Involutive morphological modifications in the rat adrenal glomerular zone after a low-sodium diet

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Summary. We have studied glomerular zone involution in the rat's adrenal gland after a period of hyperfunction brought about by a low-sodium diet. The changes observed in this zone affect those organoids that are more directly involved in steroid genesis; mitochondria, smooth endoplasmic reticulum and liposomes. The Golgi complexes appear very developed, often, showing, a positive acid phosphatase activity. Lysosomes suffered a considerable increase in their number, and carried out their digestive function on liposomes. All those changes discussed here are seen as an accomodation of this zone to the new normofunctional situation.

It has become usual to use low-sodium diet to produce experimental situations of hyperfunction in the glomerular zone of the adrenal gland[1-7]. Little attention, however, has been paid to the involution of such hyperfunctional states. Following the line started by Marx and Deane[8], we have studied the morphological changes in the rat adrenal glomerular zone after a period of low-sodium diet, followed by a short period of normal diet. We have given special attention to the role of the lysosomes in such functional states.

Material and methods. A total of 34 Wistar male rats have been studied. They were divided into 1 control group of

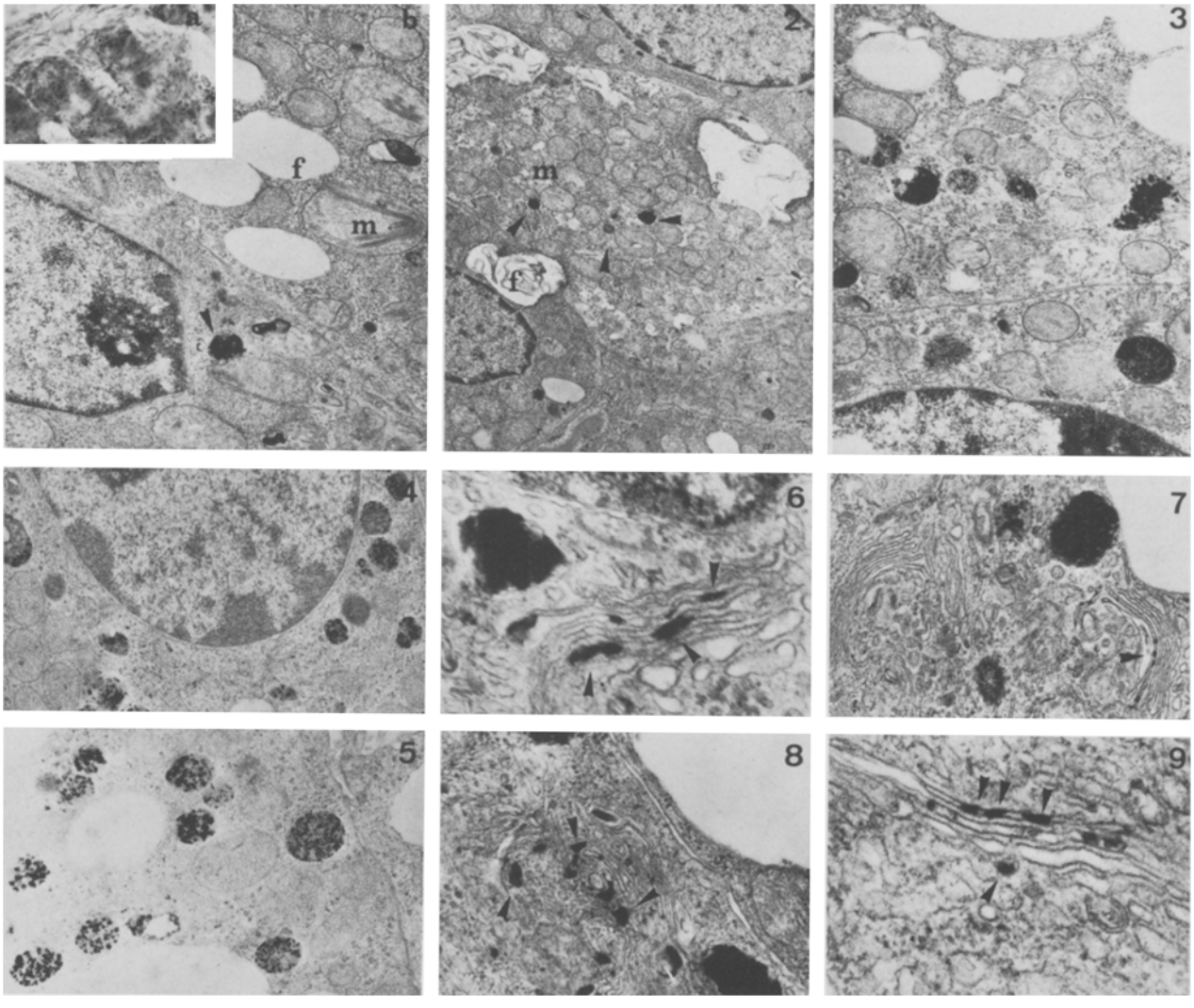

Fig. 1. *a* Acid phosphatase reaction in the glomerular zone of a control rat, frozen section. × 1000. *b* Acid phosphatase reaction in a cell of the glomerular zone of an experimental rat. See the lipid droplets (f), the pseudocrystalline arrangement of mitochondrial cristae (m), and a few lysosomes (arrows). × 8000. Fig. 2. Acid phosphatase reaction. The experimental animal had 3 days of a normal diet after 8 weeks of a low-sodium diet. Note the cytoplasmic desorganization; lipid droplets fused showing pseudomembranes in their interior (f), mitochondrial cristae disorganized (m), a few lysosomes (arrows) × 3500. Fig. 3. Acid phosphatase reaction. 3 days of normal diet after 12 weeks of a low-sodium diet. Note increase in the number of lysosomes. × 10,000. Figures 4 and 5. Acid phosphatase reaction. 5 days of normal diet after 12 weeks of a low-sodium diet. Numerous groups of lysosomes. × 8000; × 10,000. Figures 6–9. 5 days of normal diet after 8 and 12 weeks of low-sodium diet. Golgi complexes show in their dictyosomes and vesicles a material positive to acid phosphatase action (arrows). × 24,000; × 20,000; × 30,000; × 40,000.

