contained 2 ml of Earle's solution, 0.5 mg/ml each penicillin G and streptomycin; 1 mg/ml of bovine serum albumin and 1 mg/ml of glucose. Con A and/or TSH were added only to the incubation medium. The flasks were continuously exposed to an atmosphere of 95% O_2 5% CO_2.
4. Histology. Specimens for light and electron microscopy were treated as described elsewhere[5]. Observations were performed with a Siemens Elmiskop IA electron microscope.

Results. 1. Control. a) Basal. Colloid droplets were seen in 10% of cells in group A and they were not observed in group B. b) TSH-stimulated (120-min-incubation). As expected, in response to TSH, 0.1 mU/ml or 2.5 mU/ml, colloid droplet formation was markedly enhanced in both groups A and B. After 2.5 mU/ml, virtually 100% of the cells in both groups A and B had colloid droplets, frequently multiple droplets in a single cell. 2. Effects of Con A-tretment (figures 1–4). a) Without TSH. Cells from both groups A and B exposed to Con A-concentrations from 50 to 2000 µg/ml for 180 min showed ultrastructural changes in a dose dependent manner. Con A-effects were detectable regardless of whether the gland has been suppressed with thyroid powder. In response to Con A, in the concentration range of 50 to 200 µg/ml, pseudopod and colloid droplet formation was observed in the cells after 180 min of incubation (figure 2). About 40% of the cells of both groups A and B showed pseudopods and/or colloid droplets. At 200 µg/ml, Con A also induced, in a small minority of the cells, an altered distribution of microvilli (figure 3). They tended to be agglutinated. These microvilli did not show ultrastructural changes either in the membrane which covers them or in the core of microfilaments. Concentrations in the range of 1000–2000 µg/ml induced a rounding of the apical border in 50% of the cells after a 180-min-exposure to Con A (figure 1). These effects were more commonly seen in small follicles. Pseudopods or colloid droplets were seldom observed with those doses of Con A. A redistribution of mitochondria was observed in some cells when 1000–2000 µg/ml of Con A were used. They were found to be limited by and apparently separated from the rest of the cytoplasm by a membrane (figure 1, arrows). b) With TSH (figure 4). After exposure of follicular cells to both Con A and TSH (200 µg/ml and 0.05 mU/ml, respectively), a large increase in colloid droplets, not seen with those concentrations of TSH alone or Con A alone, was observed. In about 40% of the cells the cytoplasm was virtually occupied by colloid droplets after 90–180 min exposure.

Discussion. This study shows that Con A induce complex changes in the ultrastructure of dog thyroid follicular cells in vitro. The ability of Con A to round up the apical portion of follicular cells (figure 1) is in accordance with the demonstration of a complete rounding of cells in tissue culture induced by exposure to Con A[6]. The clumping of microvilli (figure 3), is also compatible with findings by other investigators. It has been suggested that agglutination of cells by Con A is a function of the presence or absence of microvilli[7]. Probably, microvilli provide large surface that easily come in intimate contact and allow Con A molecules to bind the microvilli of 2 different cells together[7]. In our case, it is possible that a similar phenomenon occurs between microvilli of the same cell.

Con A stimulated the formation of pseudopods and colloid droplets in some cells (figure 2). The droplets seen after incubation with Con A must be newly formed, since they were not observed in control thyroid slices of dogs in which thyroid glands have been suppressed. Con A also potentiated the effects of suboptimal concentrations of TSH (figure 4). These observations suggest that Con A mimicked some of TSH effects.

The present observations must be interpreted as preliminary. However, they show that Con A is capable of altering the ultrastructure of dog thyroid follicular cells in vitro in a dose-dependent manner and of affecting their response to TSH in a characteristic fashion.

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Role of the pituitary in cyproheptadine-induced pancreatic beta-cell toxicity[1]

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Summary. Hypophysectomized rats given cyproheptadine (40 mg/kg) for 10 days exhibited a loss of pancreatic immunoreactive insulin and ultrastructural changes in the cytoplasm of beta-cells. Sham-operated animals given cyproheptadine showed identical changes in pancreatic beta-cells except that cytoplasmic involvement progressed to the formation of large vacuoles. The pituitary is not directly involved with the cyproheptadine-induced depletion of pancreatic insulin but plays a role in the formation of large cytoplasmic vacuoles.

