

cytoplasmic protein fractions, Y and Z, which bind organic anions (such as bilirubin) have been isolated<sup>16</sup>. Whereas protein Y is present exclusively in the liver<sup>16</sup> and increases after phenobarbital administration<sup>17</sup>, the small intestinal mucosa only contains fraction Z<sup>16</sup>. Failure of phenobarbital in increasing conjugated bilirubin concentration in the intestinal mucosa of homozygous Gunn rats is similar to that described for the liver<sup>6</sup>. This fact and the correlation between Tm values and percent-

ages of mucosa conjugated bilirubin seem to confirm that glucuronide synthesis by the intestinal mucosa is parallel to the liver capacity<sup>1,4</sup>. We detected some conjugated radioactive pigment in the gut lumen of hepatectomized rats after the injection of labelled unconjugated bilirubin. However, the mechanism of transfer was not established<sup>18,19</sup>.

**Resumen.** Se estudió el efecto del fenobarbital sobre la conjugación de la bilirrubina en ratas Wistar y Gunn heterocigotas sometidas a la infusión continua de bilirrubina no conjugada. Los resultados obtenidos permiten suponer que el fenobarbital es capaz de estimular la conjugación de la bilirrubina tanto en el hígado como en la mucosa intestinal de ratas con deficiencia parcial de glucuronil transferasa.

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## Ultrastructure of the Parathyroid and 'C' Cells of the Thyroid in Experimental Rachitis in the Rat

In young rats submitted to a diet<sup>1</sup> characterized by an increased calcium/phosphorus ratio and a lack of Vitamin D, a decalcification of the formed bone and an absence of calcification in the growing bone was produced. These animals showed some histopathological alterations of the gastric mucosa with a significant increase of HCl. Macroscopic ulcerations in large quantities were also observed<sup>2</sup>.

Supposing that the diet directly influences the regulatory mechanisms of calcium and phosphorus, we studied the morphology of the thyroid glands ('C' cells) and the parathyroid glands since serum calcium levels were the same in the treated rats as in the non-treated which were used as the control group<sup>3</sup>.

**Material and methods.** Wistar rats, 25 to 30 days old, weighing 45 to 55 g and of aleatory sex were used and they were put on the diet immediately after weaning. Blood samples were taken by decapitation of treated and non-treated rats in basal conditions. The samples were allowed to coagulate at room temperature and were then centrifuged. The inorganic phosphate was determined by a photocolorimetric micromethod<sup>3</sup> utilizing a Beckman-DU photocolirimeter for its readings.

For the ultrastructural study, the parathyroids and thyroids were taken under anaesthesia with urethane moments before the decapitation. The fragments obtained were immediately fixated in glutaraldehyde (2 h) and osmium tetroxide (1 h), buffered by phosphate buffer to

pH 7.2 at a temperature of 4°C. Dehydrated with acetone, included in vestopal, cut with an ultramicrotome LKB, contrasted with uranyl and lead compounds and observed with a Phillips EM-200 electron microscope.

**Results.** The quantity of inorganic phosphate can be seen in the Table.

**Parathyroids.** The principal cells were characterized by a greater electronic density in the nucleus and cytoplasm, and by the emission of numerous microvilli on the surface which intercrossed with those of neighboring cells, leaving between them clear spaces (Figure 1). The number of mitochondria and of rugous endoplasmic reticulum were moderate in their development. The Golgi apparatus appeared very well developed (Figure 1). Around it were a few low-density granules surrounded by a nitid membrane. Abundance of free ribosomes and polysomes (Figure 1). The centrioles were remarkably developed so that even ciliated images were seen (Figure 1). Frequently myelinic bodies were observed in the cellular cytoplasm and in the interstitial spaces (Figure 1). The nuclei with evident nucleolus and dense chromatin, separated below double nuclear membrane, showed wide gashes. The control rats of the same age clearly showed the principal cells with their characteristic types - clear and dark - (Figure 2A).

**Thyroids:** Numerous calcitonin cells were observed with abundant secretory granules which were a size from 1200 to 2500 Å of a fine granular matrix, and surrounded by a very evident membrane. The Golgi apparatus was well-developed and showed, at its border small, very dense granules. (Figure 2B)

Serum levels of inorganic phosphorus in normal (C) and rachitic rats (R)

Groups	P	Significance
C	(9) 4.00±0.2172*	NS
R	(7) 3.97±0.2516*	NS

P = mg/100 ml. \* Mean±standard error. In parenthesis, number of determinations.

