(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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L-DOPA would have been partly due to the changes in the drying pattern of the droplets, as well as to the concentrations of DA or L-DOPA in the droplet solutions. At equivalent concentrations up to 1.5 mg/ml in the droplet solutions, both L-DOPA and DA produced a similar drying pattern of the droplets with respect to the width of the fluorescent ring, but the width of the ring of a typical droplet derived from the solution of L-DOPA 3 mg/ml was less than that of a droplet derived from DA 3 mg/ml. This may explain the apparent difference in the degree of quenching of the fluorescence between the fluorophores from DA and L-DOPA associated with a concentration of 3 mg/ml in the droplet solutions. Differences in the fading rates of the fluorophores derived from L-DOPA and DA were maintained despite the different concentrations of DA and L-DOPA in the droplet solutions and the different degrees of quenching (Table).

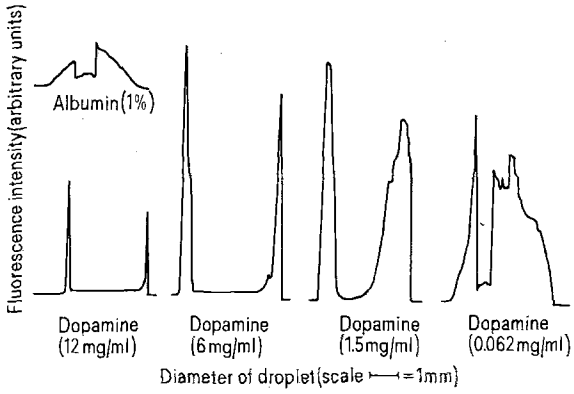

Fig. 1. The distribution and intensity of formaldehyde-induced fluorescence in dried albumin-containing 1 µl droplets, in relation to the concentration of dopamine in the droplet solution. The fluorescence intensity was recorded graphically by a pen recorder from strips 10 µm in width, which were scanned at a constant speed across the diameters of 4 typical droplets. A 1 µl droplet of a 1% albumin solution was also examined. The concentration of dopamine in each droplet solution is shown.

Photodecomposition of formaldehyde-induced fluorophores derived from L-DOPA and DA in a model system of dried albumin-containing 1 µl droplets

Concentration in droplet solution (mg/ml)		Initial intensity of fluorescence (arbitrary units)	Fluorescence intensity after 2 min of photodecomposition (% of initial intensity)
DA	12	53	79
	9	65	77
	6	74	78
	3	75	79
	1.5	67	78
	0.75	55	78
	0.375	43	81
	0.125	38	82
	0.062	34	82
L-DOPA	3	47	68
	1.5	79	65
	0.75	76	62
	0.375	52	63
	0.125	39	67
	0.062	34	71
	Nil	12	83

Each value of fluorescence intensity represents the mean of 3 readings, each from a separate droplet. The areas for fluorimetry were selected from the region of each droplet showing the most intense fluorescence. Values for control droplets, prepared from 1% albumin in 0.02 *N* HCl are also shown.

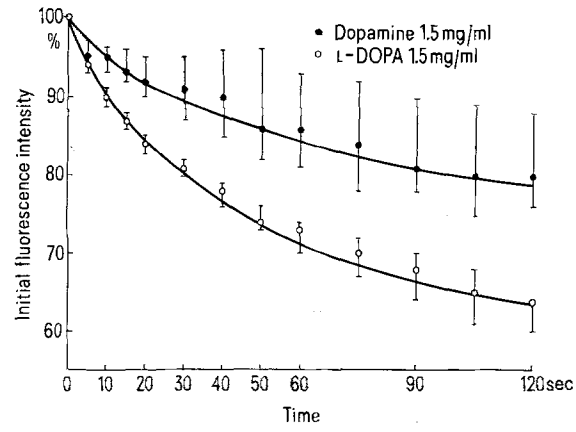

Fig. 2. Rates of photodecomposition of formaldehyde-induced fluorophores derived from L-DOPA and DA in a model system of dried albumin-containing 1 µl droplets. A point represents the mean value of 5 readings, each from a different droplet, and the range of each mean value is given. The concentration of L-DOPA and DA in the droplet solutions is shown. The areas for fluorimetry were selected from the region of each droplet showing the most intense fluorescence.

The contrast which was found in the model system was studied in tissue sections of rat brain, which had been prepared for fluorescence microscopy[1]. A sufficient i.p. dose of L-DOPA resulted in a diffuse formaldehyde-induced fluorescence in the neuropil of the cerebral cortex, which faded more rapidly than the diffuse formaldehyde-induced fluorescence in the neuropil of the caudate nucleus of a rat which had not received L-DOPA. The fluorescence in the neuropil of the caudate nucleus is almost entirely due to DA[5] while the cortical fluorescence following a dose of L-DOPA was presumably mainly derived from L-DOPA[6–8].

Résumé. On signale une méthode de différenciation de la fluorescence provenant du L-DOPA et celle de la dopamine, basée sur leur taux de décoloration lorsqu'elles sont exposées à la lumière ultra-violette. L'intensité de la fluorescence provenant du L-DOPA s'est avérée plus rapide à disparaître que celle provenant de la dopamine.

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