4 animals and 3 experimental groups of 10 animals each. The control group had a normal diet. The experimental groups had low-sodium diet, similar to that used by Hartroft and Eisenstein[9], and bidistilled, deionized water to drink for 4, 8 and 12 weeks, respectively. We sacrified 4 animals from each experimental group at the end of their respective periods of diet; the 6 animals left from each group were sacrified in pairs after 1, 3 and 5 days of normal diet. The control group was sacrified the last week of the experiment. They were all anaesthetized with chloral hydrate (300 mg/kg b. wt), previous to sacrifice. Tissue fixation was accomplished by perfusion through the aortic arch. 2 different fixation solutions were used:

1. A 3% glutaraldehyde solution in 0.12 M phosphate buffer, pH 7.4 was used for 2 animals from the control group and 5 from each of the experimental groups. The tissues were post-fixed in 1% osmium tetroxide solution in the same buffer. 2. The rest of the animals were fixed in a 2.5% glutaral-dehyde solution in 0.1 M cacodylate buffer, pH 7.3, so as to detect acid phosphatase activity. Each adrenal gland was sectioned into 2 fragments, one of them was used for the obtention of 10-µm frozen sections; from the other fragment we obtained 40-µm sections with an Smith-Farquhar tissue chopper. Both types of sections were incubated for 60 min at 37 °C in Gomori medium[10]. 1 set of each was incubated in this medium leaving out Na-β-glycerophosphate. The 10-µm sections were used as an optical control for the reaction after developing in ammonium sulphide. The 40-µm sections were washed in cacodylate buffer containing 7.5% sucrose and were post-fixed in 1% osmium tetroxide in the same buffer.

The material obtained from both methods of fixation was dehydrated with acetone and embedded in Durcupan ACM (Fluka). The ultrathin sections were stained with lead citrate and/or uranyl acetate and examined under the electron microscope Hitachi HU-12A.

Results. The adrenal glomerular zone of the animals treated with a low-sodium diet showed considerable hypertrophy and hyperplasia compared with the controls. Quantitative cytological changes in some organoids engaged in steroid synthesis were also present. The nature of these changes have been described already in our past reports[6,7]. The acid phosphatase reaction in both the control and experimental group rats, showed a glomerular zone well provided with lysosomes, when compared with underlaying zones, but no substantial differences were observed between the control and treated animals (figures 1, a and b). With respect to the animals which had 1 day normal diet, after their corresponding period of low-sodium diet, there were no differences when compared with the rest of the groups having had low-sodium diet only. Differences were observed, however, in those animals which had a 3 and 5 days normal diet after the low-sodium one; some cells in the glomerular zone exhibited disorganization in their cytoplasm (figure 2),

