Male Syrian hamsters were housed from the age of 35 days in a room lit from 06.00 h to 18.00 h by 40 W Tesla fluorescent tubes for 3 weeks prior to the experiment. During exposure to light at night, the intensity of illumination at the level of cages was around 100 lx. Hamsters killed in darkness were exposed to a dim red light for less than 1 min prior to decapitation. Pineals were removed immediately and stored in petri dishes on solid CO_2. Within 24 h, 2 pineals were homogenized in 100 µl of 0.1 M sodium phosphate buffer, pH 6.8, containing 0.25 mM/1–^{14}C/–acetyl CoA (sp. act. 1 Ci/mole) and 10 mM tryptamine, and NAT activity was determined by a modified method[14] of Deguchi and Axelrod[15]. Blanks with boiled pineal homogenates were carried through the procedure. Acetyl–/1–^{14}C/–coenzyme A (59 mCi/mmole) was purchased from Radiochemical Centre, Amersham, England.

NAT activity began to increase around 23.00 h and was maximal between 02.00 h and 05.00 h (fig.). The ratio between the highest night activity and the day activity at 16.00 h was 3.5. When hamsters were exposed to sudden light at 03.30 h, NAT activity declined within 30 min to almost 1/5 of its former value. The discrepancy between our results and those of Tamarkin et al.[13] may be due to a difference in NAT assay. Tamarkin et al.[13] homogenized pineals, in contrast to our method, only in a phosphate buffer, without acetyl CoA which stabilizes NAT[16]. However, Panke et al.[12] used the same procedure and in spite of this found the NAT rhythm. The low NAT amplitude in Syrian hamsters may be due to lower activity as compared with rats or to the lack of optimization of assay conditions. NAT was assayed at the pH optimal for the rat enzyme[15]; the pH optimal for the Syrian hamster enzyme is not known. Our demonstration in Syrian hamsters of the daily rhythm in pineal NAT activity and of the rapid NAT decline in response to light at night together with the previous demonstration of the daily rhythm in melatonin content[12,13] and of the fast drop of pineal melatonin after light exposure[13] indicates that changes in NAT activity are involved in the regulation of the melatonin rhythm in Syrian hamsters as well.

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Normal prolactin content of rat pituitary may be maintained by Nebenkern formations

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Summary. We have observed Nebenkern formations in mammotrophs of normal male rats. The ultrastructural appearance of these formations suggests that they may be part of a mechanism which by the process of autophagy disposes of older prolactin granules.

The effect of either lactation[1,2] or estrogen treatment[3–5] on pituitary mammotrophs is reflected ultrastructurally by an extensive proliferation of the protein synthetic apparatus. The most striking feature is the presence of focal proliferations of rough endoplasmic reticulum (RER) in the formation of concentric whorls; the centers of such structures have been reported to contain dense granules surrounded by agranular membranes[4], secretory granules and free ribosomes[5], vesicles, dilated buds of RER, anastomosing tubules of smooth endoplasmic reticulum and lysosomes or multivesicular bodies[6]. Haguenau and Bernhard[7] saw similar formations in mammotrophs from estrogen-induced pituitary tumors and named them 'Nebenkern'. Most authors refer to Nebenkern as structures associated with active protein synthesis[1]. Only Pantic and Genbacev[4] have suggested the possibility that Nebenkern represent a stage in the formation of bodies belonging to the lysosomal system. Pantic and Genbacev[4] reported that following estrogen treatment, Nebenkern are more developed in male than in female rats, while in untreated male rats they are not present and the RER is only slightly developed.

Materials and methods. 5 young male rats were housed in group cages in a temperature and humidity controlled sound proofed room with controlled lighting regulated to 14 h light and 10 h dark. Animals were kept in this controlled environment for 3 weeks prior to sacrifice. Pituitary glands were fixed by immersion in glutaraldehyde/osmium tetroxide and embedded in Epon 812. Sections were cut on glass knives and stained with uranyl acetate followed by lead citrate.

Results. Mammotrophs were present in small numbers and were somewhat irregular in shape, often with long cytoplasmic processes (fig. 1). Nuclei were centrally located. The mammotroph shown displays a moderately developed protein synthetic apparatus in that there are several rows of elongated RER and a distinct Golgi apparatus. The small number of secretory granules and the presence of exocytotic figures (fig. 2) suggest that the cell is in a secretory phase. Concentrically arranged RER (Nebenkern) can be seen enclosing a dense granule and vesicle, free ribosomes and what appears to be a granule in a partial state of digestion (fig. 2). In other mammotrophs a lipid droplet was some-

