

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 sincitiotrofoblasto 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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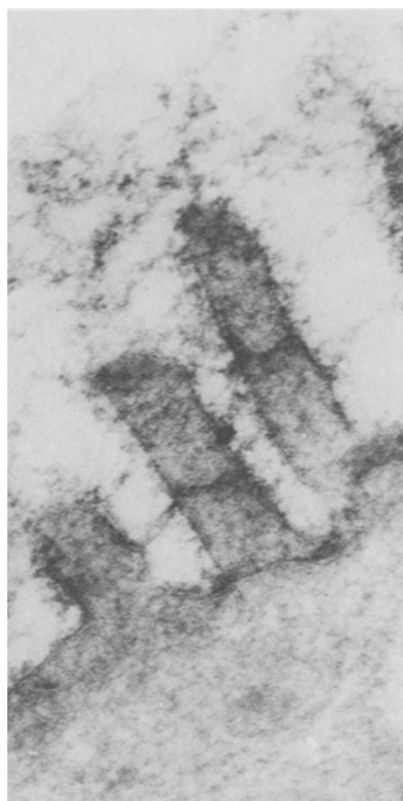


Fig. 4. Human placenta of 2nd month of pregnancy. Ruthenium red has revealed a meshwork of filaments extending from the surface of microvilli.  $\times 88,000$ .

<sup>18</sup> A. R. MIDGLEY JR. and J. B. PIERCE JR., *J. Exp. Med.* **115**, 289 (1962).

<sup>19</sup> R. B. DRESKIN, S. S. SPICER and W. R. GREENE, *J. Histochem. Cytochem.* **18**, 862 (1970).

<sup>20</sup> B. MONIS, A. GOMEZ, A. CANDIOTTI and N. IBANEZ, *Life Sci.*, Part 2, **11**, 699 (1972).

<sup>21</sup> This investigation was supported by a grant of the Consejo Nacional de Investigaciones Científicas y Técnicas (Argentina).

## 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

<sup>1</sup> R. BARER and K. LEDERIS, *Z. Zellforsch.* **75**, 201 (1966).

<sup>2</sup> D. ZAMBRANO and F. DE ROBERTIS, *Z. Zellforsch.* **86**, 14 (1968).

<sup>3</sup> H. D. DELLMANN and P. A. OWSLEY, *Z. Zellforsch.* **94**, 325 (1969).

<sup>4</sup> J. KRSULOVIC and G. BRUCKNER, *Z. Zellforsch.* **99**, 210 (1969).

<sup>5</sup> Y. NAKAI, *Z. Zellforsch.* **110**, 27 (1970).

<sup>6</sup> J. A. KIERNAN, *J. Anat.* **109**, 97 (1971).

explantation. The fixation was carried out in glutaraldehyde, the postfixation in osmium tetroxide, the embedding in Epon 812.

**Results.** 5 days after the explantation, neurosecretory fibres undergo a massive and progressive degeneration due to the experimentally induced disconnection of the neural lobe from the hypothalamic nuclei. Normally such fibres occupy a large part of the hypophyseal neural lobe, so that 10 days after the beginning of this experiment

wide interstitial spaces show only degenerating or degenerated fibres and granules residua (Figure 1 and 2). Irregularly-shaped cells appear randomly interspersed among such residue and show many peripheral cytoplasmic processes (Figure 1), quite frequently coming into contact with each other (Figure 1 and 2). Lipid-like droplets occupy most part of the cytoplasm (Figure 1, 2, 3 and 4). Many roundish or elongated dense bodies accumulate in certain cytoplasmic areas (Figure 2 and 3).

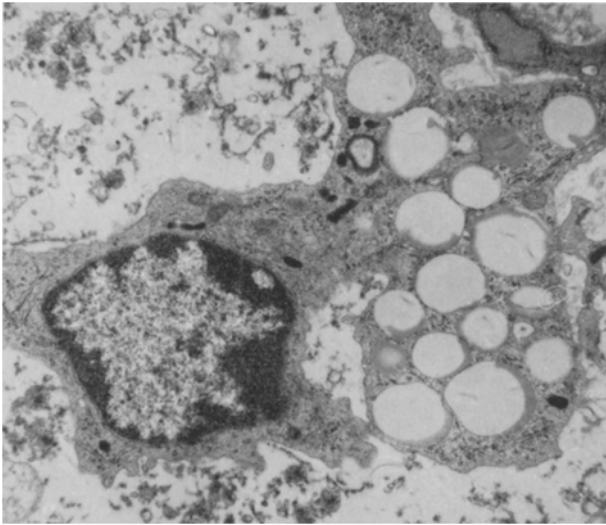


Fig. 1. One irregularly-shaped pituicyte-like cell with an eccentric nucleus, scattered lipid droplets and peripheral cytoplasmic processes. The cell is surrounded by fibres residua.  $\times 12,300$ .