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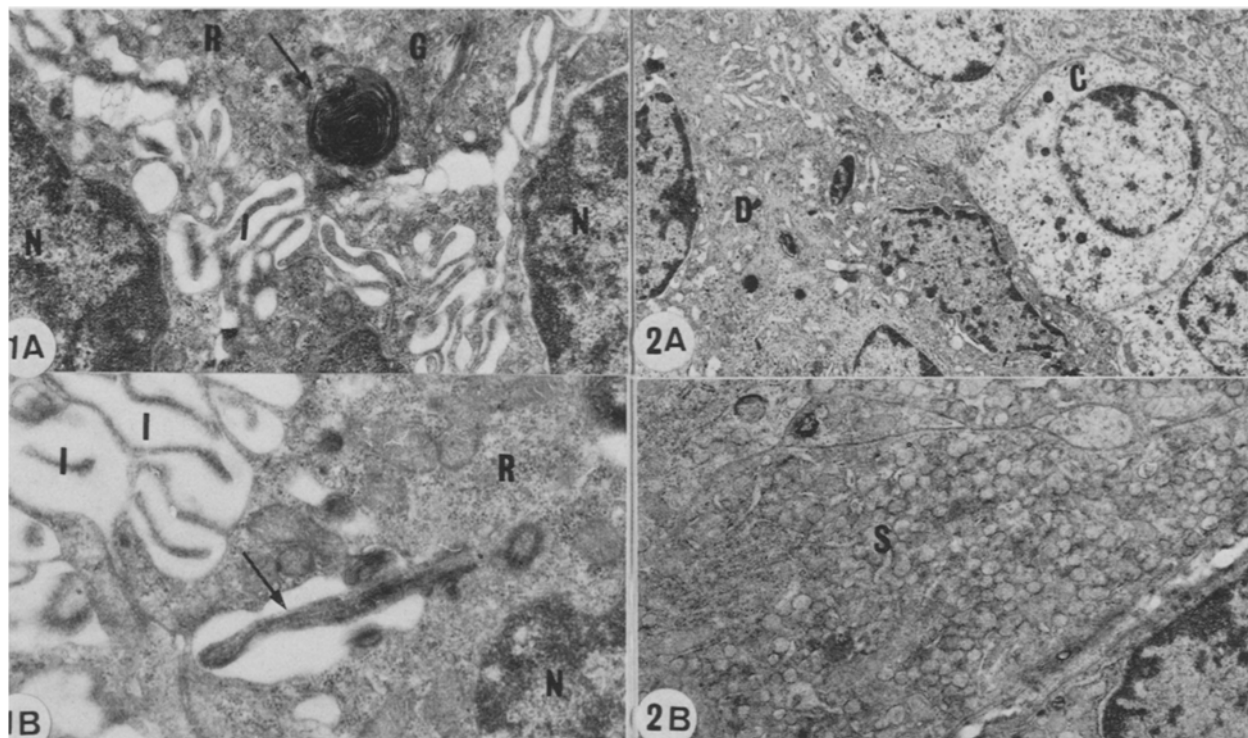


Fig. 1. Parathyroid cells. A) Myelinic body (arrow); Golgi apparatus (G); free ribosomes (R); clear spaces and microvilli (I). Nucleus (N). B) Centriolus and ciliated image. (Arrow). Fig. 2. A) Parathyroid normal gland (control rats): clear cell (C) and dark cell (D). B) Thyroid 'C' cells in rachitic rats: secretion granules (S).

**Discussion.** The studies of normal parathyroids and their verified changes under hypo- and hypercalcaemia have been numerous<sup>4-6,18</sup>. In every case of hypocalcaemia, it is observed that cellular interdigitations are found, the cellular cytoplasm is darkened and the nuclear folds are increased. In our studies, these phenomena are much more marked. All of this was possible probably because our experiments were done with adolescent instead of adult rats.

The calcitonin cells, whose variations and morphology have also been amply studied<sup>7-14</sup>, were filled with granulations, the significance of which is difficult to interpret since they point to two possibilities: 1. storage in the granules due to the lack of liberation, 2. they were in a period of formation for their ulterior secretion.

The fact that the serum levels of calcium and phosphorus were not affected might be due to the intervention of the regulatory hormones of the endogenous calcium metabolism. This is possible because the diet used by us impedes the absorption of exogenous calcium<sup>15,16</sup>.

A hypocalcaemia at the beginning of the diet could be the stimulus for the releasing of PTH and thus permanently compensate the hypocalcaemia, given that the life span of the PTH in blood is about 20 min<sup>17</sup>, and its continual secretion would bring about the picture described above which becomes nothing more than an exaggeration of the gland's morphology by hypocalcaemia-producing stimuli. A hypocalcaemia is not probable<sup>12</sup> after successive discharges of PTH so the intervention of thyrocalcitonin is not needed. This hormone has, however, been continuously synthesized<sup>10</sup> and its storing could explain the variegated picture of secretory granules which are observed by the electron microscope. We believe this possibility to be the more likely of the two given here.

**Zusammenfassung.** Bei Ratten wurde experimentell eine Rachitis erzeugt und dabei die Veränderungen der Zellen der Schilddrüse und der Nebenschilddrüse untersucht und die Werte von Ca und P im Blut. Bei jungen Tieren sind die Veränderungen wesentlicher als bei alten Ratten. Es werden die Einwirkungen der Hypocalcämie diskutiert.

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