cellular organelles were not remarkably changed in the dark and clear cells. Many secretory granules were observed in the nerve endings as well as in the intact ones. The cores in the granules were, however, rarely found. No significant morphological alteration was observed in the pineals of the rats at different post-operative periods.

Discussion. Interpretations of the predominant types of pineal cells of the rats, 'the clear cell' and 'the dark cell', which are identified by electron density and cellular contour, are controversial. GUSEK[4] reported that the clear cell is in an activated condition and the dark cell in a resting or exhausted condition, whereas WOLFE[5] presumed that the clear cell is a parenchymal cell and the dark cell is a stromal one. We have presumed that most of the dark cells are not the exhausted cells but the reserve or resting ones, because of abundant existence of mitochondria in these, except the dark ones in a hypophysectomized rat of the longest period of the experiment.

Increase of the dark cell in bilateral cervical ganglionectomy[6], which abolishes most of the gonadal response to light by interference with the transmission of light information to the pineal gland[7], and absence of the dark cell in the pineal of a young rat[8], which is generally believed to be in the stage of development, have been found.

In the present study, decrease of the clear cells and increase of the dark cells were observed in the ovariectomized and hypophysectomized rats; the changes were more remarkable in the latter. Furthermore, decrease of the secretory granules was observed in the hypophysectomized rats, whereas it was not significant in ovariectomy as compared with the intact animals. These findings suggest that the function of the pineal is depressed in hypophysectomy, and a similar but lesser change is induced in ovariectomy.

Our observations on the pineal lipid content were similar to those reported by ZWEENS[9]. As a valid correlation between lipid granules and secretory activity seems to be absent, the significance of this increased lipid in the pineal is unknown at present.

Zusammenfassung. Die dunklen Pinealzellen waren nach Hypophysektomie beträchtlich vermehrt, während die Vesikeln in den sympathischen Nervenendigungen vermindert waren. Auch nach Ovariektomie waren die dunklen Pinealzellen in geringerem Ausmass vermehrt, ohne dass die Vesikeln vermindert waren. Die osmiophile Granula war sehr spärlich.

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Maturation of Human Ovarian Follicular Oocytes in vitro

In recent years, in vitro maturation of ovarian follicular oocytes has been observed in several mammalian species and oocytes cultured in vitro after liberation from the follicles have been reported to mature at a rate similar to that observed in the ovary after stimulation by gonadotropins[1–6]. However, since the early studies of ROCK and MENKIN[7], relatively few reports on human follicular oocytes have appeared[3,4,8–11].

In the previous study of monkey follicular oocytes[12], although the time required for maturation through polar body extrusion was variable, after 46–48 h in culture, postdictyate stages of meiosis were observed in 79.7% and 26 of 47 ova revealed a polar body, suggesting that the nuclear stages preparatory to fertilization were completed in vitro. The fertilizability of such oocytes was assessed by transferring them into the fallopian tubes of inseminated recipients[13].

The present study was designed to explore the morphological characteristics of human ovarian follicular oocytes when cultured under certain in vitro conditions.

The whole ovary or a wedge of ovarian tissue was excised from the patients who were laparotomized for elective gynecological surgery. Immediately after excision, the ovarian tissue was washed in warmed tissue culture medium and placed in a sterile watch glass containing medium. The comparatively large follicles were dissected out intact and punctured to liberate the oocytes under dissecting microscope (10–30 ×). Oocytes which were devoid of granulosa cells, believed to be recovered from atretic follicles, were discarded. Representative oocytes from each ovarian tissue were examined at the time of recovery as whole mounts under the phase-contrast microscope and stained for detailed examination after fixation. The remainders, in cumulus, were transferred to depression slides containing medium TC 199 supplemented with 10% fetal calf serum. After collection the depression slides were gently agitated and the medium

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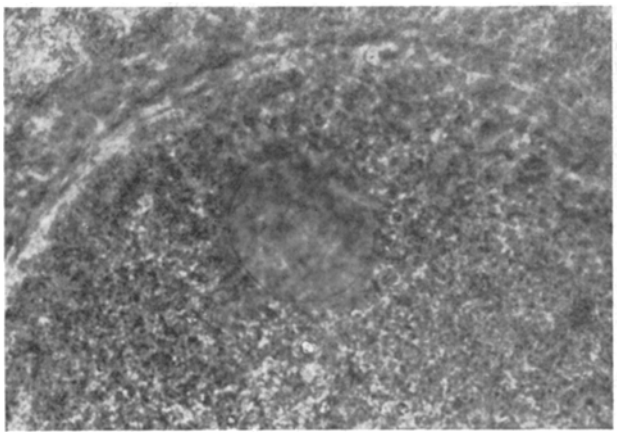

Fig. 1. An oocyte observed under phase-contrast microscopy prior to culture. An eccentrically located nucleus can be seen in the oocyte.

