for 24 h at 32°C. Nucleic acids (DNA and RNA) were stained by methylene blue (Merck, Darmstadt; C. I. Nr. 52015; PISCHINGER[7,8]; 0.002 *M*, pH 4.8, 15 min). After RNase treatment (2 mg/ml, 20 U, 3 h, 37°C) the DNA: RNA ratio of nuclei of differentiated cells (mesencephalon, stage 32) was estimated to be approximately 1:1. On the basis of the Feulgen-cytophotometric measurements of DNA content[4] and the described DNA/RNA ratio, the RNA content of nuclei could be calculated by measuring the total nucleic acid content. For determining the nucleic acid and nonhistone protein content, the stain intensity was measured in 70 individual nuclei from each slide with a Barr and Stroud integrating microdensitometer (Glasgow, Type GN 2).

Results and discussion. In all cases the measurement data, which were collated to so-called karyograms, show a doubling of dye content during the cell cycle. This means that nuclei double their RNA and nonhistone protein content from G_1 to G_2 phase, as is known for DNA and histone. Further, it became evident that the RNA and nonhistone protein contents are not constant in the course of development, but vary with stage specifically. At the onset of gastrulation, no considerable changes in nuclear RNA content can be found until stage 11b (curve B). Then a remarkable increase occurs with a maximum in the late gastrula (stage 12c, about 96% over the definitive value). In the following stages, the RNA content decreases rapidly and reaches minimum values with the formation of the neural plate (stage 14, 15). A second drastic increase in nuclear RNA content (about 70%) can be detected in the late phase of neurulation, followed by a continuous reduction to the tailbud stage.

These changes in nuclear RNA content are correlated with significant changes in the Feulgen stainability of nuclei in the appropriate stages (curve A). The comparison of the 2 curves (A, B) shows that in both phases the DNA content reaches maximum values first, to be followed by the RNA maximum about 2 h later. There is a good temporal correlation between the increases of DNA and RNA during gastrulation. During neurulation, the accumulation of RNA extends over the whole period, whereas the additional DNA increase is of short duration. These results support our assumption that the stage- and region-specific increases of Feulgen dye content[4] are an expression of enhanced gene activity during early development.

The changes of nonhistone protein content in the course of development are illustrated in curve C. With the beginning of gastrulation, the nonhistone protein content increases slightly and is then rapidly diminished from stage 11b to 12b. Because it is not possible with cytochemical methods to distinguish between chromosomal and non-chromosomal proteins, the measurement data always represent the changes of the sum of both protein classes. By measuring nuclear sizes (JANSEN, unpublished results), it was found that nuclear volume decreases continuously from stage 10 to 12b. From this fact, we should also expect a continuous reduction of nuclear protein content, as is shown for stage 11b to 12b. Assuming that the decrease of nuclear size is due to a loss of non-chromosomal protein, the slight increase of nonhistone protein indicates an accumulation of chromosomal proteins prior to the beginning of RNA synthesis (curve B), which is in agreement with findings that nonhistone chromosomal proteins are responsible for tissue-specific gene activation[9,10]. At a time when RNA content shows maximum values, a drastic increase in protein content (about 90%) takes place and is correlated with a spontaneous swelling of nuclei. This confirms the generally accepted idea that nucleic acids always occur in close association with protein. The parallel decrease of RNA and protein is, therefore, probably caused by the transport of ribonucleoprotein from nucleus to cytoplasm. During neurulation, similar correlations between RNA and protein content exist. Before the onset of RNA synthesis, the nonhistone protein content of the early neurula is increased. At the end of neurulation, RNA and protein have highest values which were slowly reduced in the following stages to the final amount of the tailbud stage.

The temporal correlation of our results with early embryonic induction on one hand, and the differentiation of the neural tube on the other, raises the question of what kinds of RNA are synthesized, and whether there are stage-specific changes in the nonhistone protein pattern during gastrulation and neurulation. The fact, however, that the stage-specific increase of RNA is accompanied by a preceding synthesis of additional DNA focusses attention on the possibility of the existence of gene amplification during early development. This point is now under investigation.

1 G. S. STEIN, T. C. SPELSBERG and L. J. KLEINSMITH, Science *183*, 817 (1974).
2 K. SCHERRER, in *Regulation of Transcription and Translation in Eukaryotes* (Eds. E. K. F. BAUTZ, P. KARLSON and H. KERSTEN; Springer Verlag, Berlin-Heidelberg-New York 1973), p. 81.
3 K. LOHMANN and W. VAHS, Experientia *25*, 1315 (1969).
4 K. LOHMANN, Wihelm Roux' Arch. *169*, 1 (1972).
5 K. LOHMANN, Wihelm Roux' Arch. *177*, 285 (1975).
6 J. S. KAYE and R. MCMASTER-KAYE, J. Cell Biol. *31*, 159 (1966).
7 A. PISCHINGER, Z. Zellforsch. *5*, 347 (1927).
8 A. PISCHINGER, Z. Zellforsch. *3*, 169 (1926).
9 J. PAUL and R. S. GILMOUR, J. molec. Biol. *34*, 305 (1968).
10 R. S. GILMOUR and J. PAUL, FEBS Lett. *9*, 242 (1970).

