Table II. Effect of ascorbic acid in protecting adrenaline from autoxidation in the presence, or absence, of Mn^{++}

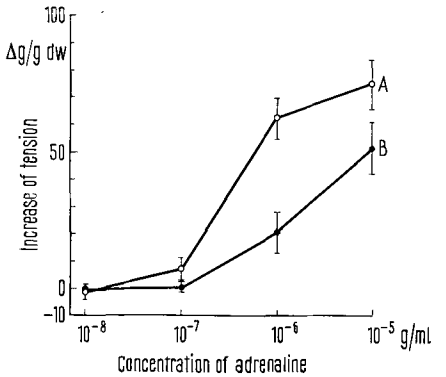
	No Mn^{++}		Mn^{++} (0.1 mM)			
Time of gassing (min)	0	20	0	1	10	20
No ascorbic acid	76.0	57.5	1.4	0.0	2.0	0.0
Ascorbic acid (10^{-5} g/ml)	85.0	81.0	67.5	35.8	13.7	7.3
Ascorbic acid (5×10^{-5} g/ml)	94.2	80.5	97.0	87.0	99.2	90.5

Data are recoveries (%) of adrenaline (4 µg/40 ml Tyrode solution). For details see legend of Table I and text.

Table III. Increase of isometric tension development of electrically driven left atria of guinea-pigs by adrenaline

	A	B
Increase of isometric tension	378.5 ± 76.4 (n = 10)	188.4 ± 47.7 (n = 10)

The Tyrode solutions containing adrenaline (10^{-6} g/ml) were gassed with a mixture of 95% O_2 and 5% CO_2 for 20 min before their application to the atria. Tension development was measured 5 min after application of the test solutions. A, adrenaline in the presence of ascorbic acid (5×10^{-5} g/ml). B, adrenaline in the presence of Mn^{++} (0.1 mM) and in the absence of ascorbic acid. Data are expressed as percent increase above control (mean ± S.E. of mean); n = number of preparations.



Effect of adrenaline on isometric tension development of electrically driven left atria of guinea-pigs. Ordinate, increase of tension in g/g dry weight (dw) above control (control = tension before addition of adrenaline: 22.8 ± 4.95 g/g dw (A) and 7.1 ± 1.41 g/g dw (B)). Abscissa, concentration of adrenaline (g/ml). Tension development was measured 5 min after addition of adrenaline. Stimulation frequency: 180/min. A, adrenaline in the presence of ascorbic acid (5×10^{-5} g/ml). B, adrenaline in the presence of Mn^{++} (0.1 mM) and in the absence of ascorbic acid. Cumulative application of adrenaline. The figures (n = 12) are expressed as mean ± S.E. of mean. In these experiments the Tyrode solutions containing adrenaline were not gassed before their application to the atria.

is perhaps caused by the experimental conditions prevailing during the adsorption procedure (pH 8.8; heavy stirring for 5 min) which may facilitate the Mn^{++}-catalyzed oxidation process. Addition of ascorbic acid (10^{-5} g/ml) to the Tyrode solution before gassing with O_2/CO_2 partially abolished the rapid breakdown of adrenaline in the presence of Mn^{++}. During 20 min of gassing the recoveries declined gradually from 67.5 to 7.3%. However, 5×10^{-5} g/ml ascorbic acid protected adrenaline completely from its Mn^{++}-catalyzed autoxidation.

In a third series of experiments the positive inotropic effect of adrenaline plus ascorbic acid and of adrenaline plus Mn^{++} without ascorbic acid has been studied in isolated left guinea-pig atria (Figure). In the presence of Mn^{++} the positive inotropic action of adrenaline was only partially inhibited. As shown in Table III, adrenaline, too, had lost only about 50% of its positive inotropic activity when the adrenaline containing Tyrode solution before application was gassed for 20 min with O_2/CO_2 in the presence of 0.1 mM Mn^{++}. In this solution no adrenaline could be detected fluorimetrically, however, 93% of adrenaline had been converted to an adrenochrome-like compound (calculated from the 'reversed blank'). Under our conditions, the lower limit of the fluorimetric estimation of adrenaline was 5×10^{-8} g/ml. Since this concentration had only a slight positive inotropic effect, if any (Figure), one can assume that this oxidation product itself has some positive inotropic activity. In conclusion, the present results demonstrate that the Mn^{++}-facilitated autoxidation of adrenaline has to be considered, when actions of adrenaline are investigated in the presence of Mn^{++}. However, degradation of adrenaline catalyzed by Mn^{++} (0.1 mM) can be abolished by ascorbic acid (5×10^{-5} g/ml) which itself does not influence the contractility of isolated guinea-pig atria[8].

Zusammenfassung. Die Autoxydation von Adrenalin in Anwesenheit von Mn^{++} wurde quantitativ untersucht. Es ergab sich, dass die durch 0,1 mM Mn^{++} bewirkte vollständige Oxidation von Adrenalin durch 5×10^{-5} g/ml Ascorbinsäure verhindert werden konnte.

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Morphological Changes in the Pineal Gland of the Albino Rat by Hypophysectomy and Ovariectomy

Little information is available on the relationship of the ovary or the pituitary to the pineal, although attention has been paid to the function of the pineal gland by demonstration of higher concentration of characteristic biogenic amines in this organ[1,2]. This paper describes morphological changes in the pineal gland of hypophysectomized or ovariectomized rats.