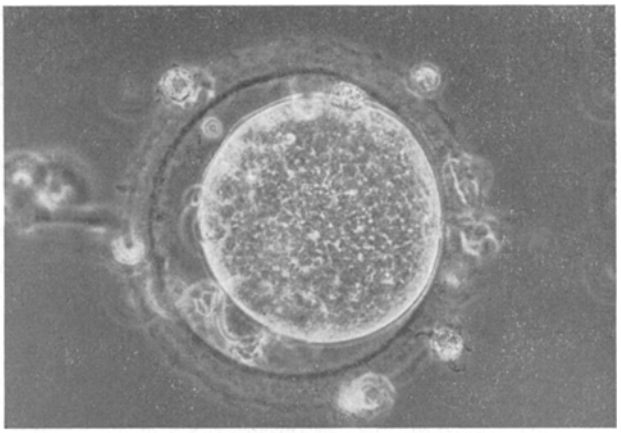

Fig. 3. An oocyte cultured for 48 h in vitro. The first polar body has already extruded in the perivitelling space. Several granulosa cells are still sticking around the zona pellucida.

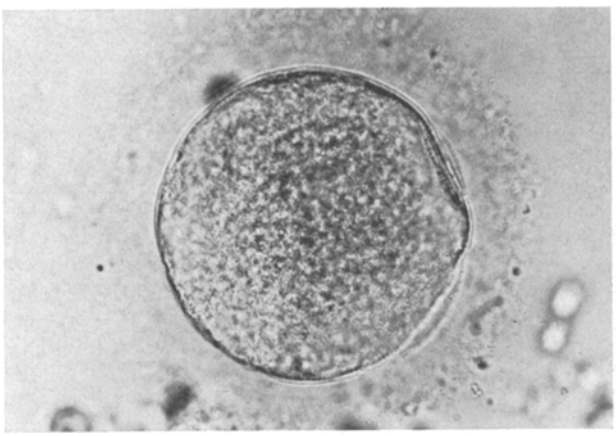

Fig. 2. An oocyte cultured for 48 h in vitro. The first polar body may be about to extrude.

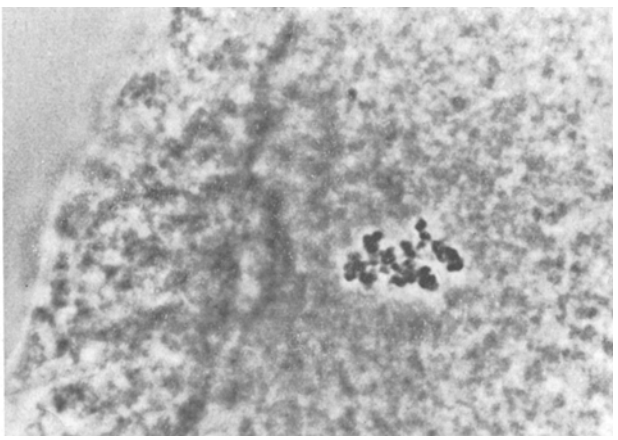

Fig. 4. An oocyte after 48 h in culture, fixed and stained with aceticlacmoid. The chromosomes are clearly visible in the cytoplasm.

was changed 3 times because of removal of debris in medium. By gently moving the slide, the ova were brought together and 2 or 3 oocytes were transferred in small drops of culture medium under light-weight paraffin oil in a plastic culture dish (Falcon Plastics; 35 × 10 mm). The oil was first mixed with sterile culture medium in a ratio of 20 to 1, and the mixture was equilibrated with 5% carbon dioxide in air before use. The dish was gassed with 5% carbon dioxide in air in an incubator at 37 °C. Randomly selected groups of oocytes were placed in the center of 4 petroleum jelly spots on a slide, and the coverslip placed over each oocyte, gently depressed until the structure within the oocyte were clearly visible under the phase-contrast microscope. Throughout these experiments aseptic procedures were followed at all times, and all glassware was sterilized and warmed before and during use.

A total of 84 ovarian follicular oocytes recovered from 18 patients were studied. 41 were examined immediately after recovery. 12 of them had already degenerated. These oocytes might be obtained from the atretic follicles. 29 oocytes had a large nucleus, the germinal vesicle. 20 oocytes were examined after 24 h in culture. Of these, 14 were still in the vesicular or resting stage. The remainder displayed some evidence of maturation. 6 oocytes were in diakinesis which was interpreted as the termination of prophase. Of 23 oocytes examined after 46–48 h in culture, 4 were still in diakinesis. The remaining 19 were in first or second metaphase.

A suitable environment is essential both for the fertilization of ova in vitro or storage in culture for longer periods. In as much as the early embryonic development in the pre-implantation stages normally occurs within the fallopian tube, the in vitro effect of tubal secretions is of some interest. Suzuki and Mastroianni[14] reported that rabbit tubal ova could be fertilized in vitro in tubal fluid with a relatively high success rate, suggesting that tubal fluid contained all of the ingredients prerequisite to fertilization. Brinster[15–17] has reported that energy for development of two-cell mouse ova could be supplied by lactate, pyruvate, oxyloacetate or phosphoenolpyruvate.

