The reason for the gradual decline in plasma neurophysin levels in the later stages of dehydration is open to conjecture. The most likely explanation is that the rate of release depends on the amount of secretion products present in the gland, and declines as the neural lobe is depleted. A further possibility is that maximal biosynthetic activity cannot be maintained for longer than a few days.

In summary, data obtained by radioimmunoassay show that water deprivation in rats causes a marked increase in neurohypophysial secretion. This increase is probably paralleled by elevated biosynthesis of the neurosecretory products which is nevertheless insufficient to maintain neural lobe stores. Moreover, the elevation in plasma neurophysin levels which accompanies the progressive fall in neurohypophysial neurophysin accords well with the view that granule contents are secreted from the neural lobe by an exocytotic process[15].

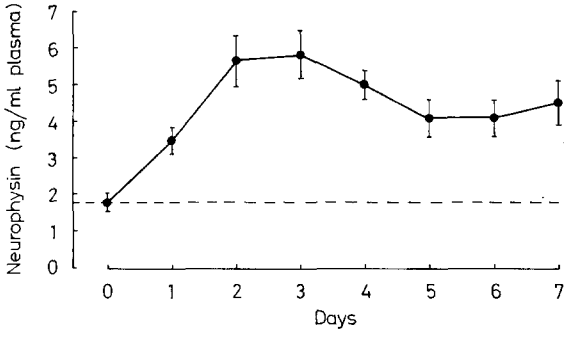

Fig. 2. Immunoreactive neurophysin concentration in unextracted plasma from water deprived rats. Each point is the mean ± S.E.M. of the plasma levels from the individuals of each group of rats.

Résumé. Au cours de la privation d'eau potable chez le rat, on observe une diminution du contenu en ocytocine, vasopressine et neurophysine immunoréactives du lobe postérieur de l'hypophyse et une augmentation du taux plasmatique de neurophysine. Ces résultats sont en accord avec l'hypothèse que la sécrétion neurohypophysaire s'effectue par exocytose du contenu des grains de neurosécrétion.

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Ovulation and the Role of the Ovarian Surface Epithelium

Already 300 years ago, Regnier de Graaf[1] gave a detailed and surprisingly accurate description, in 'De Mulierum Organis Generatione Inservientibus Tractatus Novus', of ovulation and the passage of the egg to the uterus by way of the uterine tube. However, the actual mechanism of follicle rupture is even today obscure[2–4]. To elucidate this basic event in reproduction we have followed preovulatory morphologic changes in all tissue layers[5–10] (Figure 1) that separate the egg from the outside of the follicle, including those in the often neglected surface or 'germinal' epithelium. The rabbit is particularly suitable for studies of follicle rupture, being a reflex ovulator. It ovulates regularly 10 to 12 h after mating or i.v. injection of luteinizing hormone (LH) or human chorionic gonadotrophin (HCG) and can thus provide material from accurately timed stages before follicle rupture.

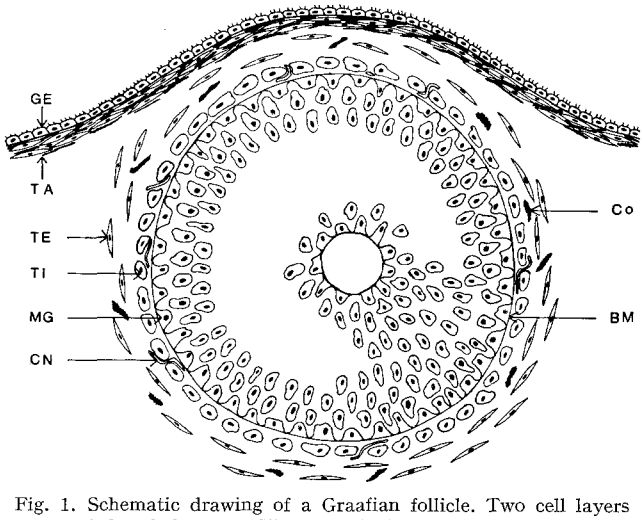

Fig. 1. Schematic drawing of a Graafian follicle. Two cell layers surround the whole ovary. These are the 'germinal' epithelium (GE) and tunica albuginea (TA). The follicle wall beneath these layers is composed of the theca externa (TE), the theca interna (TI) and the membrana granulosa (MG). The granulosa cells are at the periphery seated on a distinct basement membrane (BM) and a blood capillary network (CN) is found just outside this membrane. Tunica albuginea contains a lot of collagen (Co).

