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-

gic nerves in the myocardium earlier in gestation that it was previously thought they existed[9,10]. Unfortunately ultrastructural studies are not available to corroborate and extend these light microscopic studies. Therefore an electron microscopic study was undertaken to examine the types of nerve fibres present in the developing myocardium of fetal and neonatal rabbits.

Materials and methods. New Zealand white rabbits were used for this study. Animals were prepared for examination at 20, 24, 26, 28 and 30 days of gestation and 1, 4, 7, 10, 15, 21 and 35 days post partum (3–6 animals at each stage). The animals were obtained as previously described[11]. For electron microscopy, atrial and ventricular tissue was fixed by 3 methods: a) fixation for 2.5 h in a mixture of 2.0% paraformaldehyde–2.5% glutaraldehyde in 0.1 M cacodylate buffer at pH 7.3, containing 0.04% $CaCl_2$ with postfixation in 1% osmium tetroxide for 2 h; b) fixation for 1.25 h in 1.0% paraformaldehyde–1.25% glutaraldehyde in 0.1 M cacodylate buffer at pH 7.2, containing 0.04% $CaCl_2$ (this tissue was processed for the demonstration of acetylcholinesterase by a modification[12] of the Karnovsky method[13]); and c) fixation for 45 min in 3.5% potassium permanganate in phosphate buffered Krebs Ringer solution, pH 7.0[14]. All tissue was subsequently embedded in epon, sectioned, and then examined with a Siemens Elmiskop I electron microscope. Some postnatal animals were injected i.p. with 5-hydroxydopamine (5-OHDA), 100 mg/kg, prior to fixation in aldehydes. Tissue from some prenatal animals was treated with 5-OHDA by incubating (prior to fixation in aldehydes) slices of tissue in Krebs-Henseleit solution containing 1 mg/ml 5-OHDA, pH 7.4, at 37 °C for 30 min. The solution was saturated with 95% O_2–5% CO_2 during the incubation period.

Observations. All the structures to be described as efferent axon terminals contained a population of synaptic vesicles of a certain type, were closely adjacent to cardiac muscle cells and were, at least partially, if not totally, free of Schwann cell coverings. Several different types of such terminals were observed in the atria and ventricles of fetal and neonatal animals. The most common type of terminal encountered was that generally described as adrenergic. The adrenergic terminals, when fixed with permanganate, contained mainly small dense-cored synaptic vesicles[15] (40–50 nm) (figure 1a). These terminals were first seen in animals of 20 days' gestation; subsequently the frequency of observing such terminals increased with increasing age of the animal. Adrenergic terminals were more evident in animals pretreated with 5-OHDA and then fixed in aldehydes. By 24 days of gestation adrenergic axons were capable of taking up this exogenous amine analog (figure 1b). In such axons a mixture of small (40–50 nm) and large (70–100 nm) dense-cored vesicles was present as well as elements of the tubular smooth endoplasmic reticulum (TR). Some elements of the TR were also seen to contain

Figure 1. *a* Axon profile (A) and cardiac muscle (M) from the atrium of a rabbit of 20 days gestation. Note the many small dense-core vesicles (arrow) in the terminal. Permanganate fixation. ×22,000. *b* Axon terminal near muscle (M) in atrium of a 24-day fetal rabbit treated with 5-OHDA. Small (S) and large (L) dense-core vesicles are evident. Some profiles of TR have taken up the 5-OHDA and show electron-dense material in their lumen (arrow). Aldehyde fixation. ×30,000. *c* Tissue from the ventricle of a 24-day-old fetus processed for the demonstration of acetylcholinesterase. Axon profile (C) contains many small clear synaptic vesicles and is acetylcholinesterase-positive (sites of reaction deposit indicated by arrows). M, muscle cell. Treated with 5-OHDA. × 19,000. *d* Atrial tissue from a 1-day-old rabbit treated with 5-OHDA. 2 axon profiles are evident near the cardiac muscle (M). Adrenergic axon (A) contains small and large dense-core vesicles. Cholinergic axon (C) contains mainly small clear synaptic vesicles. Aldehyde fixation. × 17,500.

