Variations in the Golgi Complex of Mouse Somatotrops at Different Times in a 24-hour Period[1]

Circadian rhythms have been reported for growth processes[2]. On the other hand, growth hormone secreting cells (STH cells, somatotrops) of the pars distalis of the mouse pituitary are easily recognizable with the electron microscope[3–7].

It has also been demonstrated that some aspects of pituitary function undergo circadian variations[8]. Furthermore, the participation of the Golgi complex in the secretion mechanism of the cell is a well-known fact, also the correlation between its development and the variations in the intensity of that process.

The present experiment was devised to look for variations in those aspects of ultrastructural morphology that are related to the functional state of the cells of the pars distalis of the mouse. In the present report the different degrees of development of the Golgi complex of STH cells are described according to the time of day at which the animals were killed.

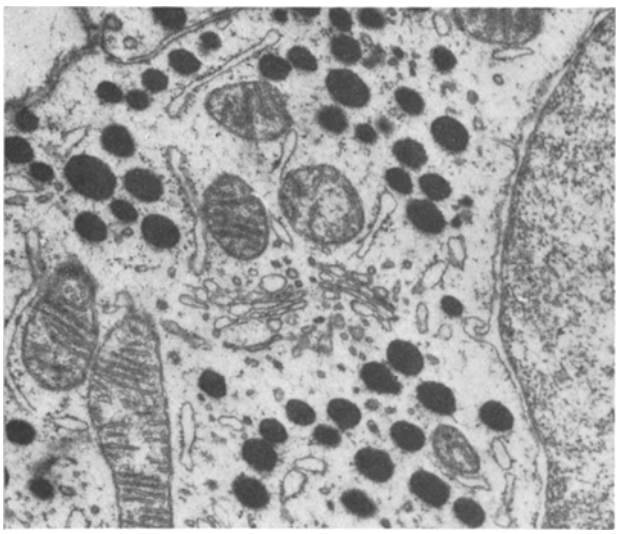

Fig. 1. STH cell of an animal killed at 24.00 h. The Golgi complex is small and well defined. × 16,000.

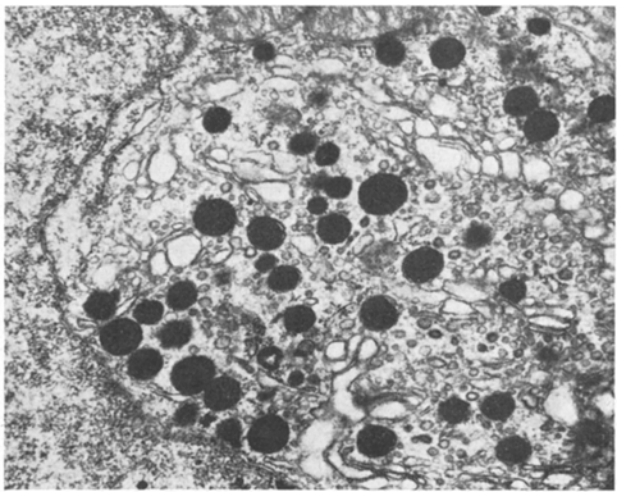

Fig. 2. STH cell of an animal killed at 18.00 h. The Golgi complex is hypertrophied and mostly composed of dilated vacuoles and numerous microvesicles. Some dense granules are also present in the Golgi zone. × 14,000.

C3H-S male mice were used. This strain, provided for our laboratory by J. W. Wilson (Brown University, Providence, Long Island, USA), has been maintained by inbreeding since 1966. The animals were weighed weekly after the time of weaning and those animals with abnormal growth curves were eliminated. When the animals were 9 weeks old they were standardized for periodicity analysis[9], single caged in a room ad hoc, at a temperature of 25 ± 1 °C, with water and food ad libitum and illumination from 06.00 h to 18.00 h (fluorescent light, 40 W) alternating with 12 h darkness. When they were 12 weeks old they were weighed and killed in 3 groups of 4 animals at noon, 18.00 h and 24.00 h. After the animals were killed, the skull was opened, the brain removed and the hypophysis exposed by eliminating the meingeal covers. A drop of cold osmium fixative[10] was then poured onto the sella turcica. After a few seconds, the gland was removed and the lateral wings of the pars distalis separated with a razor-blade in another drop of fixative and sliced into small pieces.

After fixation in cold Millonig fixative, the material was dehydrated in increasing concentrations of ethanol and embedded in Araldite[11]. Sections were cut on glass knives with a Porter Blum ultramicrotome, mounted on cupper grids and stained with permanganate[12] or lead citrate[13]. A Siemens Elmiskop I electron microscope operating at 60 kV was used to examine and photograph the sections.

The STH cells, very numerous in the lateral wings of the pars distalis, were easily identifiable among the other cellular types. They are round or oval in shape and contain abundant and very dense secretory granules which range in size from 350–400 nm. The endoplasmic reticulum is formed by parallel rows of flattened sacs with a large number of ribosomes attached to the outer surface. The Golgi complex is generally well identifiable. No significant differences existed in the aspect of the endoplasmic reticulum and the secretion granules between the animals killed at the different times of day.

