of basement membrane in the first group of animals, at most a moderate widening of this structure in the second group. No basal-like, fibrine or fibrinoïd deposits were seen. The epithelial cells were enlarged and showed fusion of the foot processes (Figure 1); at these sites there was some densification of the cytoplasm. An increased number of organelles was encountered, as well as vacuoles containing an electron dense material.

In the proximal convoluted tubules, beside many abnormal, dense inclusions, necrotic cells were seen, with destruction of the brush border. There were numerous cellular fragments in the lumens, pointing to an apical necrosis (Figure 2).

Control rabbits did not show any definite lesions.

In the first group of animals, plasma concentrations of inulin ranged from 36 to 62 mg/100 ml.

Discussion. The present experiments show that inulin produces in rabbits definite alterations of glomerular as well as proximal tubular ultrastructure. The most striking of them were: presence of numerous macrophages in the capillary lumens, endothelial tumefaction and hyperactivity, enlargement of epithelial cells with fusion of foot processes; necrosis of the proximal convoluted tubules. When the substance was given daily for two weeks, the same type of lesion was encountered, but more pronounced.

It should be pointed out that these changes were produced by doses of inulin below the maximal amounts which have been given to man or animals. Plasma levels as large as 565 mg/100 ml have been reported in dog, and 400 mg/100 ml in man[2].

The pathogenesis of the ultrastructural lesions, shown in the present study, remains conjectural and it would be premature to attempt any comparison with other experimental or spontaneous alterations. A first approach to an understanding of changes due to inulin, however, should refer to the physical chemical properties of this polysaccharide. Inulin forms supersaturated solutions, liable to precipitate in certain conditions. Its molecular weight exceeds 5000, which results in low diffusibility. Moreover, the diffusion coefficient is considerably less than would be expected, due to an elongation of the molecule, and is equivalent to a molecular weight of approximatively 15,000.

The present findings indicate that the more or less complete filtrability of a substance does not exclude the possibility of glomerular as well as tubular alterations. The above-mentioned small intrarenal storage of inulin might bear some relationship to these alterations. The tendency to precipitate is favoured in the tubules by the process of urine concentration. However, one cannot rule out some transient storage of inulin in the glomeruli, where cellular reactions to the polysaccharide seem to occur. Further studies should definitely establish whether inulin administration is contraindicated.

Résumé. La microscopie électronique a permis d'observer des lésions du glomérule et du tube proximal, après administration intraveineuse d'inuline chez les lapins: nombreux macrophages dans les anses glomérulaires, tuméfaction endothéliale, tuméfaction épithéliale avec soudure des pédicelles; nécrose des cellules épithéliales du tube proximal.

La poursuite de cette étude devrait établir définitivement si l'emploi de l'inuline est contre-indiqué dans l'exploration fonctionnelle rénale.

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The Osmiophilic Granules of the Pineal Body in Rats

The presence of osmiophilic granules in the cells of the pineal body has been reported by some authors (GUSEK and SANTORO[1]; PELLEGRINO, DE IRALDI, and DE ROBERTIS[2]; CLEMENTI, FRASCHINI, MULLER, ORNESI, and ZANOBONI[3]) and has been considered as evidence of secretory activity. This paper reports the results of an histological and histochemical study performed on the pineal body of young and adult rats.

Material and Methods. 30 Wistar male rats were divided into two groups. The first was formed by animals 20–30 days old and the second one by rats 120–180 days old. The pineal bodies were removed.

Five glands of each group were fixed in osmium tetroxide buffered solution at pH 7.2 according to PALADE[4] and embedded in a mixture 9:1 of *n*-butyl and *n*-ethyl methacrylate. Sections 700–2000 Å thick were cut on a Porter Blum type microtome equipped with a glass knife. Part of the sections was examined with a phase-contrast microscope according to the FABBRINI and GIACOMELLI technique[5], another part was stained with PASM[6] and observed with phase-contrast and light microscopes.