Figure 2. *a* Atrial tissue from a 21-day postnatal animal treated with 5-OHDA and processed for demonstration of acetylcholinesterase. A bundle of axons contains both adrenergic (A) and cholinergic (C) profiles. Both types of axons are acetylcholinesterase-positive (sites of reaction product indicated by arrows). M, cardiac muscle. × 17,000. *b* Axon profile (A) from the right atrium of a 35-day-old animal treated with 5-OHDA. The axon contains many large vesicles which have moderately electron-dense cores. Aldehyde fixation. × 19,000. *c* Atrial tissue from a rabbit fetus of 20 days gestation. Axon profile contains many large vesicles with moderately electron-dense cores (arrows). Permanganate fixation. × 18,500. *d* Large nerve fibre from the right atrium of a 15-day-old rabbit treated with 5-OHDA. This fibre contains many mitochondria, lysosome-like bodies (L), microtubules (arrows), and various size vesicles and is enclosed by Schwann cell cytoplasm (S). Nearby are the adrenergic axons (A) and a muscle cell (M). × 17,500.

small electron dense-cores indicating uptake and storage of 5-OHDA[16] (figure 1b). Elements of the TR were infrequently observed in adrenergic axons of postnatal animals.

A 2nd type of axon terminal contained mainly small (40–60 nm) agranular vesicles and was acetylcholinesterase-positive (figure 1c). Terminals of this type were presumed to be cholinergic since the morphology of their vesicles did not change after treatment with 5-OHDA. Such cholinergic terminals were commonly encountered in animals of 24 days' gestation and older. In addition, adrenergic and cholinergic terminals were frequently observed adjacent to the same cardiac muscle cell (figure 1d). Heart tissue pretreated with 5-OHDA and then processed for demonstration of cholinesterase revealed that some adrenergic terminals were also acetylcholinesterase-positive and had travelled in the same axonal bundle with cholinergic axons (figure 2a).

In tissue from both fetal and neonatal animals fixed by either aldehyde or permanganate a 3rd type of nerve fibre was infrequently observed which contained large opaque vesicles (100–200 nm) (figures 2b and 2c). These large vesicles contained moderately electron dense-cores which nearly filled the entire vesicle. Furthermore, the morphology of these cores did not change after treatment of the tissue with 5-OHDA.

Finally a 4th type of nerve profile was encountered (only in the atrium and mainly in older animals). This type of profile was large, nearly always completely invested in Schwann cell cytoplasm, and contained many mitochondria, lysosome-like bodies, large granular vesicles and microtubules (figure 2d). Such nerve fibres resembled those described as sensory nerve terminals by others[17–19].

Discussion. Several previous studies have shown that the small dense-core vesicles in permanganate-fixed autonomic axons are storage sites for catecholamines[15,20], and 5-OHDA has also been shown to be an effective marker for amine-storage vesicles in adrenergic nerves[21]. On the other hand, axon terminals containing agranular synaptic vesicles[15] and showing a positive reaction for acetylcholinesterase are generally considered to be cholinergic. With the use of these methods this study has shown that adrenergic and cholinergic nerve terminals are present in the rabbit myocardium at the early part of fetal development. In addition, at 24 days of gestation small and large dense-core vesicles and elements of the TR were clearly evident in adrenergic nerves of the rabbit myocardium. After treatment of this tissue with 5-OHDA the cores of the small and large vesicles became highly electron dense and dense-cores were noted in the TR. Dense-core vesicles are generally considered to be the vesicular storage sites for noradrenaline in adrenergic nerves and the TR has been proposed as an extra-vesicular storage site for noradrenaline in adult[22] and developing[23] adrenergic nerves and also as a site of origin of the small amine-storing vesicles[16,24]. Assuming these interpretations from the literature are correct, the findings of the present study indicate that the amine uptake and storage mechanism of adrenergic nerves in rabbit myocardium must be functional by 24 days of gestation. Consequently the information derived from this study contrasts with that of previous light microscopic studies of cardiac innervation during ontogenesis which indicated that innervation does not begin until late in development[2–7]. This apparent difference in data may be explained by the fact that previous workers did not employ electron microscopy or use amine analogs to mark synaptic vesicles in developing axons. Also, if the content of transmitter substance or enzyme is low in the developing autonomic axons, then light microscopic histochemical methods may not be sensitive enough to detect it.

The present study revealed that in tissue treated with 5-OHDA and processed for the demonstration of acetylcholinesterase some adrenergic axons were also acetylcholinesterase-positive. Similar data have been reported by previous workers for the pineal gland[25], myometrium[26], heart[27] and iris[28] in adult animals. This information is significant in relation to the hypothesis of Burn and Rand[29] that there is a cholinergic link in adrenergic transmission.

The axons fixed in aldehydes and containing large moderately-electron-dense cores are identical in structure to those described previously as purinergic[30]. In addition, similar large vesicles in axons fixed in permanganate resemble the large opaque vesicles described in the nerve fibres of the retractor penis muscle of the bull as non-adrenergic, non-cholinergic inhibitory nerves[31]. According to Burnstock[32] some adenyl compounds have negative inotropic and chronotropic effects on isolated mammalian heart and thus they could possibly be released from inhibitory purinergic axons in the vagus nerve. Additional experiments are necessary to further characterize such axons as purinergic nerves in the heart.

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