Coated Vesicles in the Rat Adrenal Glomerular Zone After a Low-Sodium Diet

G. PALACIOS and M. LAFARGA

Department of Histology, Faculty of Medicine, Autonomous University, Hospital de San Pablo, Barcelona-13 (Spain), 24 March 1975.

Summary. In rats subjected to a low-sodium diet, a great activity was observed of the coated vesicles at Golgi complex and cell surfaces of glomerular adrenal zone. These findings are related to the function of these organoids in the uptake and transport of necessary substances under stimulating conditions of the zone.

The presence of coated vesicles in the adrenal cortex cells of various species has been confirmed in recent investigations[1-5]. In these previous studies these elements were discovered in relationship with the Golgi complex and the cellular membrane. It is recognized that these vesicles participate in the transport of protein substances

1 J. A. LONG and A. L. JONES, Am. J. Anat. *120*, 463 (1967).
2 J. A. LONG and A. L. JONES, Lab. Invest. *17*, 355 (1967).
3 N. S. MCNUTT and A. L. JONES, Lab. Invest. *22*, 513 (1970).
4 G. G. NUSSDORFER, Z. Zellforsch. *103*, 382 (1970).
5 D. WASSERMANN and M. WASSERMANN, Cell Tissue Res. *149*, 235 (1974).

to the interior of the cell and of hydrolytic enzymes from the Golgi complex to the lysosomes[6]. During an experimental study concerning the rat adrenal cortex with low-sodium diets, a large activity of these coated vesicles was observed in the glomerular zone, mainly in the Golgi regions and on the cell surface.

Material and methods. 36 adult male Wistar rats, weighing between 250 and 300 g, were divided into 3 groups: a) A first group of 6 rats received a normal diet and served as control. b) A second group of 24 rats was given a low-sodium diet for 6 weeks. This diet followed a formula similar to that of HARTROFT and EISENSTEIN[7]. Each week of the experiment 4 animals of this group were sacrificed. c) A third group of 6 rats, also serving as control, which for 6 weeks received the same diet as a second group but with a normal proportion of sodium. These animals were sacrificed at the end of the 6th week.

All animals were subjected to perfusion fixation with 3% glutaraldehyde in a 0.12 *M* phosphate buffer at pH 7.4 for 15 min. The adrenal glands were extracted, sectioned to fine slices, submerged in the same fixative for 2 h, and finally post-fixed in 1% osmium tetroxide in the same buffer. After dehydration in increasing concentrations of acetone, the material was included in Durcupan ACM (Fluka). Ultra-thin sections were stained with lead citrate and examined with a Hitachi HU-12 electron microscope.

Results. The cells of the glomerular zone in the control animals appeared to be forming compressed cords (Figure 1A). The cell surfaces were separated by spaces of about 200 Å. Some coated vesicles could be seen on these surfaces (Figure 1B). Some characteristic organoids of these cells could be seen in the cytoplasm, such as lipid droplets, smooth endoplasmic reticulum, and tubular crest mitochondria (Figure 1, A and B).

In the experimental animals, from the second week until the end of the experiment much activity on the part of the coated vesicles in the Golgi regions could be observed (Figures 2 and 3). Moreover, a large number of vesicles of this type invaginated in the cell membrane and passed to the superficial zone of the cytoplasm (Figure 4). On the other hand, during the course of the experiment an important increase of other organoids could be verified, such as mitochondria, smooth endoplasmic reticulum, and lipid vacuoles.

Discussion. In the present study, great activity of coated vesicles was shown in the glomerular zone cells of rats on a low-sodium diet. Similar findings were not described by other authors who studied this zone under the same experimental conditions[8–12].

In other conditions of the adrenal cortex stimulation, such as after the employment of ACTH or the administration of prostaglandins, a larger coated vesicle activity was also described[13,14].