Initially, we studied the ovarian surface epithelium in the light microscope and then soon found that paraffin embedded material was unsuitable for detailed examination of the cells. Epon sections (1 µm) proved superior and revealed several distinct changes, e.g., large, dark, cytoplasmic bodies in the surface epithelium during the

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preovulatory period, both after i.v. injection of HCG[5] and after mating. To elucidate their biological significance and possible relation to ovulation, more penetrating studies were required.

By scanning electron microscopy, follicle growth and alterations to the ovarian surface could easily be followed both in broad outline and in considerable detail[6]. The cells covering preovulatory follicles increased clearly in size up to 8 h after injection of HCG and showed an increasing number of large, intracellular structures with prominent rounded contours (Figure 2). By transmission electron microscopy large, electron dense, lysosome-like

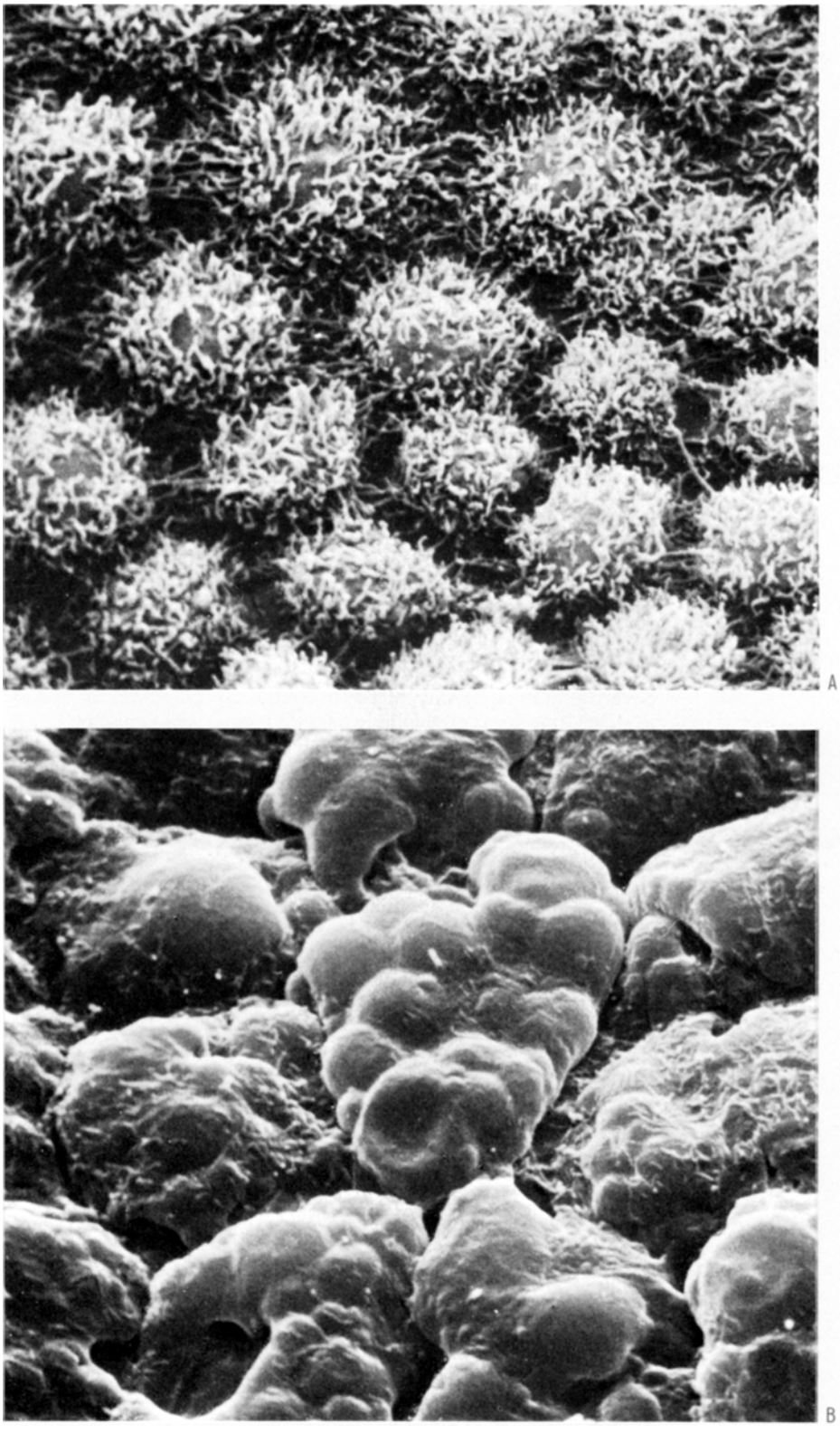

Fig. 2. Scanning electron micrographs of 'germinal' or surface epithelium of rabbit ovaries. A) Normal surface epithelial cells before the ovulatory stimulation. The cells are approximately cubic with a convex surface covered by microvilli. ×2,250. B) Changed surface epithelial cells on the apex of a follicle 10 h after i.v. injection of 25 IU HCG. The cells are considerably larger than earlier and they show only a few microvilli. Several protruding intracellular bodies can be seen bulging against the cell surface. ×2,250.

bodies were found in some cells already 4 h after HCG (Figure 3). These membrane-surrounded structures increased in size up to 8 h after HCG but then decreased markedly. Obviously, they corresponded to the round bodies observed by light microscopy and scanning electron microscopy. Using histochemistry combined with electron microscopy, we recently found that many of the dense, cytoplasmic bodies of the surface epithelium represent lysosomes.

