

Fig. 3. Fluorescence of a neuroglial 'clump' isolated in, and immediately examined in, 0.9% NaCl solution.

differed from these quoted, in that 1. the present cells, the whole of the cytoplasm fluoresced, compared with less than 18% in the latter, 2. the lipofuscin in our cells was not confined to granules, 3. the present neurons and neuroglia were unfixed and isolated, 4. in the animals were generally younger, and 5. the preparation carried out in the present experiments was much simpler than that in the experiments quoted.

**Résumé.** Nous avons démontré l'autofluorescence dans des neurones et neuroglies détachées du noyau de Deiters du lapin. Les cellules furent isolées et examinées dans une solution de 0.9% NaCl, sans fixation, deshydratation ou addition de fluorochromes. La longueur d'onde de la fluorescence était 5400–5700 Å, et la fluorescence apparaissait d'habitude dans le cytoplasme et le nucléole. On a conclu qu'elle provient de la lipofuscin.

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## Cytochemical Changes of a Glycocalyx of Human Placenta with Maturation

Diastase-resistant, PAS-positive material was found in the free surface of the syncytiotrophoblast of the chorionic villi of the immature human placenta<sup>1</sup>. Electron microscopy<sup>2,3</sup> and further characterization of carbohydrates by light microscopic histochemical techniques<sup>3</sup> indicated the presence in the syncytiotrophoblast of the human immature placenta of a cell coat or glycocalyx which contained carbohydrates. Thus, it was shown that this surface coat contained sialic acid and that it was fluffy in appearance under the electron microscope<sup>3</sup>. We shall report now cytochemical variations of plasmalemma-bound carbohydrates of the syncytiotrophoblast of human placenta with age.

**Materials and methods.** Light microscopy. Tissue blocks of placenta of the 2nd, 5th and 9th month of pregnancy were fixed in 10% formalin containing 2% calcium acetate, dehydrated and embedded in paraffin. Sections were stained with the following procedures: a) Periodic acid-Schiff, with and without prior diastase digestion<sup>4</sup>. b) Colloidal iron<sup>5</sup>. c) Alcian blue (pH 1.0 and 2.5)<sup>5</sup>. d) Alcian blue (pH 1.0 and 2.5)-PAS sequence<sup>5</sup>. e) Aldehyde fuchsin<sup>6</sup>. f) Aldehyde fuchsin-alcian blue sequence<sup>7</sup>. g) Alcian blue-safranin<sup>8</sup>. h) Periodic acid-*p*-diamine procedure<sup>9</sup>. i) Periodic acid-phenylhydrazine-Schiff<sup>8</sup>. j) Alcian blue with graded increases in magnesium chloride (0.1, 0.2, 0.5, 0.8 and 1.0 *M*)<sup>10,11</sup>. k) Azure A at pH 1.0, 2.0, 3.0, 4.0 and 5.0<sup>8</sup>. l) Methylation at 37°C and 60°C<sup>12–14</sup>. m) Methylation (37°C and 60°C)-saponification sequence<sup>8</sup>. n) Sialidase digestion and AB procedure<sup>15</sup>. o) Two-step PAS<sup>16</sup>.

**Electron Microscopy.** Tissue blocks were fixed in glutaraldehyde-Ruthenium red and osmium tetroxide-Ruthenium red<sup>17</sup>. Sections were stained with lead citrate and uranyl acetate.

**Results and discussion.** As shown in the Table, the PAS reaction of the syncytiotrophoblast free surface, less intense at the 2nd month, increased progressively, reaching greatest intensity at the 9th month, in contrast with the basement membrane in which no significant changes were noted (Figure 1–3).

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Histochemical reactions in human placenta

