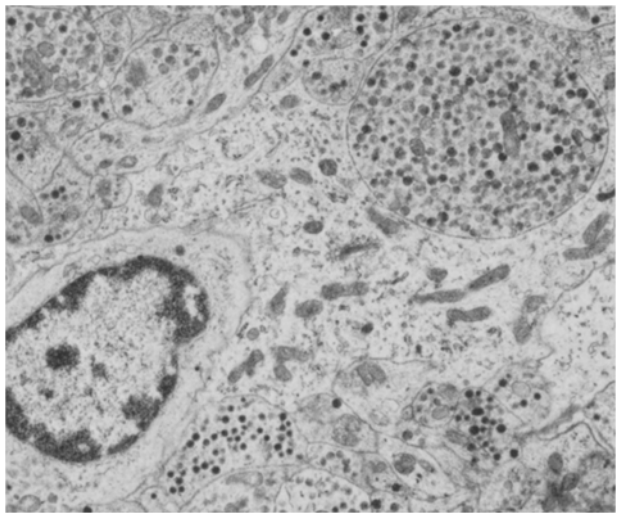

Fig. 3. Neural lobe. At the left a small, scarcely-differentiated cell shows a clear matrix and few organelles. At the right, a neuro-secretory ending is invaginated within the pituicytic cytoplasm. × 9750.

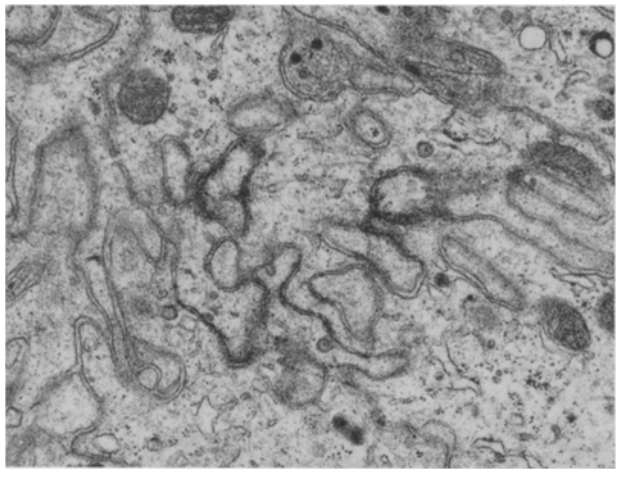

Fig. 4. Neural lobe. Many junctional complexes are interposed among pituicytic processes interweaving with each other. × 22275.

The neuro-secretory fibres surround the pituicyte and often appear invaginated in it (Figure 3). At some point it is possible to observe the synaptoid contacts already described (Figure 2)[4,5]. The interstitial space which forms, in the neural lobe of rodents, a particularly rich trabecular network[6], comes into contact with pituicytic surface only in certain circumscribed zones.

Interpituicytic contacts are often interposed between peripheral confluencing pituicytic processes: these processes reciprocally interdigitating assume in such zones a labyrintic configuration (Figure 4). The interpituicytic contact points are characterized by the presence of variably-shaped junctional complexes generally lacking the filamentous contribution[7].

Furthermore, small scarcely-differentiated cells are observed which show a dense nucleus and a clear cytoplasmic matrix, and often are in contact with typical pituicytes (Figure 3).

The pituicyte is considered as an interstitial glia-like specialized cell of the hypophyseal neural lobe[8–10]. The possibility has been suggested of a bidirectional trophic action between pituicytes and neuro-secretory fibres: one morphological expression of such action may be given by the synaptoid contacts already described[4,5], but another may be represented by the pituicytic processes which, being so extensively developed, enormously increase the surface contact and the metabolic exchange between the two structures.

The labyrinthic interweaving of peripheral processes interconnected by variably-shaped junctional complexes seems to be a linking interpituicytic device, both from a mechanical and metabolic point of view. The existence of different types of pituicytes[10] has been supposed. These observations, although not confirming such a hypothesis at least in the rat show the existence of rare and scarcely-differentiated cells lacking the typical features of pituicytes but possibly differentiating in that direction under normal or peculiar conditions.

