material of higher electron density and have different sizes. The smaller ones are mostly seen close to perivascular and intercellular spaces, while those of larger size have a more peripheral localization.

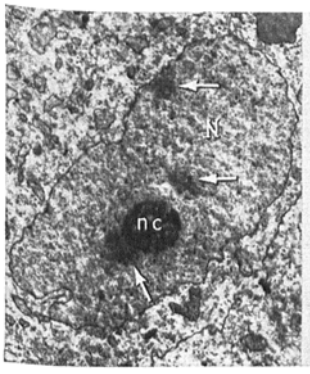

Fig. 3

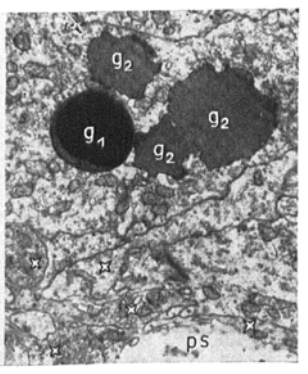

Fig. 4

Fig. 3. Adult rat pineal body. The picture illustrates that the nucleus of a pinealocyte (N) is greater than those observed in Figure 1. The nucleolus (nc) is larger and eterochromatin is well visible. Magnification × 3300.

Fig. 4. Adult rat pineal body. In the cytoplasm of a pinealocyte four granules are present. One of them (g_1), of regular round shape, appears to contain an electron dense droplet. Other granules (g_2) have a more irregular shape and lower electron density. Intersecting cytoplasmic processes (★) are visible around the perivascular space (ps). Magnification × 4300.

The granules of pinealocytes are supposed to contain lipids, serotonin, melatonin and other various biogenic amines (Csillik[13], De Martino, De Luca, Minio Paluello, Tonietti, Orci[14]).

Conclusions. In previous investigations, carried out during different ages, the ^{32}P uptake, the nuclear and cytoplasmic volumes of pinealocytes (De Martino, Peruzy, Pavoni, Capone, and Lintas[15]) and the number of osmiophilic granules (De Martino, De Luca, Minio Paluello, Tonietti, Orci[14]) appeared to increase in the adult rats.

Our present ultrastructural findings give further support to the opinion that neurosecretory activity of the pineal body increases during the adult life[16].

Riassunto. Sezioni di pineale di ratto impubere e adulto sono state studiate col microscopio elettronico. I reperti ottenuti depongono per una maggiore attività neurosecretoria della ghiandola nell'animale adulto.

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Action of Plastid Phospholipase D on Free and Lipoprotein-Bound Phospholipids

We have previously reported that phospholipase A (phosphatide-acyl hydrolase EC 3.1.1.4) from snake venom[1] and human or ox pancreas[2] splits lipoprotein-bound phospholipids in egg yolk and serum at a higher rate than in their total lipid extracts or than purified ovolecithin. In the present study we investigated whether phospholipase D (phosphatidylcholine phosphatidohydrolase EC 3.1.4.4) from spinach chloroplasts shows a similar preference for lipoprotein-bound phospholipids.

Spinach chloroplasts, prepared according to Kates[3], were suspended in distilled water to the amount of 26–33 mg dry weight per ml, and stored for a maximum of 4 days. The chloroplast suspension was acted on egg yolk and human serum, on their total lipid extracts and on purified ovolecithin, prepared as described previously[1]. Both the egg yolk and egg yolk lipid extracts were diluted with half volume of distilled water. The purified lecithin was suspended in distilled water at a concentration of 50 mg per ml, corresponding to the concentration of lecithin in the diluted egg yolk[4]. Phospholipase D activity was measured in the presence of acetate buffer of pH 4.7[3]. The incubation temperature was 37°C in the absence of ether, and 25°C whenever ether was added to the system. Hydrolysis of the substrates was followed by both the release of free choline, determined by precipitation with ammonium reineckate[5], and by qualitative chromatography on silicic acid impregnated paper[5].

As shown in Figure 1, an amount of enzyme, effecting a rapid release of choline from egg yolk, produced only slight hydrolysis of egg yolk lipid extract and of ovolecithin. The discrepancy between the hydrolysis of egg yolk and that of purified ovolecithin is further illustrated in Figure 2 by the decrease in the lecithin spots. Essentially similar results were obtained when comparing the rates of hydrolysis of lecithin in human serum and in serum lipid extract.

These results suggested that, similarly to what had been found for phospholipase A[1], the integrity of the lipoprotein structure is essential for optimal activity of phospholipase D. Indeed, plastid phospholipase D showed a

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decreased activity when tested on egg yolk in which the lipoprotein complexes were denatured by repeated freezing and thawing.

Ethyl ether is known as the most potent stimulator of phospholipase D activity[6,7]. Predictably, in the presence of ethyl ether, the rates of hydrolysis of both free and lipoprotein-bound substrates were greatly increased and, moreover, they became practically equal (Figure 3). In this respect phospholipase D differs from the phospholipase A from both snake venom and pancreas, which, although being ether-activated[8], splits lipoprotein-bound phospholipid preferentially even in ether-saturated systems[1]. In agreement with the observations of KATES[3], the stimulation of plastid phospholipase D was constant when the amount of ether in the system was reduced from 3 ml to 0.4 ml. Unlike ether, addition of 1 ml 0.025 *M* sodium desoxycholate to egg yolk lipid extract and ovolecithin, although stimulating the hydrolysis of each, failed to equalize the respective rates. Addition of 0.5 ml 1 *M* $CaCl_2$, or of 1 ml 3.2% w/v ovoalbumin solution to all substrates tested, had no influence on the rate of hydrolysis. The inefficiency of Ca^{++} in levelling the hydrolysis rates of the various substrates is not surprising since the amount of ionized calcium present in chloroplasts almost satisfies the enzyme requirements[9].