		PAS	Diast.-PAS	Two-Step PAS	Coloid. Iron	AB pH 1.0	AB pH 2.5	AB pH 1.0-PAS	AB pH 2.5-PAS	Ald. Fuchls.	Ald. Fuc.-AB	AB-Safranin	PA- <i>p</i> -Diamine	PA-Ph-Schiff	AB-Mg Cl <sub>2</sub> <i>M</i> pH						Azure A pH			Meth. 37°C	Meth. 60°C	M-Sap. 37°C	M-Sap. 60°C	Sialidase	Control
															0.1	0.5	1.0	1.0	3.0	5.0	AB				AB				
Age of Placenta	Structure																												
2nd month	SCS	R2	R2	R2	B4	B1	B4	P2	PB3	V2	B4	B4	D3	R2	B4	B3	B1	0	0	0	P4	B3	0	B4	B1	B3	B4		
	BM	R4	R4	R2	B4	B4	B3	PB3	PB3	V3	V4	B3	D2	R1	B3	B2	B1	0	0	0	P4	B2	0	B2	0	B3	B3		
5th month	SC	R3	R3	R2	B3	B1	B3	P2	PB3	V2	B3	B1	D2	R3	B3	B2	0	0	0	P1	P1	B2	0	B3	B2	B2	B3		
	BM	R4	R4	R2	B2	B2	B1	PB3	PB3	V1	V1	B1	D1	R1	B2	B1	B1	0	0	0	P1	B1	0	B1	0	B1	B1		
9th month	SC	R4	R4	R3	B2	0	B2	PR3	P3	V2	B3	B1	D1	R3	B2	B1	0	0	0	0	P1	B1	0	B1	B1	B2	B2		
	BM	R4	R4	R2	B1	B1	B1	PB3	PB3	V1	V1	B1	D1	R1	B1	0	0	0	0	0	P1	B1	0	B1	0	B1	B1		

The abreviations used are: SCS, surface coat, syncytiotrophoblast; BM, basal membrane; R, red; B, blue; P, purple; D, dark-gray; PR, purple-red; PB, purple-blue; V, violet; VB, violet-blue. Numbers indicate degree of reaction.

The electron microscope revealed that Ruthenium red enhanced the electron opacity of the syncytiotrophoblast surface coat, in which a filamentous material was observed (Figure 4).

Procedures for the demonstration of anionic groups such as colloidal iron, alcian blue, and aldehyde fuchsin combined with blocking procedures and neuraminidase digestion indicated the presence of sialic acid in the free cell surface of the syncytiotrophoblast of the human placenta. In addition, sulphate groups were noticed as shown by the distinct reactivity of the glycocalyx with alcian blue, pH 1.0 and alcian blue containing 0.5 M Mg Cl<sub>2</sub>. Furthermore, methylation at 60°C, followed by saponification, decreased significantly, but did not abolished, the alcianophilia of surface coat indicating that both sulfate anions and carboxyl group of sialic acid and/or uronic acids were present. Aldehyde fuchsin stains mucosubstances<sup>6</sup>. The reaction of the glycocalyx with aldehyde fuchsin did not change with age, whereas the alcianophilia did. Furthermore, if the behaviour of the cell surface after neuraminidase treatment is observed in the Table, it will be noted that sialic acid was present

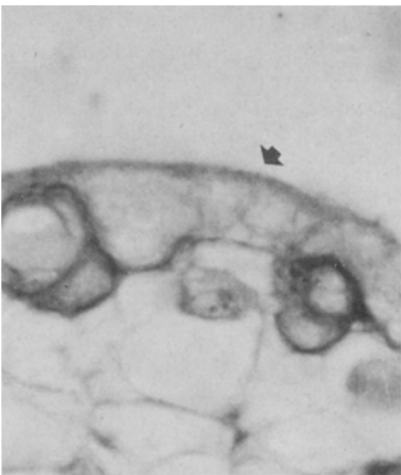


Fig. 2. 5th month.

Human placenta.

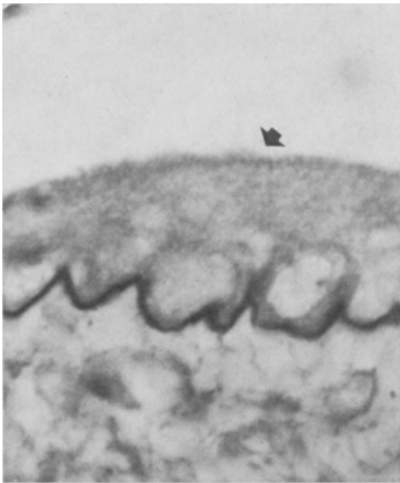


Fig. 1. 2nd month of pregnancy.

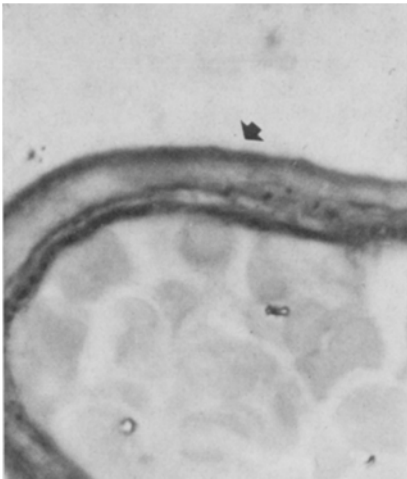


Fig. 3. 9th month, PAS. The glycocalyx of the luminal surface of a chorionic villus is more stained than the remaining cytoplasm. Note that the glycocalyx becomes increasingly apparent with maturation of the placenta (arrow). Basement membrane is deeply and distinctly stained. × 1000.

in the free surface coat of the placenta of 2nd and 5th months but not in the placenta at term. The periodic acid-*p*-diamine procedure indicated the presence of both reactive and unreactive periodate polymers<sup>9</sup>. The two-step PAS<sup>16</sup> would suggest that a glycoprotein as well as a mucopolysaccharide might be present in the surface coat. In the basement membrane, periodic acid reactive carbohydrate visualized with Schiff reagent did not vary with age. As shown in the Table, acid groups in basement membrane are largely sulphate anions, which diminished with age.