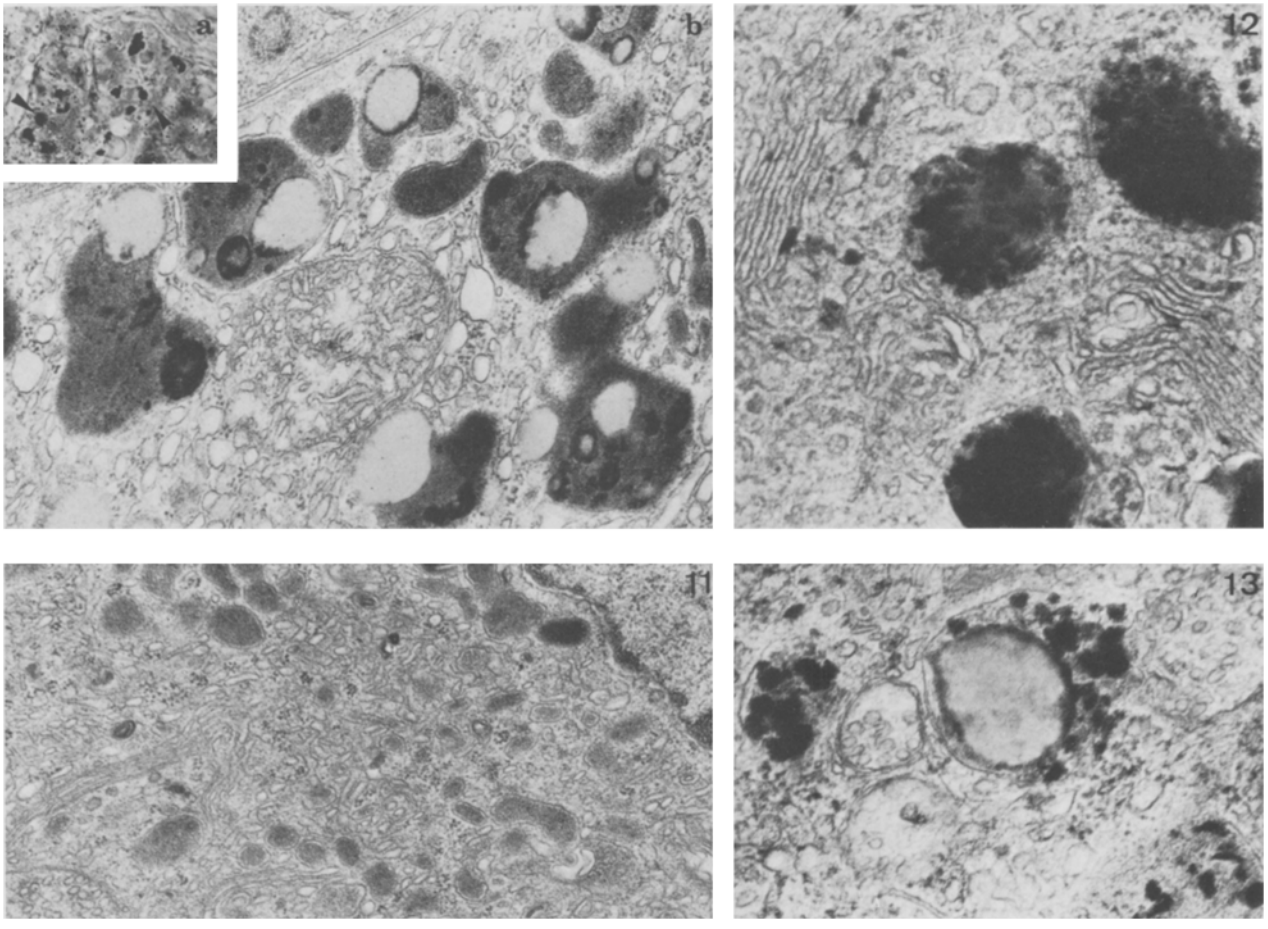

Fig. 10. *a* Acid phosphatase reaction, frozen section showing heaps of material positive to the reaction (arrows), in the glomerular zone cells of a rat which had 5 days of a normal diet after 12 weeks of a low-sodium one. × 1000. *b* Groups formed by large dense bodies of vacuolar content in the same cells. × 17,000. Figures 11 and 12. 5 days of a normal diet after 12 weeks of a low-sodium one. Golgi fields showing prelysosomes and large lysosomes positive to the acid phosphatase reaction. × 17,000; × 35,000. Fig. 13. Dense body of vacuolar content with material showing positive acid phosphatase reaction. × 20,000.