Five glands of each group were fixed in Bouin solution and embedded in paraffin and beeswax mixture. Sections 0.5–1 µ thick were stained with PASM, PAS, and hematoxiline and observed in a light microscope[9].

Five pineal bodies of each group were fixed in 10% calcium-formalin solution and embedded in a 9:1 *n*-butyl and *n*-ethyl methacrylate. The sections, 700–2000 Å thick, were examined in a phase-contrast microscope[10].

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[3] F. CLEMENTI, G. FRASCHINI, E. MULLER, A. ORNESI, and A. ZANOBONI, Atti Accad. Med. Lomb. *17*, 209 (1962).
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[6] Silver methenamine staining (JONES' technique[7,8]).
[7] D. B. JONES, Amer. J. Path. *29*, 33 (1953).
[8] D. B. JONES, Amer. J. Path. *95*, 313 (1957).
[9] The light micrographs were taken with a Leitz-Ortolux microscope equipped with wide range lenses (objective: CPl, 40:1, 170/0,17; ocular, Periplan × 8).
[10] For the phase-contrast observation Leitz lenses were used (objective Pv Fl Oel, 70:1, 170/0,17; ocular, Periplan × 8; condenser for phase-contrast in black and white and colour).

Results. The pineal bodies of impuberal rats, examined in a phase-contrast microscope, showed few or no osmiophilic granules (Figure 1). On the contrary, in adult rats they were numerous (Figure 2). The granules were soluble in xylene (Figure 3) and were stained by PASM.

The phase-contrast observation of calcium-formalin fixed specimens did not reveal the presence of cytoplasmic granules. The morphological features of the pinealocytes appeared better outlined (Figure 4).

In the light microscope the granules, stained with PASM, were visible around the perivascular spaces and the capillary walls (Figure 5).

0.1 ml of 0.05 hydroalcoholic solution of melatonin or serotonin were put on strips of Whatmann 3 mm paper.

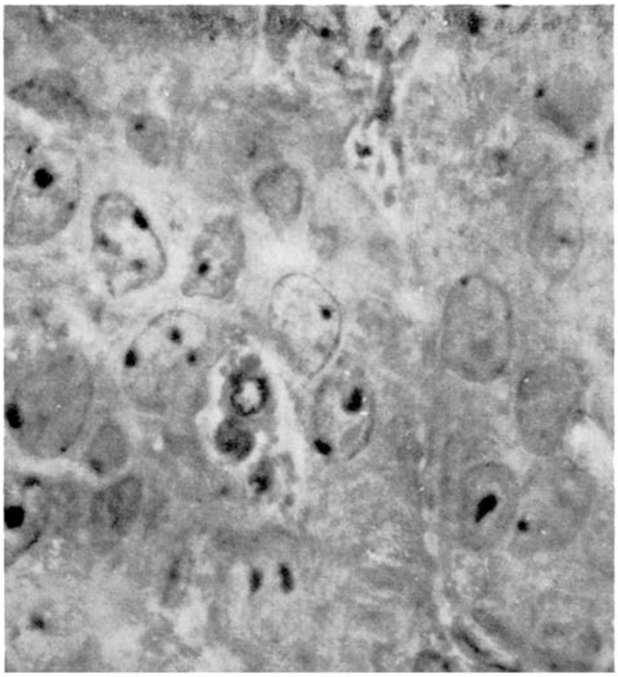

Fig. 1. Immature rat pineal body. A capillary with its perivascular space is seen. No osmiophilic granules are present in the pinealocytes. Osmium tetroxide fixation. Methacrylate embedding. Phase-contrast micrograph. Magnification ×2500.