Material and methods. 15 female rats of the Wistar strain aged 9–12 weeks were divided into 3 groups and kept for 14 h in light and 10 h in darkness at 24 h periods throughout the experiments. One week later, 8 rats were hypophysectomized[3] and 3 rats were ovariectomized. The pineals were removed under ether anesthesia 3–6 weeks after the operation and were fixed in gultaraldehyde solu-

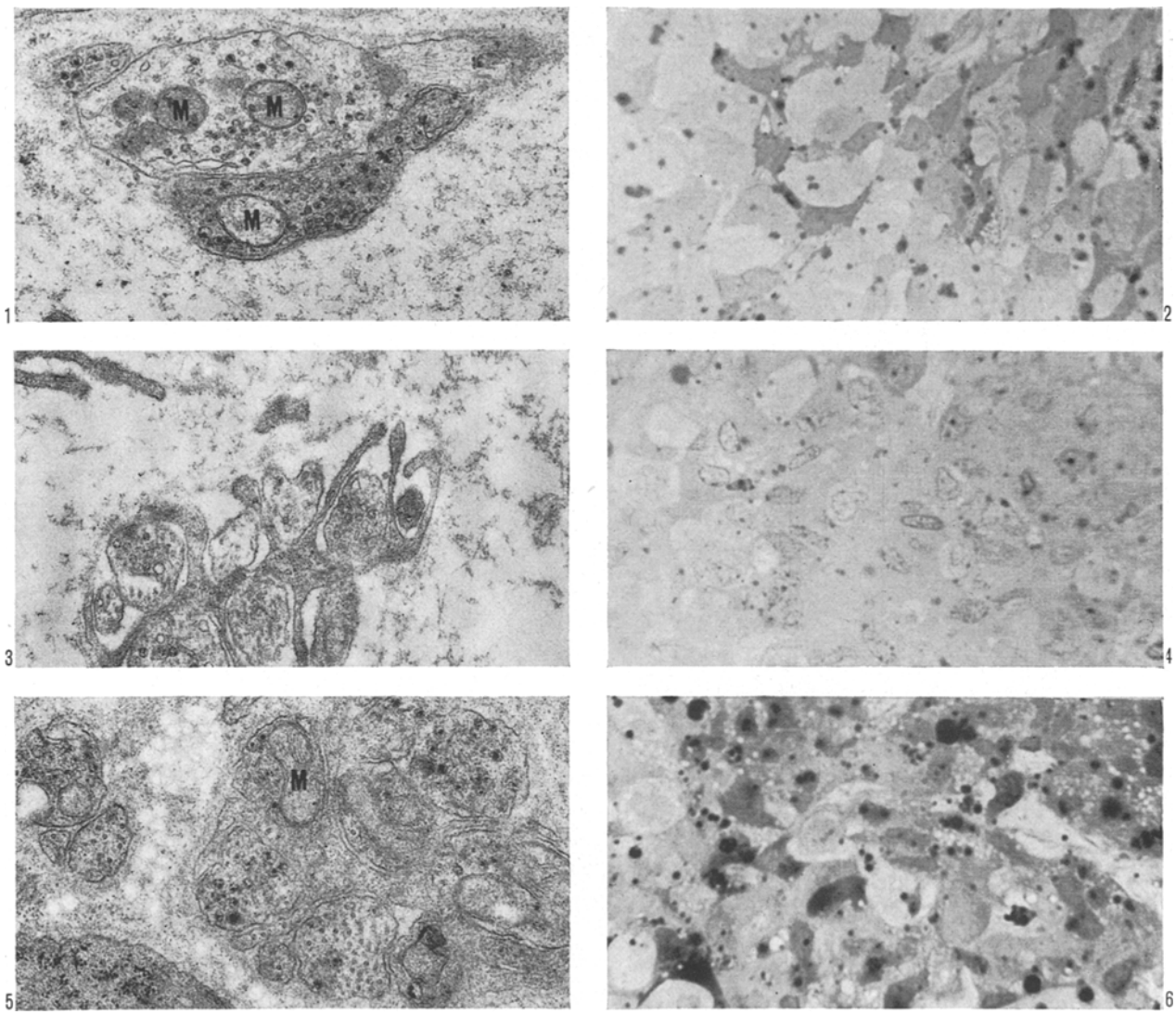

Fig. 1. Control. Nerve endings are found with mitochondria and many secretory granules, in which the cores are almost always found. M, mitochondrion. × 17,200.

Fig. 2. Control. Lipid granules are found more frequently in the clear cells than in the dark ones. Toluidine blue stain. × 1000.

Fig. 3. Hypophysectomized. Secretory granules decrease prominently in the nerve endings. In addition, the cores almost disappear. × 20,000.

Fig. 4. Hypophysectomized. Increase of the swollen dark cells is found. Lipid granules decrease in the clear cells. Toluidine blue stain. × 1000.

Fig. 5. Ovariectomized. Many secretory granules are observed in the nerve endings. The granules with cores are only a few. M, mitochondrion. × 23,200.

Fig. 6. Ovariectomized. Decrease of the clear cells and increase of the dark cells are found. Lipid granules of the clear cells increase in number and size. Toluidine blue stain. × 1000.

tion, immersed in OsO_4 solution and then embedded in Epon for electron microscopy. The thick epoxy sections were stained with toluidine blue for light-microscopy. 4 intact animals were killed at the corresponding times.

Results. Decreases of the intracellular organelles and ribosomes were observed in the dark and the clear cells, as well as decrease of the secretory granules in the nerve endings in the hypophysectomized rats, as compared with the intact animals. The cores of the secretory granules, which were distinctively observed in the intact animals, were scant. Lipid granules decrease slightly in the clear cells. No clear cell was found in one of the animals in this group which was killed 6 weeks after the hypophysectomy – the longest experimental period. In the parenchyma of this pineal, the mesh work of the dark cells was formed by connection of elongated cytoplasm.

In the ovariectomized animals, increase of the dark cells and increase of lipid granules, which were enlarged, were seen in both the dark and the clear cells. The intra-

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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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