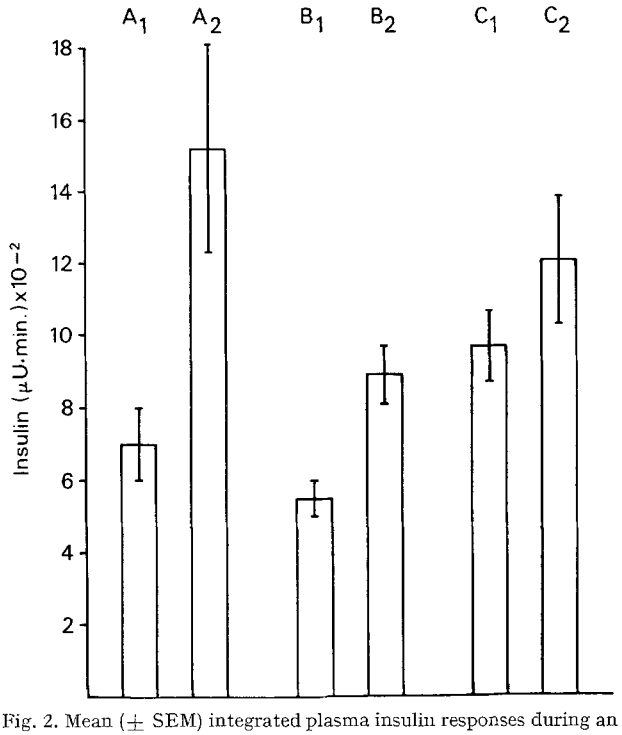

Fig. 2. Mean (± SEM) integrated plasma insulin responses during an i.v. glucose tolerance test in rabbits.
A) after 5 weeks treatment. 1. Placebo group. 2. Progesterone group ($p < 0.05$).
B) 1 week after treatment had been stopped. 1. Placebo group. 2. Progesterone group ($p < 0.01$).
C) 7 weeks after treatment had been stopped. 1. Placebo group. 2. Progesterone group (N.S.). The P-values were calculated with the permutation test[12].

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These results agree with the reported effects of progesterone on insulin responses during glucose tolerance tests[2,3]. It has been shown that progesterone treatment augments the plasma insulin response without having any apparent effect on glucose tolerance. One explanation is that progesterone induces β-cell hyperplasia while at the same time it is an insulin antagonist, causing impaired peripheral tissue utilization of glucose[2]. The fact that progesterone inhibits the insulin effect on glucose uptake of rat diaphragm in vitro[11] supports this suggestion.

Seven weeks after progesterone treatment had been stopped, there was no significant difference between the integrated insulin responses of treated and control rabbits. This shows that the progesterone effect is fully reversible. The progesterone levels were also normalized after 7 weeks (Table II). There could be a correlation between the rate in drop of progesterone levels and the normalization of the insulin responses but no attempt was made to study this question.

Résumé. Un traitement pendant 5 semaines avec de la progesterone a provoqué une augmentation significative de la réponse insulinique à l'injection i.v. de glucose chez la lapine. Une semaine après la fin du traitement, les taux de progesterone et la réponse insulinique étaient encore élevés. 7 semaines après le traitement la réponse insulinique était de nouveau normale, montrant que l'effet de la progesterone était entièrement réversible.

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Water Deprivation in Rats: Elevated Plasma Neurophysin Levels

Water deprivation in rats is a potent stimulus to the hypothalamo-neurohypophysial system and leads to release of both oxytocin and vasopressin[1]. The availability of specific radioimmunoassays for oxytocin, vasopressin and their carrier proteins, the neurophysins, led us to reinvestigate the effects of prolonged water deprivation on the amounts of these substances present in the rat neural lobe. Plasma neurophysin levels were also determined in the same animals in order to ascertain whether they were elevated by the stimulus and in order to study, in an indirect way, the kinetics of neurohypophysial hormone secretion.

Adult, male rats of the SIV 50 strain (Sprague-Dawley derived) weighing between 230–310 g were kept 5 to a cage and allowed food ad libitum (Nafag 850 pellets). 28 animals used as controls had, in addition, free access to tap water. 10 groups of 10 animals each were deprived of drinking water for periods ranging from 1 to 10 days. At the end of the period of water deprivation, the animals were decapitated. Each neurohypophysis was rapidly removed, separated from adjacent tissue and transferred to a Pyrex tube containing 2 ml of distilled water for homogenization. Insoluble material in the homogenate was removed by centrifugation and the supernatant used for radioimmunoassay. Blood was collected from the bodies immediately after decapitation and centrifuged for blood haematocrit determination. The plasma was used for measurement of osmotic pressure and chloride concentration, as well as for determination of immunoreactive neurophysin levels.

