Study of the connective tissue of human fetal membranes

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Summary. No significant difference in collagen content per unit surface area was found for 3 different areas of the fetal membranes in relation to the rupture site. Electron microscopy examination revealed that tissue nearer the rupture site contained fewer collagen fibres than did the other 2 sites studied. It is suggested that collagen has been dissolved at the rupture site.

Introduction

There is little quantitative information on the connective tissue components in fetal membranes on which their mechanical properties presumably depend. Measurement of fetal membrane thickness from histological sections showed no significant difference in values for prematurely ruptured and term ruptured membranes (Polishuk, Kohane & Hadar, 1964; Polishuk, Ben-Sira & Kohane, 1965). However, Artal, Sokol, Neuman, Burstein & Stojkov (1976) used a micrometer with fresh tissue and observed a significant decrease in the thickness near the rupture site of prematurely ruptured membranes.

The tensile properties of fetal membranes have been studied and most authors have failed to show a clear relation between tensile strength and premature rupture of membranes (Danforth, McElin & States, 1953; Embrey, 1954; Polishuk, Kohane & Peranio, 1962; Polishuk *et al.*, 1964; Meudt & Meudt, 1967). However, Artal *et al.* (1976) and Parry-Jones & Priya (1976) measured mechanical properties of fetal membranes and found a difference between membranes that ruptured prematurely and normal ones. Work on the tensile properties of fetal membranes is of interest because of its probable relation to a functional property of collagen. In rats, collagen constitutes up to 11% of the dry weight (Harkness & Harkness, 1956).

Because the mechanism of rupture of fetal membrane is not fully understood, this study was undertaken to look for any possible alteration of either the collagen content or the collagen fibre arrangement near the rupture site in full term, spontaneously ruptured membranes.

Materials and Methods

Gestational sacs were obtained at delivery from 15 uncomplicated term pregnancies: 12 were used for chemical analysis and 3 for electron microscopy. The fetal membranes were trimmed from the placenta and spread over a plastic sheet to lie flat. The opening indicating the rupture site was taken as the point of reference and the position of samples taken is shown in Text-fig 1. Care was taken not to let the membranes slip or change shape. To make the points comparable in all placentae, a strip measuring 12 cm in length and 5 cm in width extending from the rupture site to the insertion site was cut out, spread out on a piece of hard polythene and frozen. The

frozen tissue was used because it did not slip about and change shape during the cutting so that the area was well defined. The samples were cut with a cylindrical punch of known area.

Text-fig. 1. The relation of collagen content (mean $\pm$ s.e.m. for 12 samples) of fetal membranes and the site from which the specimens were taken.

Collagen determination

The samples were placed in 20 ml glass test-tubes with 5 ml 6 N-HCl autoclaved at 172.15 KPa (25 lb/in²) for 5 h. Hydroxyproline was determined by Stegmann's method on a Technicon autoanalyser as described by Grant (1964). The collagen content was calculated by multiplying the hydroxyproline content of the hydrolysate by 6.94 (see Jackson & Clearey, 1967).

Electron microscopy

Specimens measuring about 3–5 mm long were taken from sites equivalent to those used for collagen determination and then fixed in 2.5% glutaraldehyde in 0.1 M-phosphate buffer (pH 7.4) for 2–4 h (Sabatini, Bensch & Barnett, 1963). After fixation the specimens were washed 2–3 times for 15 min in phosphate buffer, pH 7.4, and were subsequently post-fixed in 0.1 M-phosphate-buffered saline in 2% osmium tetroxide at pH 7.4 for 2–4 h at room temperature (Palade, 1952). The specimens were then rapidly dehydrated in a graded series of ethanol and finally in propylene oxide for 10 min and embedded in epon (Luft, 1961). Thin sections were cut on an ultramicrotome and mounted on uncoated grids. They were then stained with uranyl acetate (Watson, 1958) for 30 min and with lead citrate (Venable & Coggeshall, 1965) for 2 min and finally examined under a Zeiss EM 95–2 electron microscope.

Results

Quantitative measurements

The relationship between the collagen content and the site from which the sample was taken is shown in Text-fig. 1 and indicates a decrease in the collagen content per unit surface area of fetal membrane in the middle part of the membrane. However, tests for significance of the differences between the means and the homogeneity of variance indicated that there was no significant difference in the collagen content at the three points ($P > 0.25$).

PLATE 1

Electron micrographs of sections cut from Areas A (**Fig. 1**), B (**Fig. 2**) and C (**Fig. 3**) as shown in Text-fig. 1. There are dense collagen fibres in Figs 1 and 2, but near the rupture site (**Fig. 3**) the fibres are fewer. $\times 7200$.

(Facing p. 384)

Qualitative study

As shown in Pl. 1, Figs 1–3, there were many collagen fibres, often in a wave pattern, in membrane samples taken at Areas A and B (Pl. 1, Figs 1 and 2), but in tissue near the rupture site (Pl. 1, Fig. 3) there were fewer fibres and no obvious pattern.

Discussion

The tensile strength measurements so far reported (Danforth *et al.*, 1953; Embrey, 1954; Polishuk *et al.*, 1962; Meudt & Meudt, 1967) indicate the ability of fetal membranes to withstand pressure, and in most organs that are subjected to physical stress and strain collagen plays a major role in resisting such forces (Harkness 1961). Fetal membranes are subjected to continuous tension as gestation proceeds (Parry-Jones & Priya, 1976). Our results showed that there was no significant difference in the amount of collagen near the rupture site compared to other sites.

The method we used for determination of collagen gives the total amount present and not the ratio of the soluble to insoluble fractions. The observed change in the density of the collagen fibres and more homogeneous matrix near the rupture site may be interpreted as dissolution of collagen fibres.

There are no equivalent data for comparison with our collagen measurements of human fetal membranes. Morphologically, however, the pattern with collagen fibres running in curves is similar to that described by Bautzmann & Schröder (1958), but these authors did not compare the rupture site to other sites. Generally, one would expect the weakening of fetal membranes to happen at term and it is not certain what mechanisms are involved.

We did not study prematurely ruptured membranes but the fact that such membranes have less elasticity than do membranes ruptured at full term, as reported by Artal *et al.* (1976) and Parry-Jones & Priya (1976), may be interpreted in terms of change in the chemical nature of collagen rather than the amount. The possibility that other connective tissue components may be involved is also worth considering.

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