Cyproheptadine is one of several structurally related compounds which can produce unique changes in pancreatic beta cell structure and function in rats[2,3]. These alterations are characterized initially by vesiculation of rough endoplasmic reticulum, a loss of insulin containing secretory granules, and followed later by the formation of large cytoplasmic vacuoles in the beta cells[4]. Decreased pancreatic insulin levels and hyperglycemia were observed in conjunction with these morphologic changes[5]. In a morphologic study, Richardson[6] observed an absence of large vacuoles in the beta cells of hypophysectomized rats administered cyproheptadine for 10 days while sham-operated controls given the drug exhibited typical cyproheptadine-induced changes. Pancreatic insulin content, an important biochemical parameter altered by cyproheptadine administration, was not measured in that study. The present study was performed to determine

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whether removal of the pituitary protected rats from cyproheptadine-induced depletion of pancreatic insulin. The results help to clarify the role of the pituitary in the alterations in the endocrine pancreas caused by cyproheptadine.

Materials and methods. Hypophysectomized and sham-operated, male, Wistar rats (170–230 g) were obtained from Simonson Laboratories (Gilroy, CA). Animals were allowed free access to food and water at all times. The drinking water of hypophysectomized rats contained 5% glucose in 0.9% saline. Starting on the 5th day after surgery rats were administered an oral dose of cyproheptadine hydrochloride monohydrate (40 mg/kg) daily for 2, 8 or 10 days. Control animals (hypophysectomized and sham) received only the water vehicle (1.5 ml/kg). 24 h following the last daily dose, rats were sacrified by decapitation and the pancreas removed. A portion of each pancreas was taken for morphological examination at the light and electron microscopic levels as previously described[2,4] and the remainder was homogenized in acid-ethanol and extracted by the procedure of Davoren[7] for analysis of immunoreactive insulin. Insulin was determined using the alcohol precipitation, radioimmunoassay procedure of Makulu et al.[8] and a rat insulin (Novo, Denmark) standard. Criteria to verify hypophysectomy were absence of an increase in body weight and a significantly smaller weight of both adrenal glands upon postmortem examination. Cyproheptadine administration had no effect on adrenal weight.

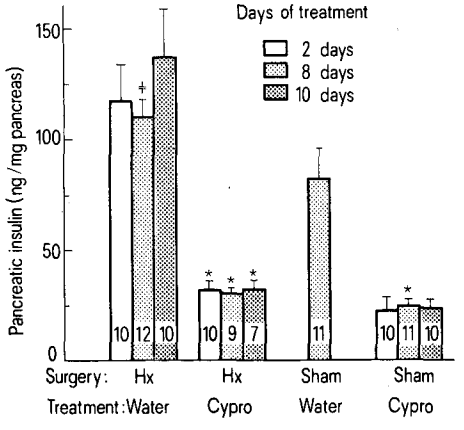

Fig. 1. Depletion of pancreatic insulin caused by cyproheptadine (Cypro) administration for 2, 8 and 10 days in hypophysectomized (Hx) and sham-operated rats.
Asterisks (*) indicate a statistically significant difference from respective water-treated control by group t-test, $p < 0.05$[13]. A dagger (‡) indicates a statistically significant difference from sham-operated, water-treated control by group t-test, $p < 0.05$. Bars show the mean ± SE and the number of animals is shown inside the bar.

Results and discussion. Cyproheptadine treatment of hypophysectomized rats for 2, 8 or 10 days produced a marked decrease in pancreatic insulin (figure 1). Approximately an 85% reduction in insulin was observed regardless of the length of drug treatment. The insulin content of the pancreas of sham-operated rats administered cyproheptadine for 2, 8 and 10 days was also reduced and not different from that of the hypophysectomized animals receiving drug for the same periods of time. The hypophysectomized rats receiving water for 8 days had significantly more pancreatic insulin than corresponding sham-operated animals. This is due to removal of the pituitary which produces a decrease in the weight of the pancreas due to atrophy of the exocrine tissue, but has no effect on the size or insulin content of islets[9–12].