Neurophysin levels were determined by radioimmunoassay using a cross-species reactive antibody (A_5IV) raised against bovine neurophysins; purified bovine neurophysin II served as standard[2]. Oxytocin and vasopressin were assayed using specific antibodies according to previously described methods[3].

After 1 day of water deprivation, the amounts of immunoreactive oxytocin and vasopressin present in the neural lobe did not differ greatly from those of control rats. A slight increase in vasopressin content and a decrease in oxytocin content were seen, but neither were statistically significant. With longer periods of removal of water, a progressive fall in the gland content of both

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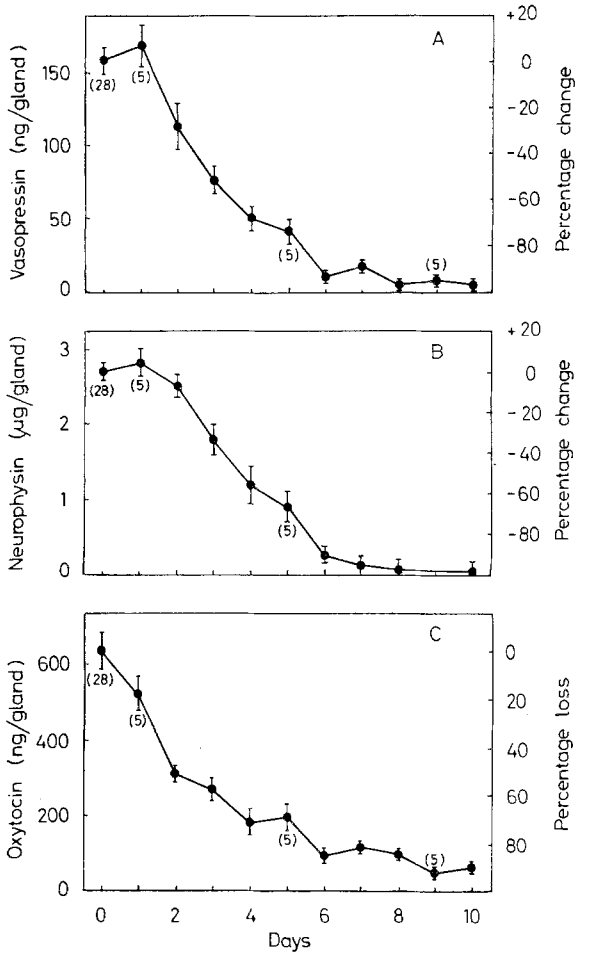

Fig. 1. Content of vasopressin (A), neurophysin (B) and oxytocin (C) in neurohypophyses from control rats (day 0) and form animals deprived of drinking water for periods of 1 to 10 days. All values were determined by radioimmunoassay; results are expressed in amount of immunoreactive material per gland, since there was no significant difference in gland weight between controls and animals deprived of water. Mean neural lobe weight was 0.9 mg (0.1 S.E., $n = 65$). Each point is the mean ± S.E.M. of 10 animals, except where the number of observations is indicated in parentheses. The numbers of observations are the same for the Table and for Figure 2.

Mean (± S.E.M.) haematocrit, plasma osmotic pressure and chloride concentration in normal rats and in rats deprived of drinking water for 1–7 days

Duration of water deprivation (days)	Haematocrit (%)	Osmotic pressure (mOsm/kg)	Plasma [Cl⁻] (meq/l)
0	43.1 ± 0.3	292.5 ± 1.0	98.0 ± 0.4
1	46.0 ± 0.8	301.1 ± 1.0	100.6 ± 1.1
2	51.2 ± 0.6	305.9 ± 1.3	104.3 ± 1.0
3	51.0 ± 0.6	308.3 ± 1.3	107.2 ± 0.8
4	53.5 ± 0.4	315.8 ± 0.7	104.8 ± 0.7
5	55.5 ± 0.8	315.7 ± 1.9	109.7 ± 1.6
6	57.3 ± 0.6	320.3 ± 2.7	109.3 ± 1.2
7	56.8 ± 1.2	333.3 ± 3.4	115.0 ± 1.8

Haematocrit was determined in heparinized capillary tubes following centrifugation at 1500 g, plasma osmolarity with a Knauer osmometer and chloride concentrations with a Zeiss flame photometer.

hormones was seen, until after 6 days the percentage loss for both hormones was greater than 80% (Figure 1, A and C). The amount of neurophysin present in the glands of rats deprived of water for 1 day also showed a slight, but statistically insignificant increase. This was followed by a progressive decline in neurophysin content (Figure 1, B).