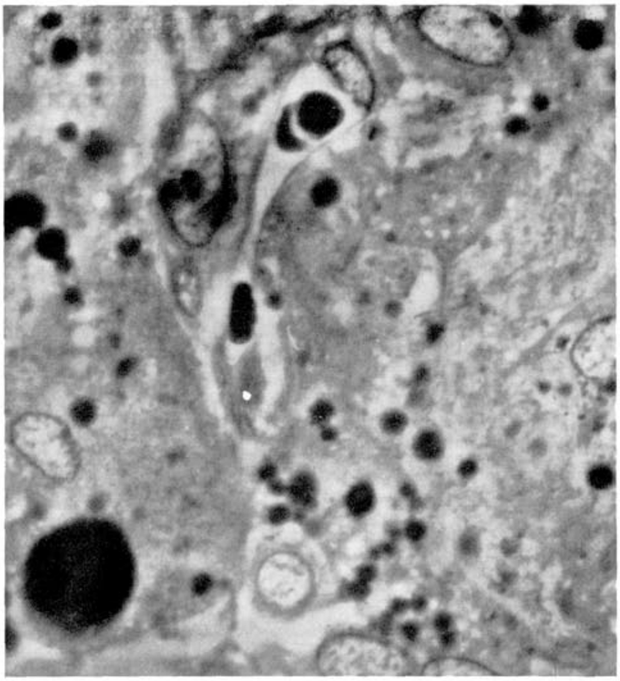

Fig. 2. Adult rat pineal body. Around a perivascular space osmiophilic granules of different size are visible. Osmium tetroxide fixation. Methacrylate embedding. Phase-contrast micrograph. Magnification ×2500.

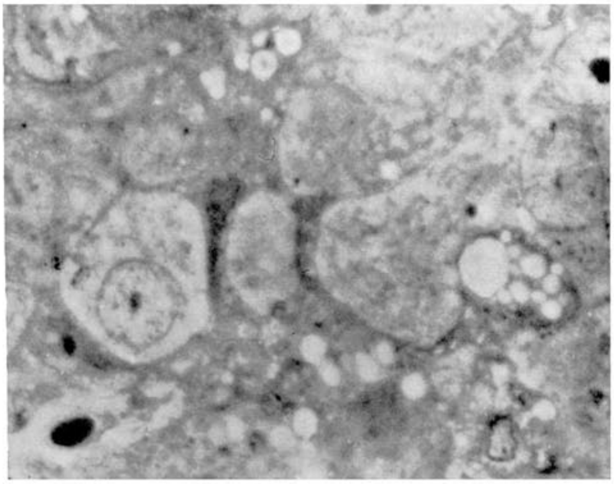

Fig. 3. The phase-contrast micrograph shows a section of an adult rat pineal body after treatment with xylene. The osmiophilic granules have disappeared. Osmium tetroxide fixation. Methacrylate embedding. Magnification ×2500.

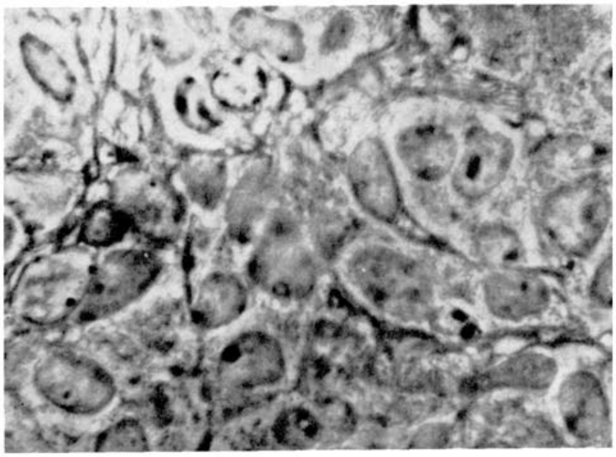

Fig. 4. Section of adult rat pineal body in which the morphological features of the pinealocytes are more evident after calcium-formalin fixation. No granules are found. Methacrylate embedding. Phase-contrast micrograph. Magnification ×1750.