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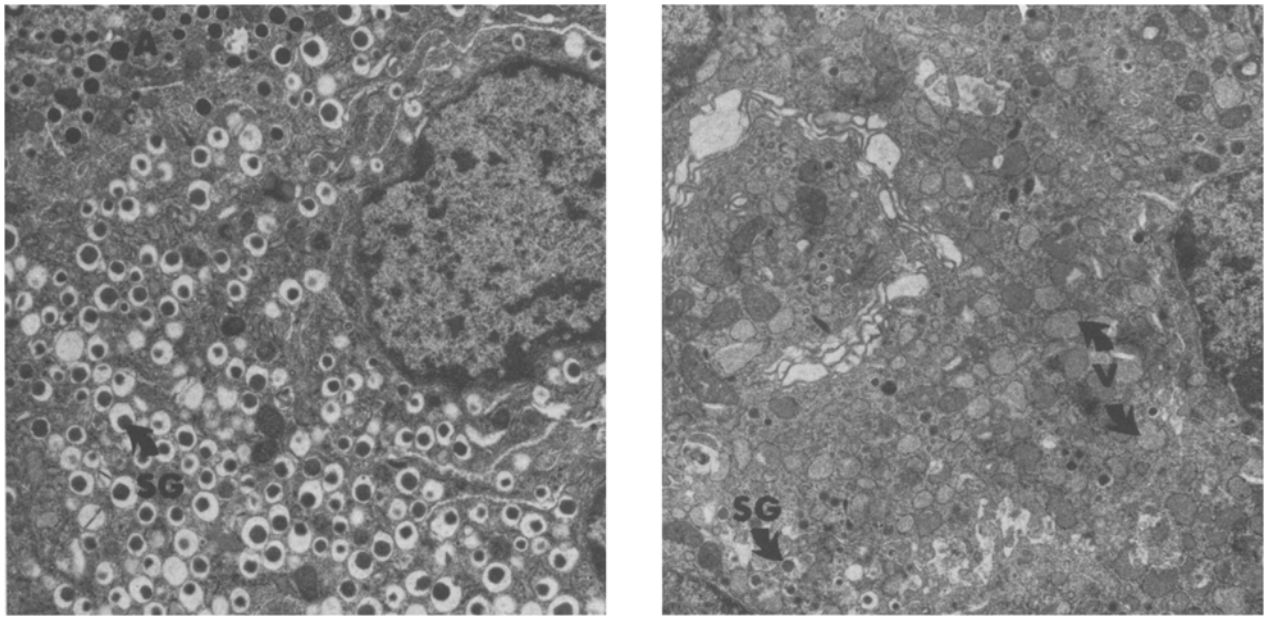

Fig. 2. Electron micrographs of endocrine pancreas from hypophysectomized rats receiving water (left panel) or cyproheptadine (right panel) for 8 days. *SG* Secretory granule, *V* vesicles, *A* alpha cell. ×8300.

Using electron microscopy, changes in ultrastructure of beta-cells were observed in hypophysectomized rats after 2, 8 and 10 days of cyproheptadine treatment. After 2 days of treatment with cyproheptadine, beta-cells were degranulated and the rough endoplasmic reticulum was vesiculated. Further alterations in the beta-cell ultrastructure were not observed in animals treated for 8 and 10 days with cyproheptadine. Representative electron micrographs of beta-cells in hypophysectomized rats receiving water or cyproheptadine for 8 days are shown in figure 2. No large cytoplasmic vacuoles were observed in islet cells from hypophysectomized rats. Sham-operated rats receiving cyproheptadine for 2 days exhibited typical drug-induced beta cell alterations such as vesiculation of the endoplasmic reticulum and degranulation. After 8 and 10 days of cyproheptadine treatment, large cytoplasmic vacuoles typical of cyproheptadine treatment were also present. These morphologic changes in beta-cells of sham-operated rats receiving cyproheptadine are not shown here because they were identical to alterations observed in drug-treated animals in previous studies[2,4,6]. No consistent alteration in other cell organelles of beta-cells was noted in hypophysectomized and sham-operated rats receiving cyproheptadine for a 10-day-period.

