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].

14 L. J. Fischer, A. B. Grow and K. L. Hintze, Diabetes *25*, suppl. 1, 343 (1976).

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.

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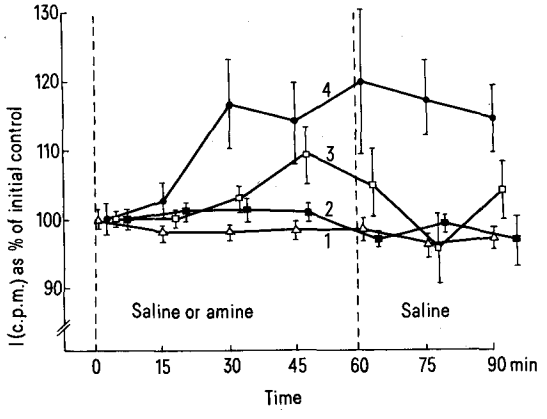
2 D. De Wied and W. De Jong, Ann. Rev. Pharmac. *14*, 389 (1974).
3 J. Tuomisto, T. Ranta, P. Männisto, A. Saaminen and J. Jeppäluoto, Eur. J. Pharmac. *30*, 321 (1975).
4 G. M. Brown, E. Krigstein, J. Dankova and O. Hornykiewicz, Neuroendocrinology *10*, 207 (1972).
5 A. Melander and F. Sundler, Endocrinology *90*, 188 (1972).
6 A. Melander, Acta endocr. *62*, 565 (1969).
7 I. Grim and S. Reichlin, Endocrinology *93*, 626 (1973).
8 H. J. Chen and J. Meites, Endocrinology *96*, 10 (1975).
9 A. González-Luque, M. L. Age, A. P. S. Dhariwal and F. E. Yates, Endocrinology *86*, 1134 (1970).
10 A. González-Luque and E. Coll-García, Acta cient. venez. *24*, 63 (1973).

For quantification of results and statistical analysis by 2-tailed t-tests, counts corresponding to the 3 initial control samples (saline a.h. or i.v.) of a single experiment were averaged. This value represents 100% at zero time. Counts obtained from the following 6 samples, corresponding to periods of amine infusion plus recovery, were expressed as percent change of the value at zero time.
The drugs employed were: DA (3,4-dihydroxy-2-phenylethylamine, HCl), Nutritional Biochem.; NA (L-arterenol bitartrate hydrate), Calbiochem.; and A (L-epinephrine bitartrate), Sigma Chem.



Effects of anterior pituitary microinfusion of saline (curve 1), dopamine (curve 2), adrenaline (curve 3) or noradrenaline (curve 4) on ^{131}I release. Each figure represents the mean ± SE of 8–9 experiments in 5–6 dogs.

Results and discussion. A.h. infusion of saline throughout the experiment only caused minor changes in the time-course of radioiodine release (figure). The curve obtained with either DA or A did not differ significantly from the control curve. In contrast to this, NA infused a.h. caused c.p.m. increase after a latency period of 30 min. Such increase remained unchanged throughout the experiment, and was significant ($p < 0.01$) in all the points of the curve when compared to controls. NA, A or DA, in no less than 8 experiments each, did not cause any change in radioactivity compared to controls when administered i.v. under the same schedule, infusion rate and dosage as for a.h. administration.
The present findings suggest that NA is an stimulatory agent for dog thyrotrophic secretion in vivo, acting in situ on the gland since peripheral effects can be ruled out from the control experiments. Perhaps NA is acting on thyrotrophic cells causing TSH release; however an indirect action in situ cannot be ruled out. It is less likely that NA is acting by diffusion on extraglandular sites since extremely low amounts of the amine are infused in relatively long periods of time (about 0.1 ng/60 min). However, it has been known that NA can release TRH from mouse hypothalamic fragments in vitro[7], and an overflow of NA from the anterior pituitary to the hypothalamus should not be overlooked. An A-induced TSH release in vitro from mouse anterior pituitary slices has already been shown[11], nevertheless we could not demonstrate this action in the dog in vivo. Differences would be attributed to species variation or different experimental conditions.

11 J. F. Wilber, G. T. Peake and R. D. Utiger, Endocrinology *84*, 758 (1969).

Effect of a sesquiterpene from *Aristolochia indica* Linn. on fertility in female mice

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Summary. A sesquiterpene isolated from the roots of *Aristolochia indica* (Linn.) was found to exert 100% interceptive activity and 91.7% anti-implantation activity in mice at a single oral dose of 100 mg/kg b.wt. No toxic effect was found at the dose levels used.

The root of the plant *Aristolochia indica* Linn. (N.O. Aristolochiaceae) is reputed to have emmenagogic[2,3] and abortifacient properties[4]. The effect of different exrtactives from the root on interception in female mice was reported earlier from this laboratory[5]. The crude petroleum ether extract was found to exert 100% abortifacient activity in mature female mice at a single oral dose of 100 mg/kg b.wt. The present communication deals with the follow-up studies with a pure crystalline product, m.p. 150 °C, isolated from the petroleum ether extract and identified as a sesquiterpene, the characterization of which is in progress in the Medicinal Chemistry Department of this institute.
Materials and methods. The colony-bred, Swiss albino normal cycling female mice weighing 20–25 g were caged with fertile males in the ratio of 2:1 at a controlled room temperature (24–25 °C). Presence of vaginal plug was marked as day 1 of pregnancy. The test sample was pasted with gum acacia powder and suspended in water for oral administration. Since the crude petroleum ether extract exerted 100% interceptive activity in mice at the dose level of 100 mg/kg b.wt, the pure compound was also given in the dosage of 100 mg/kg b.wt on day 6–7 of pregnancy. After establishing the antifertility activity, the sesquiterpene was administered at successively lower dose levels of 75, 50, 30 mg/kg b.wt for the elucidation of dose-response relationship. Proper controls were maintained. Laparotomy was performed on day 8–10 by observing vaginal changes and depression of mammary glands. The compound in the dosage of 100 mg/kg b.wt was also fed to a group of mice on day 1 of pregnancy. Laparotomy was performed on day 6 of pregnancy. Controls were treated with vehicle only.
The results indicate that the compound exerted 100% abortifacient activity in the dosage of 100 mg/kg b.wt. Subsequent lower doses showed lower percentages of activity (table). The dose response relationship is shown in the figure. Laparotomy indicated abortion to occur between day 8 and 10 of pregnancy. The uterine lumen was either empty or showed fetus in degenerated condi-