Riassunto. Nella presente nota sono sinteticamente analizzati i caratteri ultrastrutturali dei pituiciti e, in particolare, i rapporti dei pituiciti stessi con le fibre neuro-secretorie spesso realizzantisi a livello dei prolungamenti pituicitari periferici, le zone di contatto interpituicitario ricche di complessi giunzionali e l'esistenza nel lobo neurale di cellule poco differenziate che potrebbero rappresentare i precursori dei pituiciti maturi.

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On the Occurrence of Spaces Similar to Intercellular Canaliculi in the Leydig Cells of Mice

Numerous recent electron microscopic observations have confirmed the presence of a large perivascular space in different endocrine cells. Further, it is well known that the plasma membrane of the pericapillary secretory cells are generally provided with numerous irregular microvilli. These morphological aspects have been commonly related to the secretory exchanges occurring between the endocrine cells and the endothelial cells[1].

In the course of submicroscopic studies on the mouse testis, an unusual arrangement of the perivascular and intercellular spaces in the Leydig cells was observed. The present paper deals with the presence of characteristic spaces somewhat similar to 'canaliculi' existing in the

[1] A. GIROD, *Leçons sur les Glandes Endocrines* (Simep, Lyon 1968).

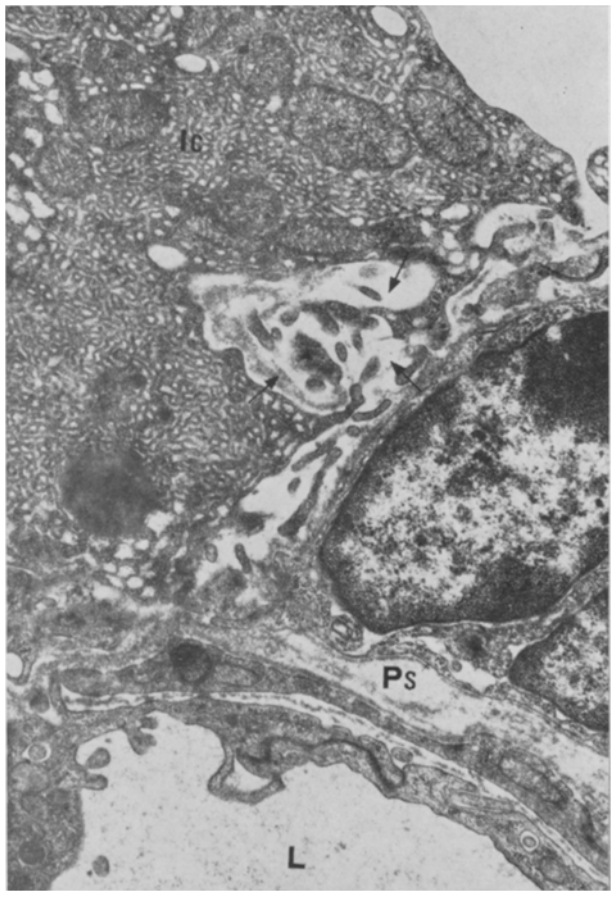

Fig. 1. Mouse testis. A large perivascular space (PS) is clearly in connection with a deep infolding (→) of a Leydig cell (IC) provided with numerous microvilli; on the right: pericapillary cell; L, lumen. ×18,000.