The collagen-rich tunica albuginea is the strongest apical barrier separating the egg from the outside. During the last couple of hours before follicle rupture, when the dense bodies of the surface epithelium considerably decreased in amount, signs of material emptying into vacuoles were found, and sometimes open communications from the vacuoles towards the underlying tunica albuginea. An extracellular oedema appeared under the epithelium with degenerated fibroblasts and disintegrated collagen in the outermost portion of the tunica albuginea. The alterations gradually proceeded inwards, and, at 9.5 h and later, marked changes were obvious, even in the inner portion of the theca externa[9]. Lysosomes, however, were sparse both in the tunica albuginea and theca externa, and even rarer in the theca interna, while in the granulosa layer they were more frequent.

The blood capillaries close to the membrana granulosa showed a gradually increasing number of small pores, and close to ovulation, the endothelial cells displayed large gaps, up to 3 µm in diameter[10]. Parallel with these changes in the vessels, the preovulatory follicles grew rapidly in size and an augmenting oedema occupied the whole ovary.

The membrana granulosa gradually dissociated, particularly the last hours before ovulation, whereupon several long processes from granulosa cells were seen to penetrate the disintegrating basement membrane[8].

It is not easy to interpret these changes and relate them to follicle rupture. Before we try to do that it may be helpful to consider some relevant and recently reported findings. The levels of oestrogen and progestin are high in rabbit ovarian follicles; they are maximal about 3 h after mating and then decrease[11]. Testosterone probably follows a similar pattern[12,13]. In contrast, prostaglandins increase and reach very high values during the latter half of the preovulatory period[14]. Sex steroids[2,3,7] and prostaglandins[7,14,15] are apparently obligatory in the intraovarian mechanism of ovulation. Prostaglandin