Various important functions have been attributed to coated vesicles: protein uptake; source of basic membrane material which can be incorporated in organoid development; acting as carriers in the transport of substances into the cell, such as hydrolytic enzymes from the Golgi complex to the lysosomes[6,15,16]. The first two functions

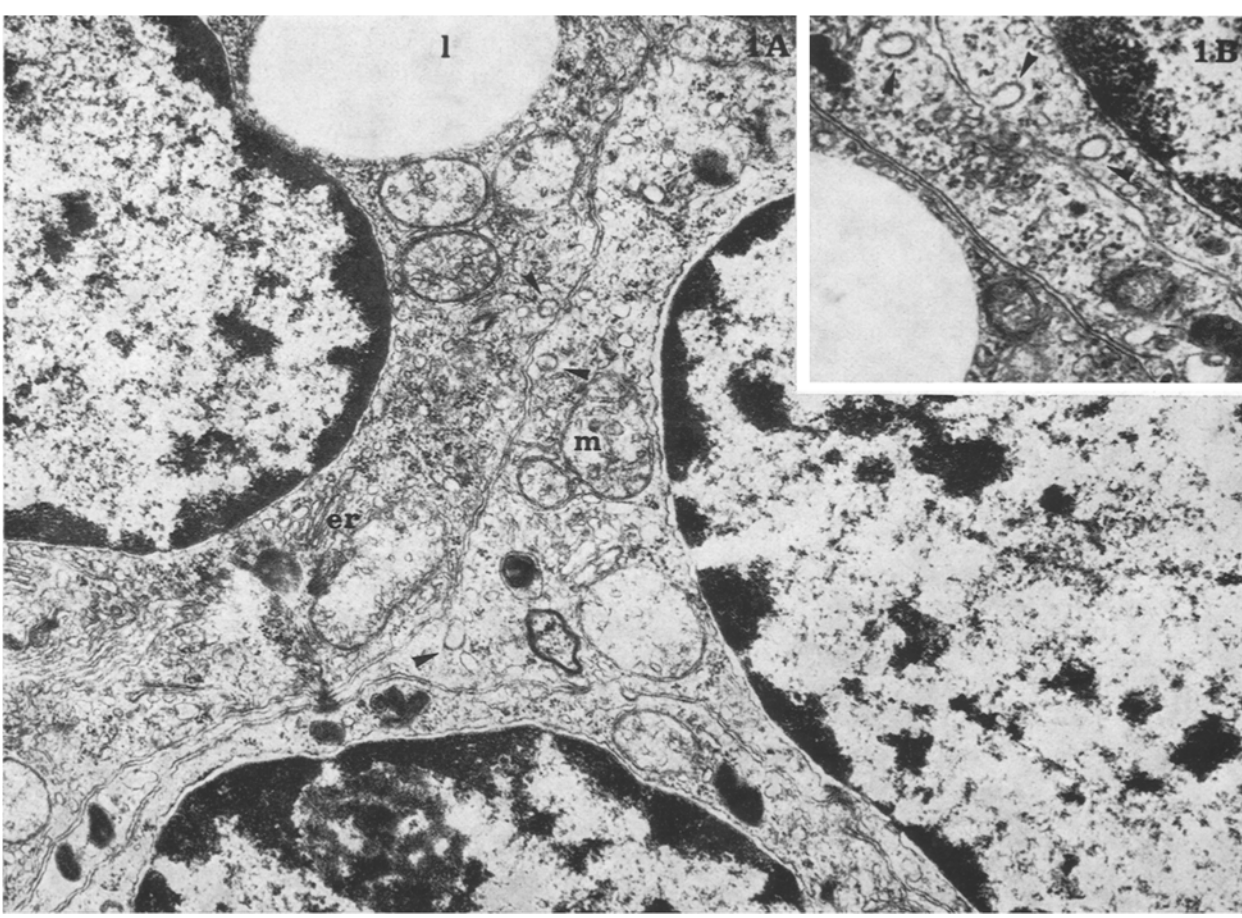
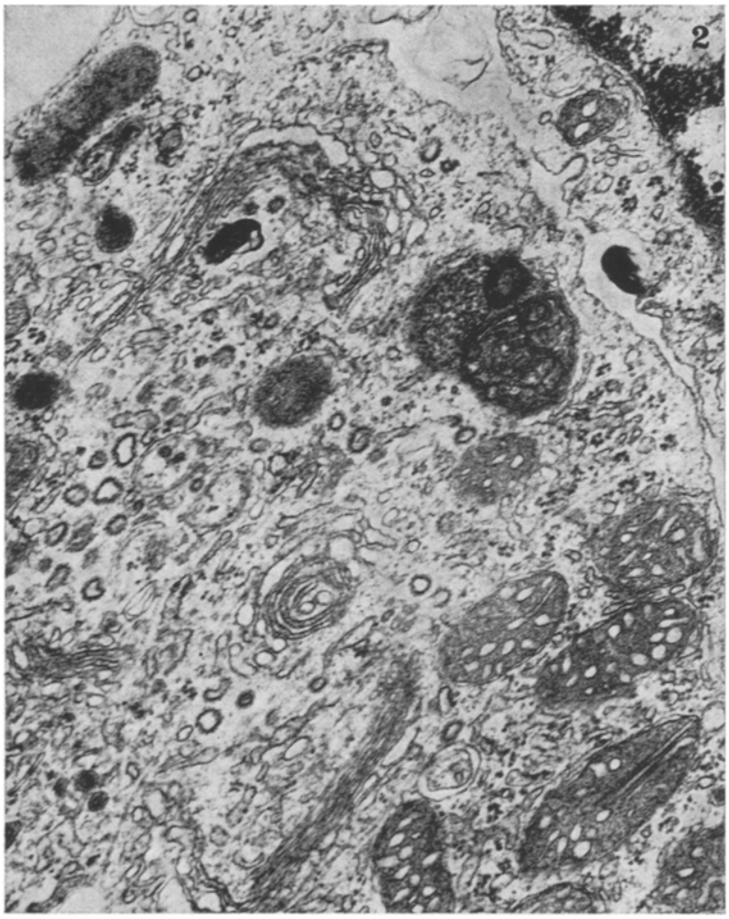
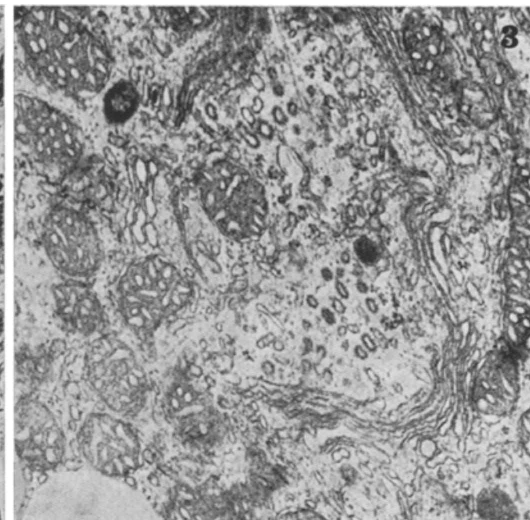