Blood haematocrit, as well as plasma osmotic pressure and Cl^- concentration obtained from the same animals show fairly linear rises during water deprivation, indicative of progressive dehydration and haemoconcentration. These results are summarized in the Table.

The neurophysin concentration in the plasma from control rats was 1.8 ng/ml (0.2 S.E., $n = 28$); it doubled after 1 day, and more than tripled 2 days after removal of water. Later, plasma neurophysin values declined, but remained above control levels throughout the remainder of the experiment (Figure 2).

The hormones, oxytocin and vasopressin, and their respective neurophysins are associated within neurosecretory granules found in the axons and neurosecretory axon endings of the neurohypophysis. There is evidence that neurosecretion from the neural lobe occurs by a calcium-dependent process of exocytosis of the contents of the neurosecretory granules[4]. Thus, during experimentally induced secretion from isolated rat neurohypophyses, neurophysins escape along with the hormones but unaccompanied by cytoplasmic marker enzymes[5]. Moreover, elevated plasma neurophysin levels have been reported in several conditions of increased neurohypophysial hormone secretion in rats[6–9]. Our data show that during water deprivation, the change in neurophysin content of the neurohypophysis closely parallels that of the hormones and follows a similar time course. These changes are accompanied by elevated plasma neurophysin levels, as would be predicted from the exocytosis hypothesis.

Two additional observations are worthy of comment. First, the fact that after only 1 day of water deprivation, there is virtually no change in gland content of either the hormones or neurophysin even though the plasma neurophysin level has doubled. Secondly, after a few days of water deprivation, the plasma neurophysin levels decline although the stimulus is still increasing in strength.

The first effect can be accounted for by increased biosynthesis. Increases in cell, nuclear and nucleolar size, in the RNA content and in the activity of enzymes have been reported in the hypothalamic supraoptic and paraventricular nuclei of rats deprived of drinking water[10–12]. After 1 day of water deprivation, enhanced biosynthesis appears to counterbalance approximately the increase in secretory activity. If both synthesis and release are augmented, the net effect on neural lobe content may vary in either direction which could account for the variation between the results given by different workers[1,13,14]. During longer periods of water deprivation, the stimulation of hormone release outweighs the increase in biosynthetic activity, leading to a progressive exhaustion of secretory products stored in the neural lobe.

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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].

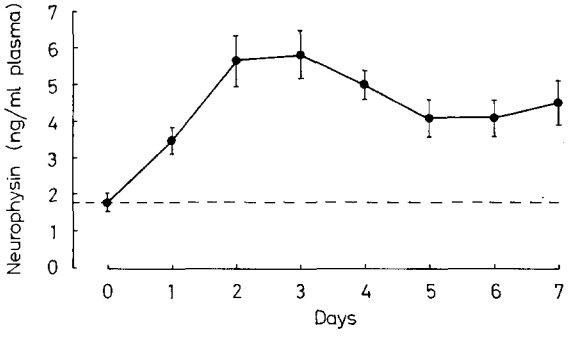

Fig. 2. Immunoreactive neurophysin concentration in unextracted plasma from water deprived rats. Each point is the mean ± S.E.M. of the plasma levels from the individuals of each group of rats.

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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[16] Research Fellow of the Belgian F.N.R.S.

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.

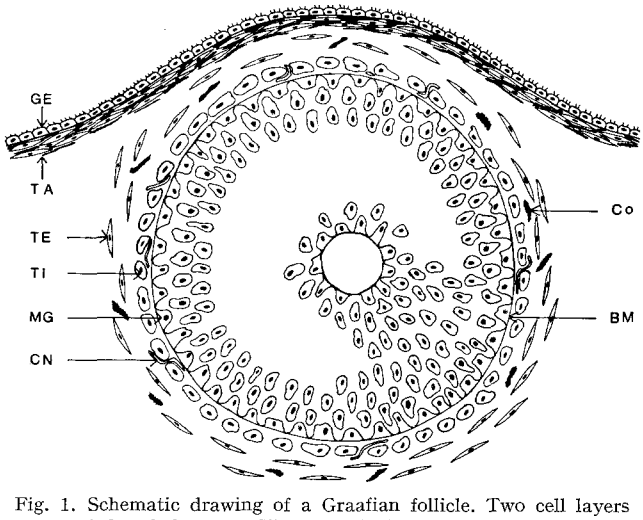

Fig. 1. Schematic drawing of a Graafian follicle. Two cell layers surround the whole ovary. These are the 'germinal' epithelium (GE) and tunica albuginea (TA). The follicle wall beneath these layers is composed of the theca externa (TE), the theca interna (TI) and the membrana granulosa (MG). The granulosa 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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