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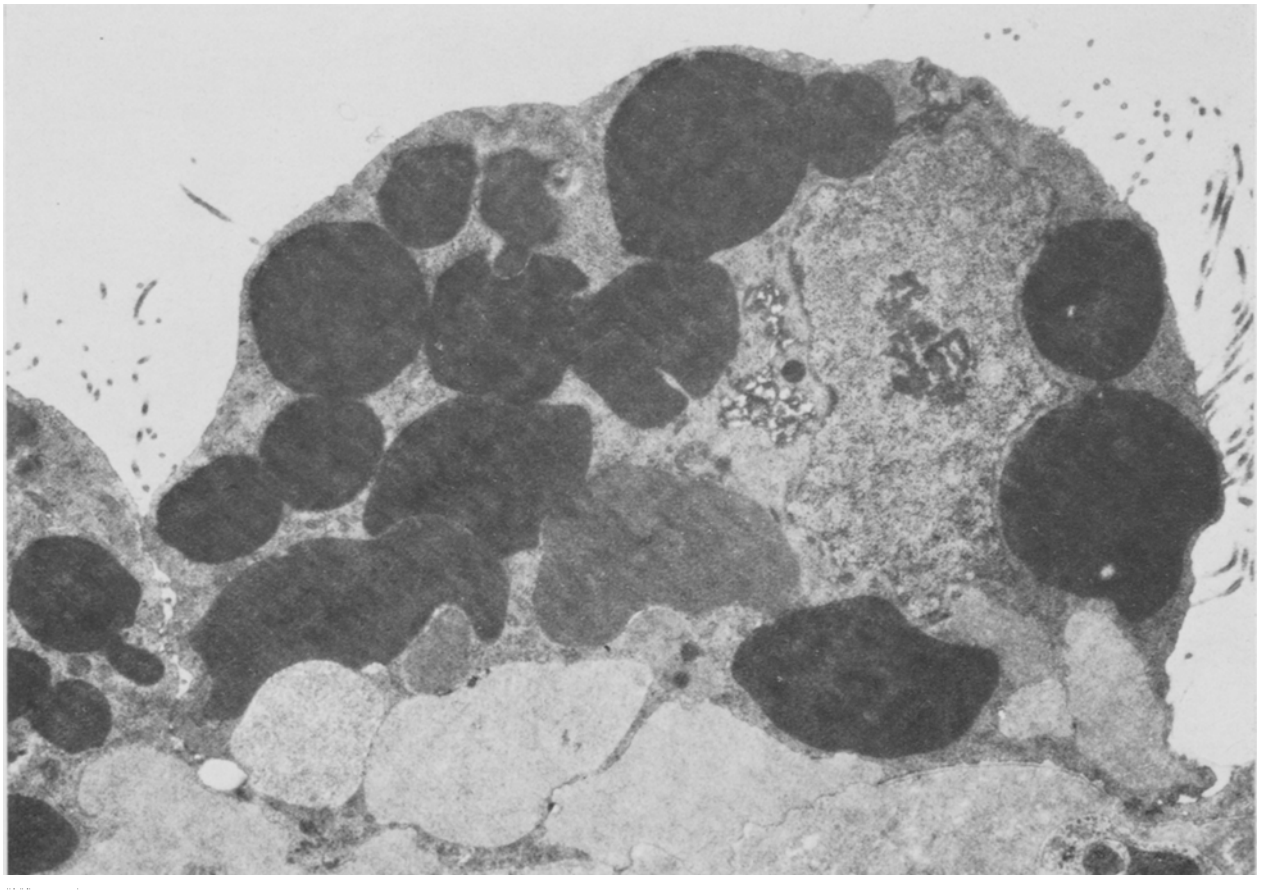

Fig. 3. Transmission electron micrograph of a surface epithelial cell on the apex of a follicle 8 h after i.v. injection of 25 IU HCG. There are several large electron-dense bodies in the cytoplasm. ×7,400.

$F_{2\alpha}$[16] and sex steroids[17] are able to labilize lysosomes, while prostaglandin E_1 is reported to stabilize lysosomes[18]. Whatever the net effect of the prostaglandins, prostaglandin $F_{2\alpha}$ appears to be essential for follicle rupture in rabbits, since intrafollicular injections of antiserum prepared against prostaglandin $F_{2\alpha}$ blocked ovulation in 24 of 25 follicles[15]. The ability of prostaglandin E_1 to promote vascular permeability should also be noted[19,20]. Lysosomal enzymes released extracellularly may be coupled to collagen degradation in various ways[21], e.g. by activating procollagenase[22]. Moreover, oestradiol[23] and perhaps even progesterone[24] may diminish the expression of collagenase activity. Therefore, a fall to low levels of these steroids might directly incite increased collagenase activity. With this background we should like to present the following working hypothesis regarding the intraovarian mechanism of ovulation (Figure 4).