Fig. 1. Control animal. A) Group of glomerular zone cells in intimate contact with each other. In their cytoplasm can be seen mitochondria (m), lipid vacuoles (l) and smooth endoplasmic reticulum (er) ×20,000. B) Detail of coated vesicles (arrows) near the cell surface ×25,000.





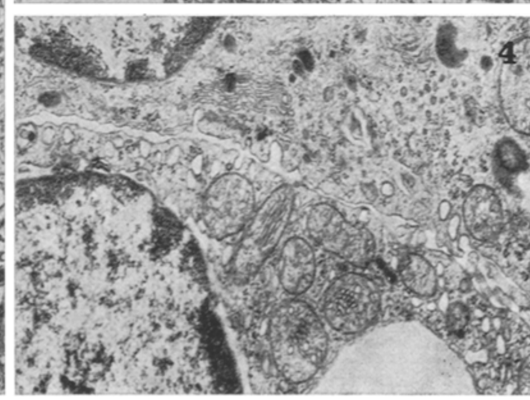


Fig. 2. Large coated vesicle activity in the cytoplasm and Golgi region in an experimental animal. ×20,000.
Fig. 3. Golgi complex showing numerous coated vesicles. Experimental animal. ×12,000.
Fig. 4. Transport of coated vesicles between the cell surface and the cytoplasmic interior. Experimental animal ×12,000.

would be very important to a cell in hypertrophic conditions which require plastic elements for the construction of its organoids. The last functional property attributed to these organoids would attend to the requirement of an increased catabolic turnover in a hyperfunctioning cell. Both of these conditions exist in the cells of the glomerular zone, due to the stimulus resulting from the utilization of these diets.

[6] D. S. Friend and M. G. Farquhar, J. Cell Biol. *35*, 357 (1967).
[7] P. M. Hartroft and A. B. Eisenstein, Endocrinology *60*, 641 (1957).
[8] J. D. Lever, Endocrinology *58*, 163 (1956).
[9] F. Giacomelli, J. Weiner and D. Spiro, J. Cell Biol. *26*, 499 (1965).
[10] J. A. Long and A. L. Jones, Anat. Rec. *166*, 1 (1970).
[11] J. H. Shelton and A. L. Jones, Anat. Rec. *170*, 147 (1971).
[12] D. T. Domoto, J. E. Boyd, P. J. Mulrow and M. Kashgarian, Am. J. Path. *72*, 433 (1973).
[13] G. G. Nussdorfer, G. Mazzocchi and L. Rebonato, Z. Zellforsch. *115*, 30 (1971).
[14] D. P. Penney, J. Olson and K. Averill, Z. Zellforsch. *146*, 297 (1973).
[15] O. A. Schjeide and R. I. San Lin, in *Cell Differentiation* (Eds. O. A. Schjeide and J. De Vellis; Van Nostrand Reinhold Company, New York 1970), p. 224.
[16] G. G. Maul and J. A. Brumbaugh, J. Cell Biol. *48*, 41 (1971).