affecting the lipid droplets which had a tendency to fuse forming pseudomembranes in their interior. The mitochondria also showed modifications losing the pseudocrystalline cristae characteristic of the preceding period (figures 2 and 3). The smooth endoplasmic reticulum was scarce, the acid phosphatase positive dense bodies increasing in number (figure 3). This was more evident in animals having had a 5 day normal diet after their low-sodium one. We also observed numerous groups of lysosomes in these animals (figures 4 and 5); the Golgi complex which was well developed in those cells, showed in their vesicles and dictyosomes a material positive to the acid phosphatase reaction (figures 6–9). In some of the histochemical control sections we saw, under optical microscope, heaps of material positive to the reaction (figure 10, a), which corresponded ultrastructurally to groups of large dense bodies of vacuolar content proceeding, in all probability, from liposomic desintegration (figure 10, b). The vacuolar residues were surrounded by dense precipitates indicating their phosphatase activity (figure 13). The Golgi fields had either heaps of small bodies of variable density (probably prelysosomes) (figure 11), or large lysosomes (figure 12).

Discussion. The administration of a low-sodium diet produces a hyperfunctional situation in the rat's adrenal glomerular zone 1–7. The return to a situation that one could consider as normofunctional causes a cytological disorganization in that zone, which affects those organoids that are more directly related to steroid genesis; liposomes, mitochondria and smooth endoplasmic reticulum. Lysosomes play a main role in those changes, its activity is chiefly directed to liposome digestion; the liposomes are the structures containing the steroid hormone precursors such as cholesterol and its esters[11], and the hormones themselves, as has been suggested[12]. Szabó[13] showes a direct lysosome action on cholesterol crystals of the fasciculate zone. We could not find, despite its having been pointed out by other authors, an increase in the number of lysosomes in hyperfunctional situations produced either by the above-mentioned diets in the glomerular zone[2], or by stimulating hormones in the fasciculate zone (ACTH, prostaglandins)[14,15]; on the other hand, a cytoplasmic atrophy of some cells in the glomerular zone has been observed, due to an increase in the number of lysosomes, in experimental states of hypofunction brought about by high content sodium diets 4. Hypophysectomy offers contradictory results; Fujita[16] makes quite evident that there is a fatty degeneration of the cells in the fasciculate zone with the apparition of dense bodies, which include lipid droplets. On the contrary, Szabó et al.[14] describe a decrease in the number of lysosomes after hypophysectomy and a recovery in number after ACTH administration. The use of drugs inhibiting cholesterol synthesis also increase the number of acid phosphatase-positive, dense bodies in the cortical cells[17,18]. The regressive changes observed by us in the glomerular zone cells, have not lead to an atrophy or cellular degeneration, such as that described in the fasciculate zone after treatment with dexametasona[19], or to a process of celular death far more programmed and dependent on hormonal factors such as that described with the term 'apoptosis' in cells of the adrenal gland[20,21]. This may be due to having used a time of involution, in our work, not long enough to bring about such changes, or it may be that the blockage on functional activity in these cells is not sufficient to produce it. As a matter of fact, during the 5 days of involution, the glomerular zone kept the same thickness as in the preceeding stage of hyperfunction. Other authors[8] needed 12 days to get the normal thickness of this zone. A complementary study, which we have already started, should clarify these points.

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Types of nerve terminals in fetal and neonatal rabbit myocardium[1]

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Summary. With the use of electron microscopy 4 types of axonal profiles were observed in the developing myocardium of rabbits: 1) adrenergic axons which contained mainly small dense-core vesicles and which presumably can store 5-hydroxydopamine; 2) cholinergic axons which contained small clear synaptic vesicles and which were acetylcholinesterase-positive; 3) axons which contained large vesicles filled with moderately electron-dense material and which resembled purinergic axons; and 4) profiles filled with mitochondria, vesicles of various sizes, lysosome-like bodies, and microtubules and which resembled sensory terminals.

A number of workers have approached the development of the innervation to embryonic, fetal and neonatal myocardium from a morphological standpoint. Generally, fluorescence histochemical studies have indicated that in many species adrenergic myocardial innervation occurs only at a late developmental phase, e.g. the first postnatal week in rats[2-4] or near term in the rabbit[5] and lamb[6]. Likewise acetylcholinesterase-positive nerve fibres could not be detected until late in development, e.g. 12th postnatal day in the rat[7] and 24th–27th day in the rabbit[8]. However, recent studies have shown that treatment of the heart with amine analogs allows the detection of fluorescent adrener-