Light microscopic examination of pancreatic islets using the quantitative methods reported previously[2] indicated that the incidence and severity of vacuole formation were reduced by hypophysectomy. This data is not shown because it confirms a previous report[6].

The large cytoplasmic vacuoles in pancreatic beta-cells of rats treated with cyproheptadine are thought to arise from a coalescence of material in dilated rough endoplasmic reticulum[4]. Removal of the pituitary apparently retards this process but not the drug effects which precede vacuole formation. The most important of these effects, loss of pancreatic insulin, was detected in this study using immunoassay and electron microscopy. Utilizing morphologic methods Richardson[6] concluded that pancreatic insulin was normal in hypophysectomized rats given cyproheptadine. The reason for this apparent discrepancy is not known but morphologic data alone, without morphometric analysis, could be misleading when an assessment of insulin content is desired. Results of the present study suggest that cytoplasmic vacuole formation in beta-cells of rats treated with cyproheptadine is not a necessary consequence of the drug-induced insulin depletion. Cyproheptadine-induced insulin depletion and vacuolization of beta-cells may require different initiating or permissive factors. It is also possible that a change in the absorption, distribution or metabolism of cyproheptadine, caused by the lack of the pituitary, could result in an alteration of some effects of the drug in the pancreas of hypophysectomized rats. It is clear from the results of this study, however, that insulin depletion is not attenuated by the lack of pituitary-related factors. This depletion is consistent with recent results which show that cyproheptadine inhibits proinsulin synthesis in an in vitro system devoid of direct pituitary influence[14].

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In situ noradrenaline-induced stimulation of dog thyrotrophic secretion[1]

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Summary. Anterior pituitary microinfusions of noradrenaline in the dog causes a significant release of TSH while adrenaline and dopamine do not.

Contradictory findings concerning the role of catecholamines (CA) on thyrotrophic secretion have been reported[2,3]. One point is that this secretion may be controlled, at least in part, by brain CA[4,7,8]. On the other hand, CA may act also directly on the thyroid gland[5,6]. We report here evidence showing that noradrenaline (NA), among the CA, selectively exerts an in situ stimulatory action on thyrotrophic secretion of the dog.

Methods. Mongrel dogs, of both sexes, about 14 kg of b.wt, were employed. Under pentobarbital anesthesia, implantation of chronic anterior pituitary (a.h.) and jugular vein cannulae was performed according to González-Luque et al.[9]. Right after the operation, the dogs were injected intravenously (i.v.) with 50 μCi I^{131}. Treatment with NA, adrenaline (A) or dopamine (DA) started 48 h later in a room at 22 °C. Microinfusions containing the amines or saline pH 7.2 (a.h. or i.v.), at a rate of 2.03 μl/min were performed by means of an infusion pump (Harvard, Model 940), over a period of 120 min. In separate experiments, the 3 CA were tested at concentrations of 10 ng/ml of the base; 5 ml blood samples were collected at 15 min intervals, starting at the beginning of the initial infusion period. Timing was adjusted to correct for dead space. Radioactivity was measured in 2 ml plasma samples and the c.p.m. were corrected for back-ground activity and decay. Changes in thyrotrophic secretion were evaluated indirectly from the curve of radioiodine release into the blood circulation[10]. At the end of the experiments, the animals were killed in order to check for the correct implantation and permeability of the a.h. cannula.

1 This study was supported by Grant No. S1-049 from the Consejo Nacional de Investigaciones Científicas y Tecnológicas, Grant No. 281 from the Consejo de Desorrallo Científico y Humanístico, Central University of Venezuela, and Fundación 'José María Vargas'.

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