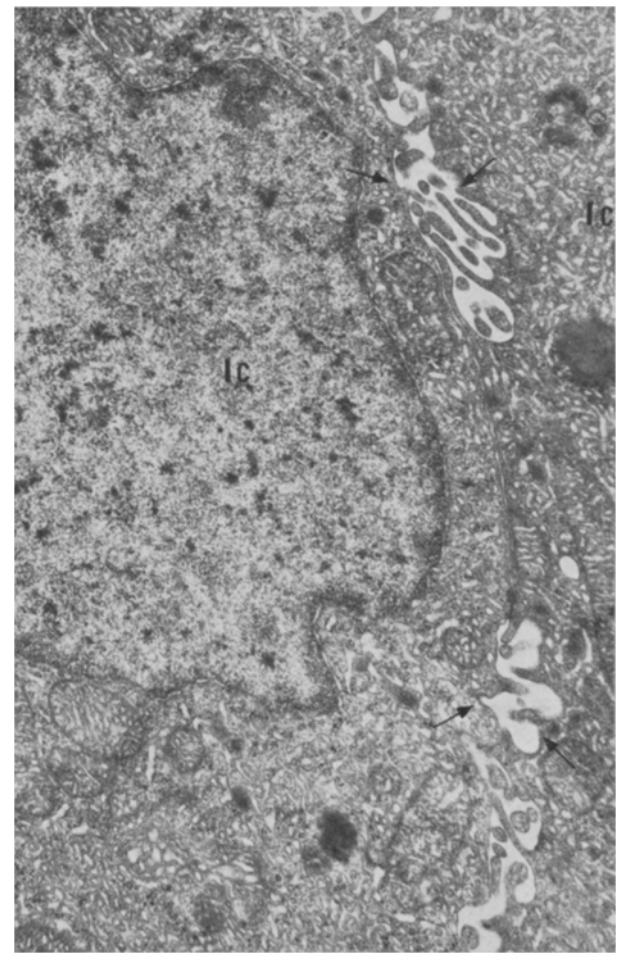

Fig. 2. Mouse testis. 2 adjacent interstitial cells (IC) form invaginations similar to 'intercellular canaliculi' (→) in which numerous microvilli protrude. ×18.000.

Leydig cells which has probably not been reported so far[2].

Material and method. The present observations were carried out on 12 mature mice of Swiss strain, kept under strictly uniform conditions and on a standard diet. The specimens were fixed in 2% glutaraldehyde for 2 h and post-fixed in 1% osmium tetroxide buffered at pH 7.2[3]. The material was embedded in Epon 812[4] after dehydratation in a series of graded ethanol. Sections cut on a Porter-Blum MT-1 ultramicrotome using glass knives were stained with lead citrate[5] and uranyl acetate[6] and observed in a Zeiss EM 9A electron microscope.

Observations. The interstitial Leydig cells of the mouse testis present in the connective tissue around the seminiferous tubules appeared in sections scattered or more usually grouped in a small number of elements. The cells arranged in groups were observed strictly associated to each other by means of 'junctional complexes', specialized 'tight junctions' and interdigitations of the plasma membrane.

The capillaries appeared frequently separated from the Leydig cells by a large space, irregularly shaped and of a variable width in sections (about 0.4–1.5 μm). An amorphous material originated from the basal lamina was noted in the pericapillary spaces. The interstitial cells towards the endothelial wall showed an irregular profile forming large evaginations and numerous microvilli (Figure 1).

In some areas between 2 adjacent interstitial cells, regular invaginations very similar to 'intercellular canaliculi' were noted. Numerous microvilli protrude from the contiguous cells in these spaces. In other sections some of the intercellular spaces were clearly observed in connection with the infoldings present in the pericapillary spaces.

Discussion. The actual findings confirmed the existence of large perivascular spaces in the Leydig cells of the mouse testis. Further, intercellular spaces somewhat similar to small channels were sometimes observed between 2 adjacent cells. A morphological pattern of such a type has occasionally been reported in previous researches on the fine structure of different steroidogenic cells

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(human luteal cells)[7–13]. Particularly GREEN, GARCILAZO and MAQUEO[14] furnished a detailed description of these spaces in human corpora lutea of the menstrual cycle and pregnancy.

The 'canaliculi' remarkably similar to bile canaliculi appeared to terminate in the perivascular connective tissue of the capillaries of the corpus luteum and were interpreted as an intercellular system of channels for the transport of luteal cells secretion from the cells to the capillary[14].

Comparing the present results with the findings of the above papers, it seems reasonable to suggest that similar large pericapillary and 'like-canaliculi' intercellular spaces might also be present in the Leydig cells of the mouse testis. Although it should be added that they never reach the same complexity observed in the human corpora lutea where the cells are grouped in large amounts.

Concluding, we propose that the small channels observed might rather be interpreted as narrow and deep infoldings of the perivascular spaces among the Leydig cells. Therefore they might probably serve to increase the secreting surface of the cells.