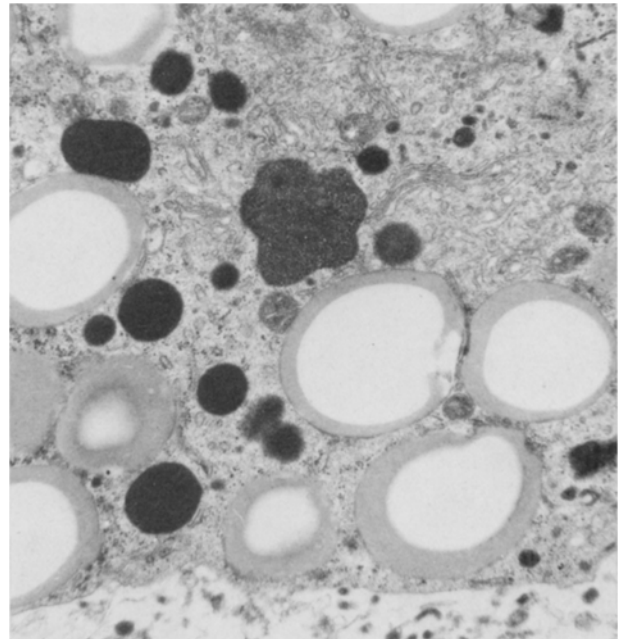


Fig. 3. A portion of the pituicytic cytoplasm shows roundish dense bodies, lipid droplets and some Golgi apparatus.  $\times 17,500$ .

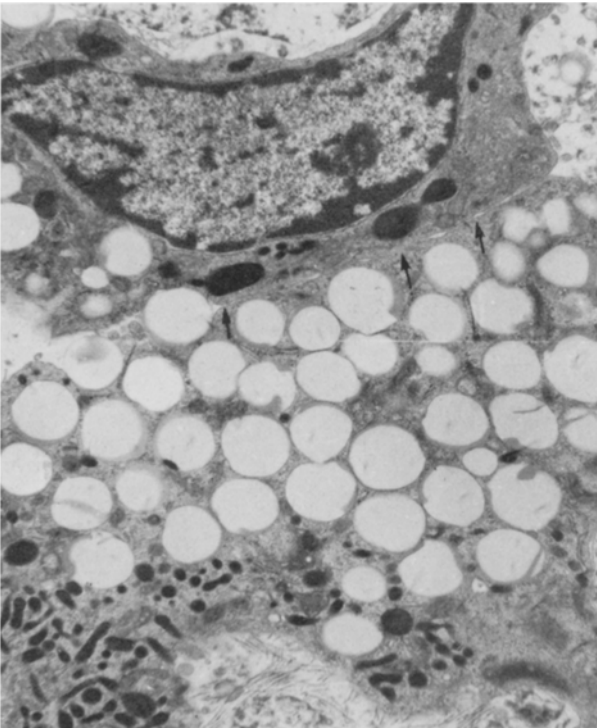


Fig. 2. Two pituicyte-like cells come into contact each other (arrows). Observe the abundant vacuolized lipid droplets and, at the low part of the figure, many small dense bodies.  $\times 10,200$ .

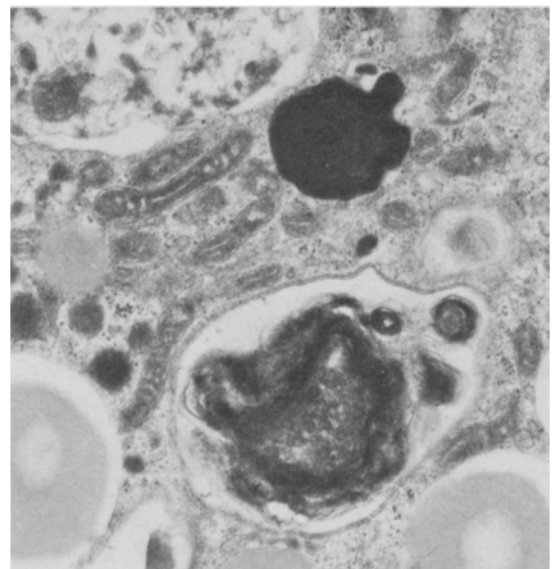


Fig. 4. A phagocytized nerve fibre is contained within a cytoplasmic vacuole of a pituicyte-like cell.  $\times 20,000$ .

Sometimes, degenerating densified neuro-secretory fibres appear, being phagocytized by pituicytes themselves (Figure 4). Few cisternae of the rough endoplasmic reticulum, small mitochondria and scarce free ribosomes are observed. The nucleus is variably-shaped, showing abundant masses of condensed chromatin (Figure 1 and 2). Tissue culture provides a simplified experimental model for morpho-physiological investigations: these preliminary observations utilize this technique to investigate organotypic cultures of neural lobes where randomly localized pituicyte-like cells survive among degenerating neuro-secretory fibres. The ultrastructural features common to in vitro and in vivo pituicytes appear to be: a) the irregularity of the cell shape due to the presence of many peripheral cytoplasmic processes; b) the abundance of lipid droplets which are peculiar to the neural lobe of rats<sup>7</sup>; c) the high number of phagocytized degenerating fibres; d) the frequent reciprocal contact between 2 or more pituicyte-like cells.