Previous studies on the hydrolysis of phospholipids by plastid-bound[6] and soluble[9] phospholipase D report very low splitting rates in systems devoid of ether. The present observations show that phospholipase D in spinach chloroplasts is able to hydrolyze lipoprotein-bound phospholipids at high rates even in the absence of ether. According to KATES[7], ether exerts its activating effect on phospholipase D by being adsorbed on both plastids and phospholipid micellae, thus rendering their surfaces lipophilic enough to allow good contact between enzyme and substrate. It might be suggested that the activating effect of the lipoprotein state is also due to facilitation of contact between chloroplasts and substrate. This is indicated by a striking difference in the dispersion of the chloroplasts in the reaction systems containing lecithin or egg yolk. Microscopic observation showed the chloroplasts aggregated in the lecithin-containing system, whereas they were homogeneously dispersed in egg yolk[10].

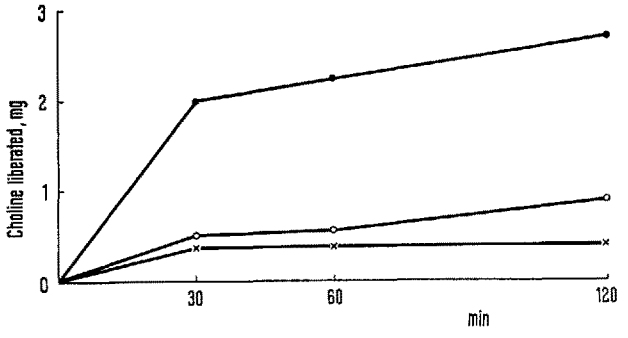

Fig. 1. Comparative assay of free choline released in the course of action of plastid phospholipase D on various substrates. The reaction system consisted of 0.5 ml substrate, 0.5 ml acetate buffer pH 4.7, 3 ml chloroplast suspension and distilled water to a final volume of 5 ml. Incubation was carried out at 37°C. ●—●, egg yolk; o—o, egg yolk lipid extract; x—x, ovolecithin.

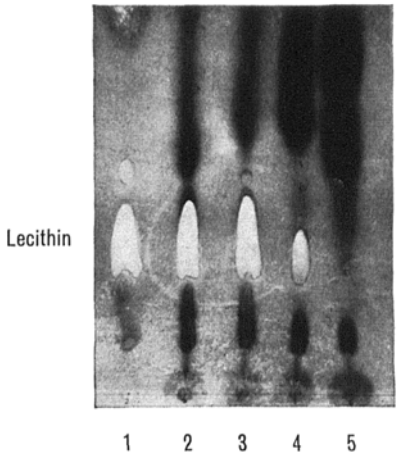

Fig. 2. Action of phospholipase D on purified lecithin and on lecithin in egg yolk. System as in Figure 1. 1, egg yolk; 2, lecithin plus chloroplasts, zero time; 3, egg yolk plus chloroplasts, zero time; 4, lecithin plus chloroplasts after 2 h incubation; 5, egg yolk plus chloroplasts after 2 h incubation.

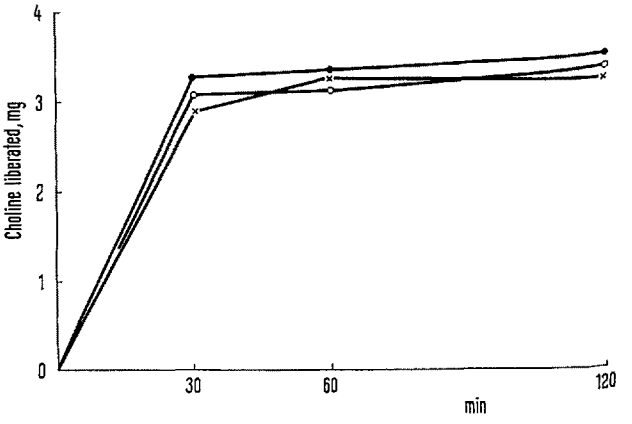

Fig. 3. Effect of ether on choline release from free and lipoprotein-bound substrates by phospholipase D. 3 ml diethylether added to the system of Figure 1. Incubation was carried out at 25°C. ●—●, egg yolk; o—o, egg yolk lipid extract; x—x, ovolecithin.

Résumé. Les phospholipides en état de lipoprotéine sont hydrolysés à un rythme plus accéléré par la phospholipase D des chloroplastes que ne le sont les phospholipides purifiés. La remarquable affinité de l'enzyme pour les soustrats en forme lipoprotéique se manifeste en l'absence de l'éthère éthylique qui est l'activateur indispensable à l'hydrolyse des phospholipides purifiées.

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