Riassunto. Tra le cellule interstiziali del testicolo del topo sono state osservate al M/E strette e profonde invaginazioni simili a «canalicoli intercellulari». Le fessure intercellulari sono in continuità con gli ampi spazi pericapillari e probabilmente servono ad aumentare la superficie secernente delle cellule di Leydig.

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Histochemical Differentiation of the Formaldehyde-Induced Fluorophores Derived from Dopamine and L-DOPA

The fate of administered L-3, 4-dihydroxyphenylalanine (L-DOPA) has been investigated in animals by the formaldehyde-fluorescence method[1]. One difficulty in this type of study is that histochemical differentiation of L-DOPA from dopamine (DA) has been impossible as their fluorophores have similar properties[2]. In the present study a method of differentiating the fluorophores derived from L-DOPA and DA is reported, based on their rates of photodecomposition during irradiation with UV-light.

Material and methods. A range of concentrations of L-DOPA and dopamine hydrochloride (Koch-Light) were prepared by dissolving these substances in 0.04 *N* HCl. Each solution was then added to an equal volume of 2% aqueous bovine serum albumin. (Merck). The final solutions contained a range of 6 concentrations of L-DOPA from 0.062 to 3 mg/ml, and a range of 9 concentrations of DA from 0.062 to 12 mg/ml. 1 µl droplets from each of these final solutions were air dried prior to exposure to gaseous formaldehyde, generated from paraformaldehyde stored at a relative humidity of 58%, for 1 h at 80 °C. The reaction vessel contained 5 g paraformaldehyde/l. The droplets were then mounted in Entellan (Merck) and examined with a Zeiss large fluorescence microscope incorporating the EMI 6256B photomultiplier. The light source was a stabilized HBO 100 Osram mercury lamp and a combination of the exciter filters UG5 and BG 38/2.5 mm (Schott) was selected together with the barrier filter 47 (Zeiss). The droplets were examined by vertical illumination using a X 40 oil immersion objective with a numerical aperture of 1.0. The intensity of the exciting light was much reduced by an iris diaphragm during the selection of the areas for fluorimetry, and the fluorescence intensity during photodecomposition was measured for 2 min from circular areas each with a diameter of 10 µm. One area was chosen from the most intense region of fluorescence of each droplet to be examined.

Results. The dried droplets had approximately circular outlines with diameters ranging from 3–4 mm. There was no consistent variation in droplet diameter in relation to DA or L-DOPA concentration in the droplet solution. The drying process led to the fluorophore being concentrated in a ring surrounding a central area which showed minimal fluorescence. The resulting fluorescent rings showed differences in width, depending on the concentration of DA or L-DOPA in the droplet solution. (Figure 1).

The initial maximal intensity of fluorescence and the degree of fading of the fluorophores derived from the different concentrations of DA and L-DOPA in the droplet solutions are shown in the Table. Further droplets were examined and the results are shown in Figure 2.

In Figure 2, the 2 sets of final values for ther percentage of the initial intensity were significantly different at a level $P=0.008$ (Mann-Whitney U-test, 2-tailed). The mean values for the initial fluorescence intensity, in arbitrary units, were 80 (DA) and 77 (L-DOPA). As the preparations of DA and L-DOPA had similar molecular weights the droplets examined for Figure 2 were derived from 2 solutions of approximately equimolar concentration. There was no obvious difference between the drying patterns of the droplets derived from the 2 solutions.

Discussion. The fluorophore derived from DA is a 3,4-dihydroisoquinoline which is in a pH-dependent equilibrium with the corresponding tautomeric quinoidal form. The latter exhibits fluorescence with an emission maximum at 480 nm, while the former has an emission maximum at 510 nm[3]. The reaction conditions for fluorophore formation in a model system of albumin-containing droplets may differ from those associated with tissue sections, where the quinoidal form predominates[3].

The results in Figure 1 and in the Table suggest that concentration-dependent quenching[4] of the fluorophores had taken place, as the concentration of DA or L-DOPA in the droplet solutions was increased. Differences in the concentration of fluorophore in the fluorescent rings of the droplets derived from different concentrations of DA or

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