Finally, the organotypic culture of the neural lobe makes it possible to obtain a relatively pure population of pituicytes, due to the degeneration of the interstitial

neurosecretory fibres: such a fact may be quite useful to evaluate the morpho-physiological behaviour of pituicytes under peculiar experimental conditions.

*Riassunto.* Nella presente nota preliminare sono analizzati gli aspetti ultrastrutturali di lobi neurali post-ipofisari in coltura organotipica ove si è evidenziata la massiccia degenerazione delle fibre neurosecretorie e la presenza di cellule simil-pituicitarie attivamente fagocitanti le fibre stesse.

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<sup>7</sup> F. RAVIOLA and G. RAVIOLA, *J. Histochem. Cytochem.* 11, 176 (1953).

### Ultrastructural Features of Pituicytes in the Neural Lobe of Adult Rats

The fine structure of pituicytes has been investigated mainly under experimental conditions<sup>1-3</sup>. The concern of this report is to re-investigate synthetically the ultrastructure of pituicytes under physiological conditions, so far as the general features of this type of cell are concerned.

The hypophyseal neural lobe of adult normal Wistar rats of both sexes – fed with standard diet and water ad libitum – was prefixed with glutaraldehyde in phosphate or cacodylate buffer, postfixed in osmium tetroxide, dehydrated in ethanol and embedded in Epon.

Pituicytes occupy a large part of the neural lobe being interposed among the neuro-secretory fibers originating from hypothalamic nuclei. They show a roundish nucleus with a clear nucleoplasm and a large cytoplasmic area

consisting of a large perinuclear halo (Figure 1) and of peripheral elongated processes (Figure 2). A variable number of lipid droplets are scattered throughout the cytoplasm (Figure 1 and 2). At its periphery, the pituicyte assumes contact with the following structures: a) with the neuro-secretory fibres (for most part of its edge), b) with the interstitial space often represented by basement membrane material (in certain limited areas), c) with other pituicytes.

<sup>1</sup> S. L. PALAY, *Anat. Rec.* 121, 348 (1955).

<sup>2</sup> J. KRSULOVIC and G. BRÜCKNER, *Z. Zellforsch.* 99, 210 (1969).

<sup>3</sup> C. OLIVIERI-SANGIACOMO, *Experientia* 28, 1362 (1972).

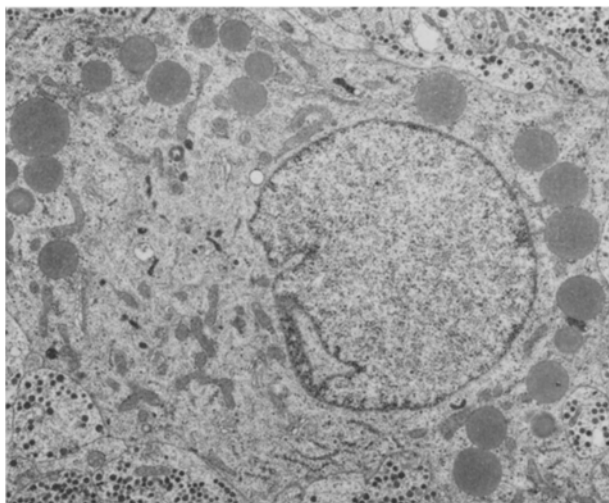


Fig. 1. Neural lobe. A large pituicytic area, recognizable from the typical lipid droplets, is surrounded all around by neurosecretory fibers.  $\times 5850$ .

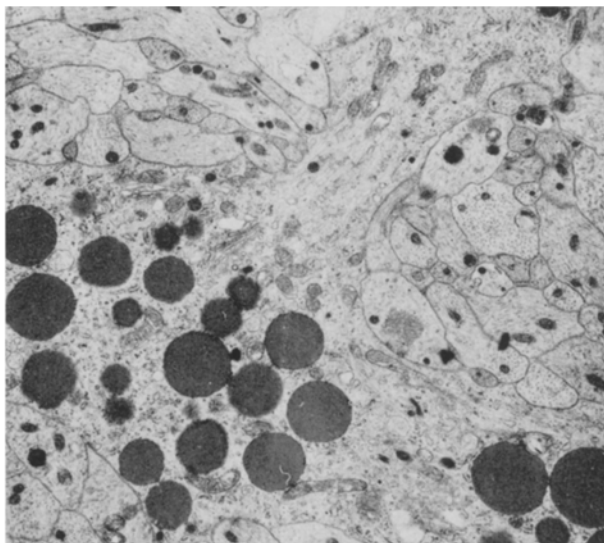


Fig. 2. Neural lobe. A small pituicytic process is emanating from a bigger one rich in lipid droplets. Note, to the right, a synaptoid contact.  $\times 7700$ .