The strips were then exposed to osmium tetroxide vapours. The staining reaction was prompt and intense for melatonin[11] and slow for serotonin[12] (Figure 6). The strips were decolorized by treatment with periodic acid 10% solution.

Discussion. These results show that osmiophilic granules are present in the rat pineal gland and that their number increases with age. The high content of the pineal body in serotonin and melatonin, and the staining of these two substances after exposure to osmium tetroxide vapours, supports the view that serotonin and melatonin are components of the osmiophilic granules. The granules seem to contain also lipids, because they are soluble in xylene. The positive PASM staining of the granules observed in the phase-contrast microscope might suggest that the PASM material visible in the light microscope is the same material which is stained by osmium tetroxide.

Finally, the perivascular localization of the granules induces speculation that they enter the blood stream as a product of secretory activity.

Fig. 5. Argyrophilic material is localized around and inside the perivascular space and around the capillary wall. Bouin fixation. Paraffin and beeswax embedding. PASM, Schiff and hematoxiline stainings. Light microscopy. Magnification ×1700.

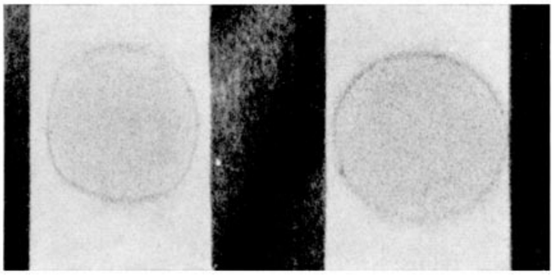

Fig. 6. Serotonin and melatonin staining after 1 h exposure to osmium tetroxide vapours.

Riassunto. Sezioni di pineale di ratto impubere e adulto sono state esaminate al microscopio a contrasto di fase ed al microscopio ottico dopo colorazione secondo la tecnica di JONES. Gli autori confermano l'esistenza di una attività secretoria che aumenta con l'età e prospettano la possibilità che nel secreto siano contenute serotonina e melatonina.

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[11] N-acetil-5-methoxytryptamine.
[12] 5-Hydroxytryptamine.

Differential Implantation of Twin Blastocysts in *Megaderma* (Microchiroptera)

While examining the reproductive physiology of the bats of Rajasthan desert, we came across a gravid female *Megaderma lyra lyra* Geoffroy during the month of April last year having two different sized foetuses.

Normally *Megaderma* does not carry two conceptuses at a time but there have been occasional instances of twinning in *spasma*[1]. So far as we know, there has been no record of twinning in *lyra*.

This note is not only intended to record the occurrence of occasional double foetuses in *lyra* but also to report, for the first time, the more important fact of the difference in size between the two. The larger female foetus (Figure) was present in the left horn of the uterus while the smaller male occupied the right. The placentae are separate, and each embryo has its own umbilical cord and blood vessels.

Neither of the ovaries showed a corpus luteum in the above gravid female. All the same, we are led to infer that both ovaries are active in *lyra*, and in both horns of the uterus progestational changes are brought about. In order to explain the difference in size of the twins, it may be argued that the left ovary ovulated first, the fertilized egg implanted in the left horn and a placenta formed; the right ovary ovulated after the left and the sperm sojourning in the right tube were activated, one of them fertilizing the egg. It is rather doubtful if after the formation of a corpus luteum in the left ovary, the right would ovulate; the sperm may be assumed to be viable as has been described in some wintering bats[2]; HAMLETT[3] thinks that retention of sperm in the tract is likely in Megadermidae. However, examination of the non-pregnant horn of the uterus of two gravid females during the middle of March this year disclosed the absence of sperm in the tubes; in these the pregnant left horn measured 16×19 mm and 21×18 mm respectively. These probably represent midterm (?) uteri as parturition in these bats is usually noticed in the middle of May. At any rate, this does not preclude sperm having entered the right horn and having been digested prior to the date of capture of the two pregnant *lyra* by us. The argument advanced above also

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