The surface of the villi in contact with the maternal blood which is in active exchange with the fetus blood is very extensive, suggesting that large amounts of glycocalyx components are present in human placenta. This, as well as the noted variations in histochemical

reactions and the progressive increase in the thickness of the syncytiotrophoblast glycocalyx, would suggest that the surface mucous coat or glycocalyx of the luminal surface of chorionic villi might play an important physiological role.

Human chorionic gonadotrophin (HCG) was localized in the free maternal surface of the syncytiotrophoblast both at the light microscopic<sup>18</sup> and ultra-structural levels<sup>19</sup> using immunocytochemical techniques. These observations would link the HCG, a glycoprotein, with the mucosubstances of the syncytiotrophoblast surface. Yet the exact relationship or identity of some components of the surface coat with HCG is so far unknown.

It has been suggested that the complex carbohydrates of certain glycocalyxes become part of the product of activity of secreting cells<sup>8</sup>. For the Tamm Horsfall mucoid, data in support of its origin in the luminal cell surfaces of transitional epithelium have been reported<sup>20</sup>.

On the basis of the histochemical findings, it can be concluded that the mucous coat of the free maternal surface of the syncytiotrophoblast of the human placenta contains polyanionic complex carbohydrates, that is, components which in the histochemical nomenclature are called acidic mucosubstances. The chemical heterogeneity of this glycocalyx seems indicated. Thus, acid mucopolysaccharide and a sialic acid-containing glycoprotein might be present, though the possibility of a sialic acid-containing mucopolysaccharide, such as keratan sulphate, cannot be ruled out<sup>21</sup>.

*Resumen.* En la superficie libre, en contacto con la sangre materna, del sinciciotrofoblasto de la placenta humana se observó un glicocáliz en el que se han caracterizado carbohidratos complejos, grupos sulfato y ácido siálico. En este glicocáliz se observaron cambios citoquímicos con la edad de la placenta. Con el microscopio electrónico, se describe la apariencia filamentosa del glicocáliz de placenta humana.

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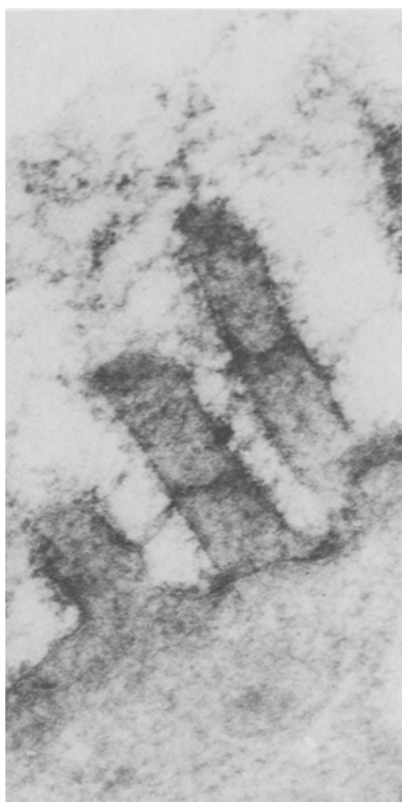


Fig. 4. Human placenta of 2nd month of pregnancy. Ruthenium red has revealed a meshwork of filaments extending from the surface of microvilli.  $\times 88,000$ .

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## Preliminary Observations on Pituicytes in Tissue Culture

The fine structure of pituicytes has been already investigated both under physiological and experimental conditions<sup>1-6</sup>, but knowledge about the functional role of them is still lacking. These preliminary observations deal with the ultrastructural features of pituicytes as grown in organotypic cultures of neural lobes in adult rats.

*Materials and methods.* Tissue culture technique: hypophyseal neural lobes of adult male rats were placed into organ tissue culture Falcon dishes. The culture medium used was Eagle's Minimum Essential Medium.

Electron microscopic technique: the explanted neural lobes were fixed partly 5 and partly 10 days after the

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