200 µg/kg in a dose-related manner, with a zenith at 30 min after injection (table). Plasma thyroid hormone levels did not change significantly after tuftsin injection (table). The plasma ir-TRH and TSH responses to cold were significantly enhanced by tuftsin (fig.). The plasma TSH levels after TRH administration were 1120 ± 67 ng/ml at 10 min and 1099 ± 56 ng/ml after tuftsin injection, respectively. The inactivation of TRH immunoreactivity by plasma or hypothalamus in vitro after tuftsin injection was around 35% with plasma and 47% with hypothalamus and did not differ from that of the control.

Discussion. Tuftsin, which was isolated by Najjar et al.[1], stimulates the phagocytic activity of blood polymorphnuclear granulocytes[2]. However, the effect of tuftsin on hormone release has rarely been studied[3]. The present experiments demonstrate that tuftsin increases plasma TSH levels. The increase in plasma TSH levels might be the result of an action of tuftsin at the hypothalamus level or at the pituitary level. The present study revealed that the hypothalamic ir-TRH decreased significantly, whereas its plasma concentration tended to increase after tuftsin injection. The hypothalamic ir-TRH content and its plasma concentration may be expressed as a balance among TRH release, synthesis and degradation. The inactivation of TRH immunoreactivity by plasma or hypothalamus in vitro after tuftsin injection did not differ from that of the control, suggesting that tuftsin may well affect TRH release or synthesis. The plasma ir-TRH and TSH responses to cold, which are known to be mediated by TRH activity[8], were enhanced by tuftsin. It has been reported that plasma TSH levels were significantly elevated after 15 min, peaked after 45 min cold exposure, and remained significantly elevated for up to 4 h[9]. Therefore, plasma TSH levels were observed at 45 min after cold exposure in the present experiment. The present investigations, taken together, suggest that tuftsin may act at the hypothalamus level to stimulate TRH release.

The plasma TSH response to TRH did not differ from that of the control, indicating that tuftsin may not act at the pituitary level. Since plasma thyroid hormone levels did not change after tuftsin injection, tuftsin may not act at the thyroid gland. However, thyroid hormone metabolism is very slow. Moreover, Onaya et al.[3] reported that tuftsin potentiated TSH-induced thyroid hormone secretion. Thus, a longer observation period is necessary to verify this point.

These findings suggest that tuftsin acts at the hypothalamus level to stimulate TRH release in rats.

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Bovine pituitary intraglandular colloid fraction F_5 localized in the rat endocrine pancreas[1]

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Summary. Bovine pituitary intraglandular colloid thought to be a waste product, is the holocrine secretion of intermediate lobe cells. It is housed in the intraglandular lumen (residual lumen) and is extruded into the venous circulation of the cavernous sinuses via clefts in the capsule of the gland aligned with the intraglandular lumen. Intraglandular colloid, fraction F_5 (mol.wt 34,000), radiolabeled with (^{125}I)Na and injected (0.15 ml) into the right internal jugular vein of male Wistar rats, accumulated in the endocrine pancreas. Autoradiographs showed that the material had specifically localized in the capillary network of the endocrine pancreas. Since the intermediate lobe is poorly vascularized, intraglandular colloid is considered to be the transport medium for intermediate lobe materials.

Key words. Transport; pituitary; pancreas; intraglandular colloid; radiolabeling; autoradiography.

It is not clear how intermediate lobe materials, i.e., the ACTH/βLPH family of peptides produced by intermediate lobe cells gain the systemic circulation since the lobe is poorly vascularized[3]. In addition there is some question regarding the final post-translational modification of these peptides, the form(s) in which they leave the cell(s) and the route(s) by which they reach the circulation. The post-translational processing of peptides within the intermediate lobe cell(s) (adrenocorticotropic hormone (ACTH), α-melanotrophin (α-MSH) and ACTH (18–39)-like peptides, β-lipotropin (β-LPH), β-endorphin (β-EP)-like and β LPH-like peptides)[4–6], suggests high levels of cellular activity. Studies of a large series of mammalian pituitary glands, showed that the bovine intermediate lobe best reflects this activity and is thus being used as a model in these studies. There is a constant breakdown of cells in the marginal half of the intermediate lobe resulting in the formation of intraglandular colloid, the holocrine secretion of intermediate lobe cells housed in the intraglandular lumen (residual lumen). The intraglandular colloid appears in the form of either a clear fluid, semifluid or gel. The marginal layer of intermediate lobe cells is restored by the direct division of cells in the deep layer of the intermediate lobe[7,8]. Clefts in the capsule of the gland aligned with the intraglandular lumen, provide an efficient entrance of intraglandular colloid into the venous circulation of the cavernous sinuses[8,9]. Indeed, intraglandular colloid is usually thought to be a waste product[10] and has been over-

looked as the holocrine secretion of intermediate lobe cells[8]. It is suggested that intraglandular colloid transports the end products of intermediate lobe cellular activity.

