Schober *et al.* [6], who also used an inflation apparatus, found similar results.

II. Results

When the membranes ruptured, they tended to rupture near the apex. The outer reticulated portion often burst before the smoother inner portion, but there was little time between bursting of the two layers. However, this two-stage bursting may have caused the deviation from the power-law curve

TABLE I

DETAILS OF EXPERIMENTAL DATA. COLUMNS: PLACENTA NUMBER (EXP.), YIELD STRESS PER UNIT WIDTH (TμH) — N/M, YIELD STRESS (Tμ) — KPA, LINEAR ELASTIC CONSTANT (μ) MPA, LINEAR ELASTIC CONSTANT PER UNIT WIDTH (μH) —- N/M , BURSTING PRESSURE (P) KPA, BURSTING VOLUME (V) NORMALIZED BY DIVIDING BY CUBE OF INFLATION APERTURE RADIUS, THICKNESS (H) — MM , NORMALIZED ELASTIC CONSTANT (K), NUMBER OF PIECES FROM EACH PLACENTA TESTED.

Exp.	Tμh	Tμ	μ	μh	P	V*	h	k	pieces
5	300	466	96	62	23.9	1.26	0.643	7.5	3
6	270	314	141	120	16.7	1.03	0.866	6.8	4
7	288	418	167	118	17.7	0.94	0.754	6.8	3
8	428	769	210	107	23.7	1.32	0.595	6.7	5
9	136	528	255	60	8.3	0.97	0.268	7.0	2
10	273	497	198	110	16.1	1.00	0.567	6.7	3
11	240	439	213	116	26.8	1.18	0.547	7.0	1
mean	276	490	183	0.99	19.0	1.10	0.61	6.9	3
S.D.	86	140	53	0.63	6.3	0.15	0.19	0.3	1.3

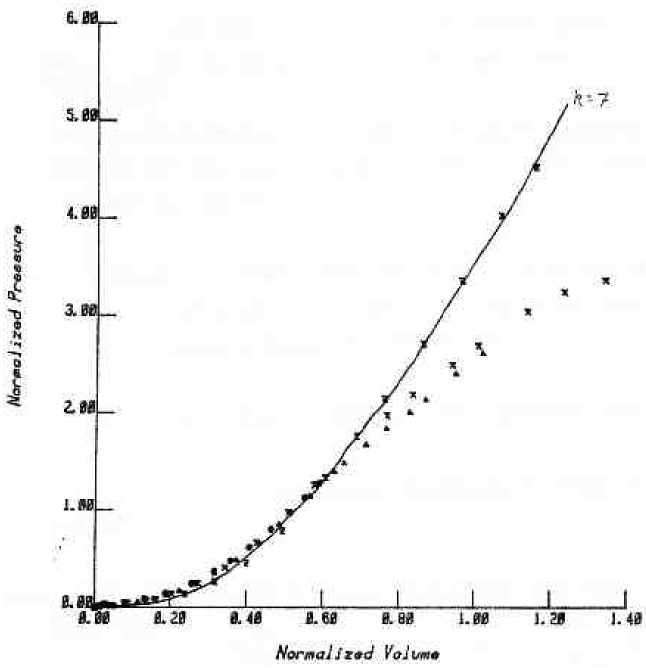

Fig. 4. The Pressure-Volume curves for three pieces of an actual sample, along with the k=7 curve. Two of the samples showed significant deviation from the predicted PV relationship at higher inflation pressures.This appeared to occur with rupture of one component of this two-layer membrane.

shown in (Fig. 4). Table I shows the measured variables: mean thickness, bursting volume and pressure, with the derived elastic equation constants and bursting tension at the apex.

III. DISCUSSION

There were trends for increased thickness and stiffness to be associated with lower bursting volume and pressure, but the correlations were not particularly steep and were affected by one outlier, sample #8, which had higher bursting strength than any others.

A. Clinical correlation

The measures were performed by me before I had any clinical information on the placentas. As it turned out, there were no premature deliveries among these, but there were pathological abnormalities. None of these appeared to be correlated with bursting strength, although the relatively tight range of values in these normally rupturing membranes suggests that the method was reliable. It remains to be seen whether there would be more variation in the mechanical properties in a more varied clinical population. The hypothesis would be that stiffer membranes (higher μh and k) would be associated with premature rupture.

B. Implications

I needed a little more input, particularly from a statistician, when it came time to publish the results. I wish that I had obtained this input earlier, because my current reading of the literature is that measurement of the physical properties of fetal membranes is still of interest and current methods are considered inadequate. The method that Tom devised and which I implemented had several advantages:

1) Swelling was minimized by inflation in mineral oil.
2) The triple O-ring clamp avoided placing uneven pressure on any part of the membranes.
3) There was little dependence on the initial stretch.

Such a device might be considered in future studies, with the goal of answering the questions of whether premature rupture is related to a uniform defect in strength or elasticity, or rather is consequence of focal forces and damage to the membranes enclosing the fetus.

IV. ACKNOWLEDGMENTS

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