On the contrary, very marked differences were found in the Golgi complex. In mice killed at midnight, the somatotrops showed a well defined but small Golgi complex which occupied a reduced zone of the cytoplasm. It consisted of flattened sacs, scarce contracted vesicles and a small amount of microvesicles (Figure 1). In the pars distalis of the animals killed at noon and 18.00 h, most of

[1] This investigation was supported with a grant from the Comisión de Investigación de la Universidad Nacional de La Plata. Thanks are due to Miss A. C. Nessi for the technical assistance.

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the somatotrops presented a marked hypertrophy of the Golgi complex, extended over a large area of the cytoplasm. The vesicles were very numerous and the vacuoles considerably dilated. Many microvesicles were scattered in the Golgi zone and the occurrence was frequent of granules of secretion in different stages of formation, ranging from condensing secretory material to dense and well-defined granules enveloped by Golgi membranes (Figure 2). Multivesicular bodies were also found in some cells.

Some authors report that the Golgi complex of STH cells is well defined but small[3,6], as we have described here in the animals killed at midnight. Other authors report pictures coincident with the one that we have described in the animals killed at 18.00 h[7]. Perhaps the differences that we have found might help to explain these apparent contradictions in the literature.

More times of a 24-h period must be explored before attempting to correlate the changes in the Golgi complex with the circadian changes existing in growth processes.

Resumen. El aparato de Golgi de las células somatotropas de la pars distalis de la hipofisis del ratón, presenta importantes diferencias ultraestructurales en distintos momentos de un período de 24 horas. Es pequeño a mediodia y marcadamente hipertrófico a mediodía y 6 de la tarde.

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The Composition of Extracted Nuclei of Developing Frog Embryos used as Template Material for RNA synthesis in vitro

After neurulation, *Xenopus laevis* and *Rana pipiens* embryos synthesize less DNA-like RNA (D-RNA) per cell[1,2] and fewer kinds of D-RNA per cell[3,4] as development proceeds to the larval stage. In addition, the ability of isolated chromatin of *Rana pipiens* embryos to act as template for RNA synthesis in vitro without the addition of exogenous microbial RNA polymerase decreases over this same period[4–6]. However, if microbial RNA polymerase is added to the in vitro system, there is a progressive increase in RNA synthesis from chromatin of the later stage embryos[5,7]. It seemed possible that a greater amount of RNA polymerase in the non-basic residual protein fraction might account for the higher RNA synthesis in vitro without added RNA polymerase, but that increased masking of the DNA template by total protein, histones, or D-RNA might account for the lower levels of RNA synthesis with the addition of exogenous RNA polymerase at the early stages. The present study examines this problem by determining the amounts of basic and residual protein, phosphoprotein, and D-RNA of extracted nuclei of gastrulae, tailbuds and larvae (stages 10, 19, 25 of SHUMWAY[8]).

The problem involved in measuring such constituents in isolated nuclei or chromatin is that significant contamination by yolk and pigment granules cannot be avoided. However, it is possible to remove the pigment granules and yolk protein by partially deproteinizing isolated nuclei with one chloroform-isoamylalcohol (24:1) extraction in the presence of 1 *M* NaCl[9]. We feel that these preparations can be used for the composition study since they show the same increase in templating activity with development as did isolated chromatin in the in vitro system containing added RNA polymerase[5]. The preparations are devoid all pigment and are optically clear after centrifugation at 65,000 *g* for 1 h, as compared to isolated chromatin or nuclear preparations which are contaminated by small amounts of pigment. It was impossible to remove protein and D-RNA from these DNA preparations by repeated precipitation with cold ethanol or isopropyl alcohol, or by wrapping them around a rod and washing with 0.01 *M* *Tris*-HCl (pH 7.5)[7].

Materials and methods. Nuclei were isolated from gastrulae, tailbuds and larvae, and were partially deproteinized as described above. The method of MARUSHIGE and OZAKI[10] was used to determine the amounts of basic and residual protein in the extracted nuclear preparations. Aliquots of the nuclear extracts (1–4 mg) in 0.1 *M* *Tris*-0.1 *M* NaCl (pH 7.5) were precipitated and washed 3 times with cold 10% trichloroacetic acid (TCA). Basic proteins were extracted twice from the pellets by shaking at 4 °C for 1 h with 0.4 ml of 0.4 *N* H_2SO_4. The combined extracts were adjusted to pH 10 with 4 *N* NaOH and brought to a final volume of 1 ml. Protein concentration was determined by the method of LOWRY et al[11] using calf thymus histone as a standard.

Nucleic acids were removed from the residues of the acid extractions by heating at 95 °C for 20 min in 5% TCA. The residual protein was dissolved in 0.1 *N* NaOH and protein was estimated using the LOWRY[11] method with a bovine seum albumin standard. The DNA content of the TCA hydrolysate was determined by the Dische diphenylamine reaction[12], and the protein:DNA ratios were calculated.

In assaying for phosphoprotein, the nuclear extract was treated with 10% TCA, centrifuged and washed.

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