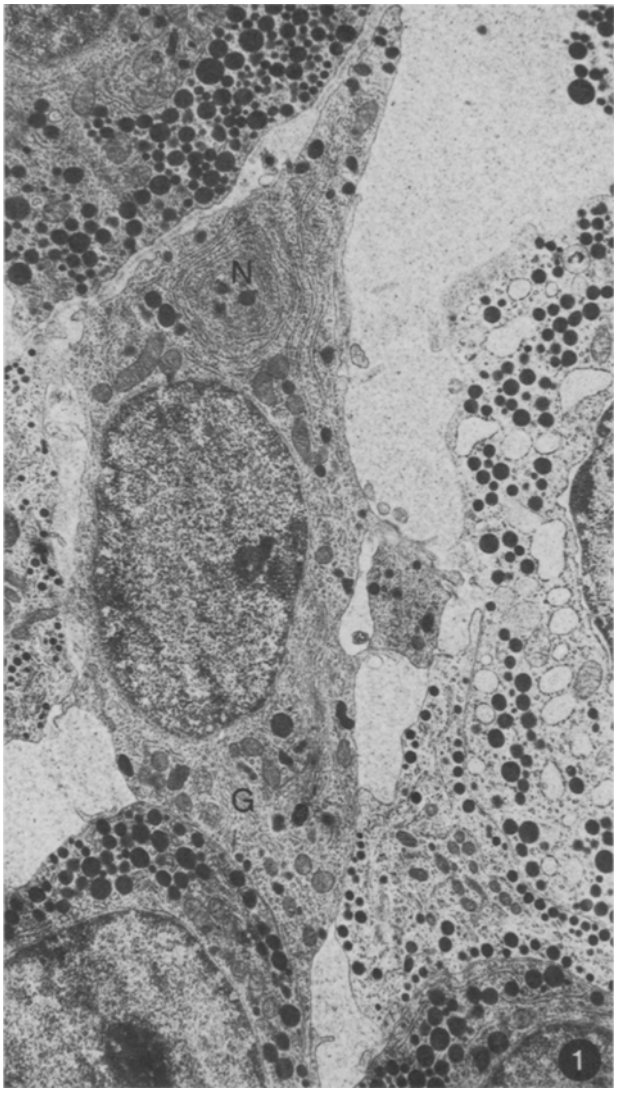

Figure 1. Mammotroph from pituitary of normal male rat. N, Nebenkern; G, Golgi apparatus. × 8275.

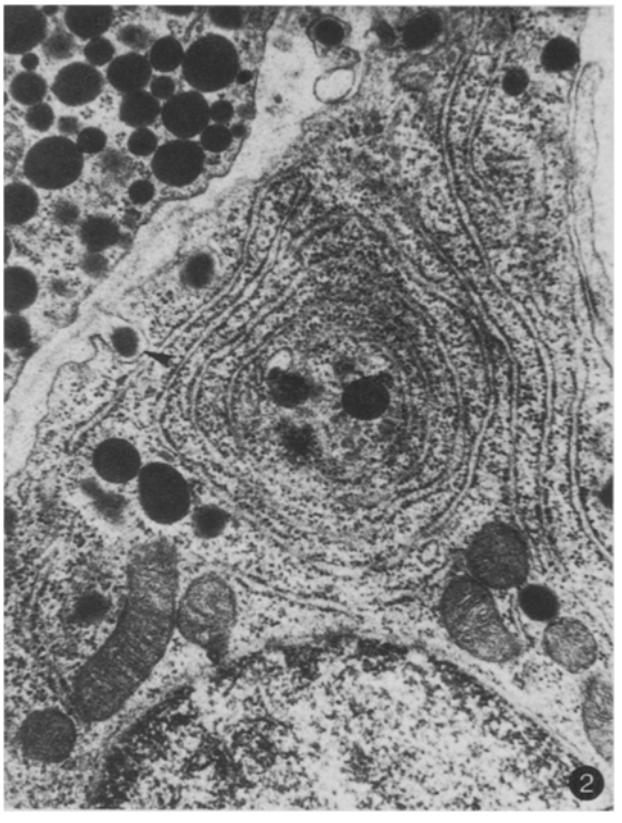

Figure 2. Same cell as figure 1 showing details of the Nebenkern and an exocytotic figure (arrow). × 19,680.

times seen within a Nebenkern. In most cases, smaller prolactin granules appear to be located peripherally and are often associated with exocytotic figures while the larger granules are usually more centrally located and positioned closer to or within either the Golgi apparatus or a Nebenkern formation.

Discussion. In normal male rats, the majority of mammotrophs have been described as having a RER that is only slightly developed[4]. On the other hand, in normal diestrous female rats, mammotrophs have a well developed RER which is sometimes in the form of Nebenkern[5]. Farquhar[8] found that secretory granules within lysosomes were progressively degraded to yield a vacuolated dense body and when the vacuole eventually separated, a free lipid droplet remained. Therefore, the presence of similar vacuoles and lipid droplets within the cytoplasm of Nebenkern may be an indication of lysosomal activity. Because newly synthesized prolactin is secreted preferentially by mammotrophs[9,10], prolactin content consists of a reserve pool (perhaps in the larger, older granules) and a mobile pool (possibly in the smaller, newly formed granules) which maintain a balance between synthesis and release. When the size of the prolactin pool is more than a mammotroph can accommodate, adjustment in prolactin content is said to be made by the process of crinophagy (the fusion of secretory granules with lysosomes)[1]. We suggest that Nebenkern formations may be responsible for disposal of excess prolactin by the usual process of autophagy which involves de novo membranous sequestration or envelopment of secretory granules by the cisternae of the RER[11]. Therefore, Nebenkern may represent a stage in the formation of lysosomes which function to control prolactin content in mammotrophs not only from estrogen stimulated pituitary gland where large amounts of prolactin are produced but also those from normal male pituitary glands where smaller amounts of prolactin are produced.

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