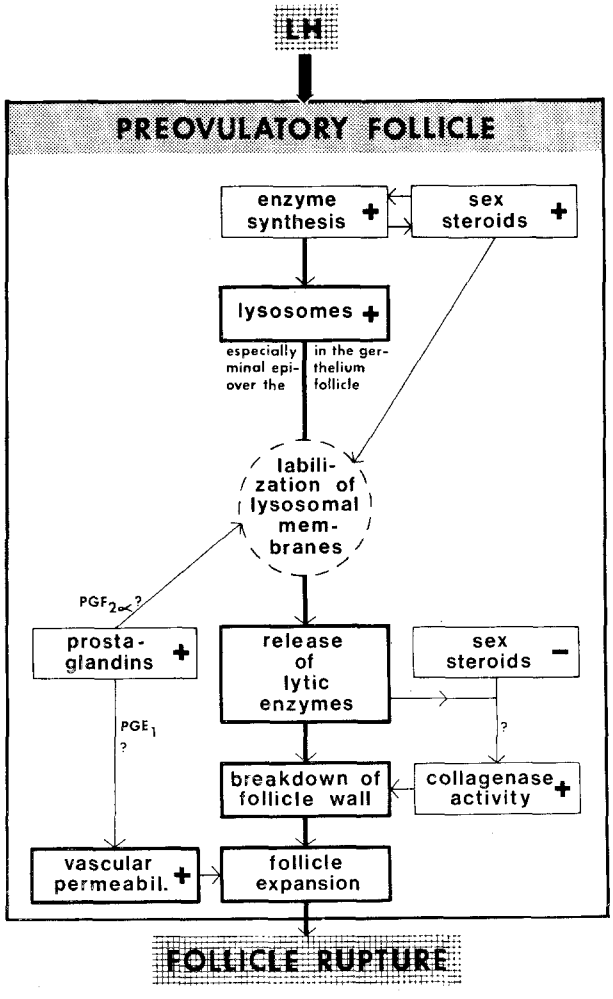

Fig. 4. Working hypothesis of the intraovarian mechanism of normal ovulation in the rabbit. Mating induces release of LH. The LH peak stimulates enzyme synthesis and steroidogenesis in the preovulatory follicle. 10 to 12 h later ovulation with expulsion of the ovum occurs. The rectangles with heavy lines specify our main morphological findings in the preovulatory follicle. Their probable relation to each other and to functional changes is illustrated. The sex steroids are maximal in the follicle about 3 h after mating but later fall, while prostaglandins F and E rise. A distinct increase in the amount of lysosomes in the surface epithelium covering preovulatory follicles is found some 4 h after an ovulatory stimulus, and, approximately 8 h after this stimulus there is a maximal accumulation of these bodies. Then the lysosomes decrease, and signs of breakdown of the follicle wall occur in the outermost portion of the tunica albuginea, and gradually proceed inwards. The blood capillaries around the follicle show an increasing number of small and large pores, the follicle expands rapidly and finally ruptures. +, increase; —, decrease.

As can be seen, we suggest that at least part of the lytic enzymes originates from the lysosomes of the surface epithelium, which should thus play a significant role in the disintegration of the follicular apex, particularly of the tunica albuginea. An important question then is what induces enzyme synthesis and the increase in lysosomes. It is well known that oestrogen stimulates an increase of acid phosphatase activity and of lysosomes in the uterine epithelium in several species, one of these being the rabbit[25]. The high local values of sex steroids may induce enzyme synthesis and lysosomal growth in the surface epithelium covering preovulatory follicles, particularly since the surface epithelium is embryologically related to uterine epithelium.

The observations that ovaries of the mature mare[26] and of the rodent *Galea musteloides* (B. J. WEIR, personal communication) have an ovulation fossa which is the only part of the ovarian surface covered by 'germinal' epithelium and the only region where the follicles rupture also indicate that the 'germinal' or surface epithelium may have a key role in ovulation.

Zusammenfassung. Nach der Paarung oder i.v. Injektion von menschlichem Choriongonadotropin nehmen die Lysosomen deutlich an Zahl und an Volumen im Keimepithel über präovulatorischen Follikeln zu. Vor dem Follikelsprung verschwinden die Lysosomen fast vollständig. Gleichzeitig wird das Bindegewebe im apikalen Teil des Follikels aufgesplittert. Die granulosazellen werden dissoziiert und im Kapillarnetz rund um den Follikel wird erhöhte Permeabilität beobachtet. Die Einwirkung von Prostaglandinen und Geschlechtshormonen, inklusive Androgenen, und die Rolle des Keimepithels für die Ovulation werden diskutiert.

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