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Recently KENNEDY and DONAHUE[18] reported that human follicular oocytes resumed meiosis in F10, a defined medium in numbers comparable to that obtained in medium containing serum. Several simple Krebs-Ringer media also supported maturation, which suggested a similarity of nutritional requirements between human and mouse oocytes. In their experiment, the cumulus was stressed to be important for supplying unique substance to the oocytes.

In the majority of recently reported experiments concerned with fertilization, ovulated ova were used and there have been few reports on the capacity of ovarian follicular oocytes for fertilization. These problems are worthy of continued attention in the future, through the study of in vitro culture of human follicular oocytes[19].

Résumé. Des œufs oocytes humains obtenus des follicules d'ovaire extraits opérativement ont été cultivés in vitro pour étudier le processus de leur maturation.

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On the Equivalence between Dense Bodies and Z-Bands

The electron-microscope studies on vertebrate smooth muscle fibres have revealed the existence of the so-called dense bodies, but their chemical nature and the role they play in contraction are still unsolved problems. LOWY and HANSON[1] suggested, without experimental evidence however, that the dense bodies in vertebrate smooth muscle fibres might be structures similar to the Z-bands in the striated fibres. For the past years, some authors[2,3] seem to have favoured this assumption, and PANNER and HONIG[4] have proposed a hypothesis on the contraction mechanism of the smooth fibres based on the analogy between the dense bodies and the Z-bands. This communication describes a dissimilar behaviour of dense bodies and Z-bands after urea extraction or extraction of actomyosin. Therefore it is suggested that dense bodies and Z-bands are not equivalent structures.

Material and methods. The muscle specimens were obtained from the normal human myometrium and from the rabbit psoas and glycerinated in the usual manner for at least 30 days before use. Some smooth and striated muscle bundles were extracted at 2 °C with a solution containing 3 *M* urea, for 1 h, according to the method described by RASH et al.[5]. Some other smooth and striated muscle bundles were extracted for 48 h with the Weber-Edsall solution, which is well-known for the removal of actomyosin. After extraction the specimens were fixed either in glutaraldehyde followed by post-fixation in buffered osmium teroxide or in the osmium fixative alone and embedded in Epon. Ultra-thin sections were stained with uranyl acetate and lead citrate and were examined in a Zeiss EM9A electron microscope.

Results. Exposure of striated fibres to 3 *M* urea, for 1 h, resulted in a complete extraction of Z-bands (Figure 1) as observed by RASH et al.[5]. Contrarywise, the urea treatment of the smooth fibres under exactly the same conditions does not effect the removal of the dense bodies (Figure 2).

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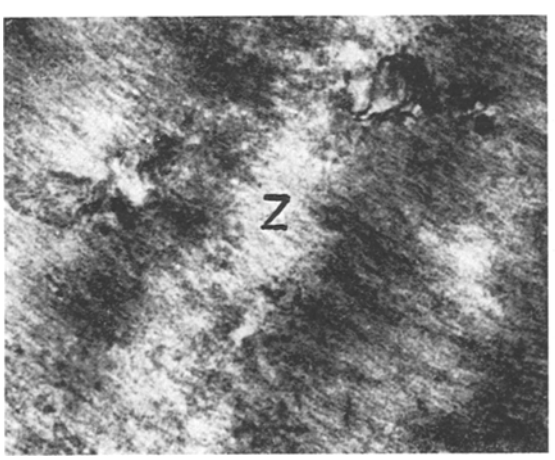

Fig. 1. Extraction of rabbit psoas using 3 *M* urea (1 h). Z-bands (Z) were completely removed. × 30,000.

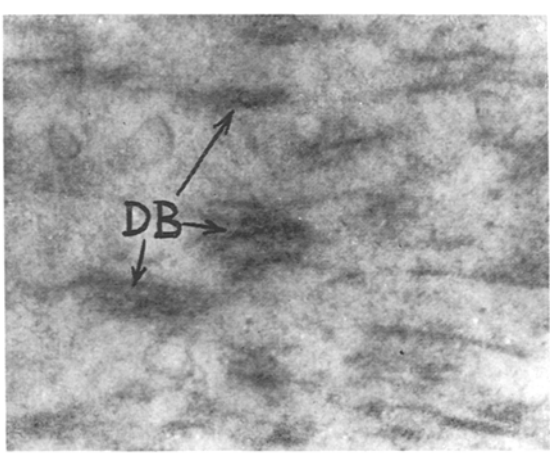

Fig. 2. Extraction of uterine smooth muscle using 3 *M* urea (1 h). Dense bodies (DB) are present. × 25,000.