This study was designed to determine whether bovine pituitary intraglandular colloid is a waste product which is quickly eliminated from the body, or whether it can be located in some previously reported anatomical site associated with known intermediate lobe materials.

Materials and methods. Bovine intraglandular colloid in the form of either a clear fluid, semifluid or gel was collected 5 min after slaughter[7], pooled in 10 ml of 0.81 M phosphate buffer, pH 6.50, homogenized and centrifuged at 3000 rpm for 5 min. The supernatant was fractionated by gel filtration in a Sephadex (Pharmacia) G-100 superfine water-jacketed column (2.6 × 70 cm), bed volume 370 ml, in 0.81 M phosphate buffer, ionic strength 1.00, pH 6.60 at a flow rate of 5.85 ml/h.

Seven primary fractions were collected and fraction F_5 (mol.wt 34,000) was randomly chosen for this study. This fraction was desalted, collected in 5-ml aliquots and labeled with (^{125}I)Na using a modification of the chloramine-T method[11]. The labeled material was desalted and the eluent was collected in 1-ml fractions. Fractions corresponding to the highest activity of the void volume peak (^{125}I)F_5 was used as the test material and those corresponding to the highest activity of the bed volume peak, free (^{125}I)Na was used as the control.

Wistar rats (230–235 g) used as experimental animals, were randomly divided into 5 experimental and 5 control groups (5 rats per group) based on a prescribed time schedule, i.e., 2, 4, 6, 12 and 24 h periods. Under pentobarbital anesthesia, experimental and control rats were injected into the right jugular vein (27 gauge needle) with 0.15 ml of either (^{125}I)F_5 or free (^{125}I)Na. At the prescribed time schedule, rats in each group were killed and tissues from the CNS, thorax, abdomen and pelvis were retrieved. The radioactive decay rate of each piece of tissue (mean weight 0.62 g) was counted on a gamma counter and recorded on a printer. Background activity was determined before counting. Tissues from rats in which the activity following an injection of (^{125}I)F_5 was greater than that following an injection of free (^{125}I)Na were considered to have retained the (^{125}I)F_5 fraction. Using the radioactivity of 1 g of blood as 100% at each time period, the significant radioactivity per g of tissue was expressed as a tissue/blood percentage and graphed. There were 5 replications of each of the 5 treatments (time periods) in every experiment including the controls[12]. Tissue which had retained the (^{125}I)F_5 at 24 h as well as tissue from the control series was processed for autoradiography[13].

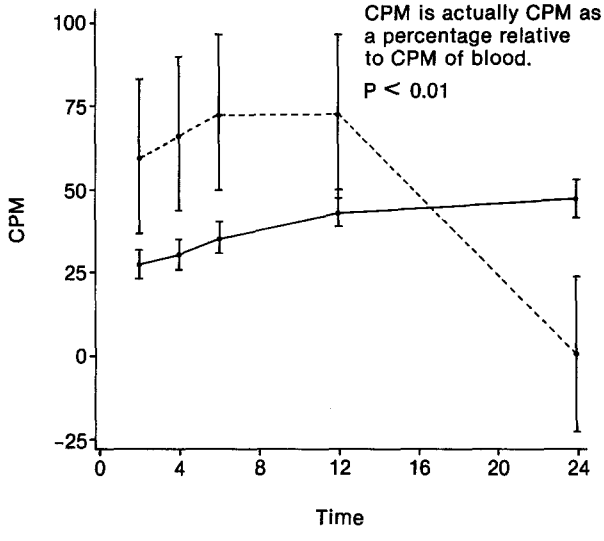

Figure 1. Comparative activity in pancreatic tissue of adult male Wistar rats following an i.v. injection of intraglandular colloid (^{125}I)F_5 used as experimental material (——), and free (^{125}I)Na used as control material (.....). There was an accumulation of radiolabeled (^{125}I)F_5 in the experimental tissue at 24 h. The reason for the level of activity in the control tissue for the first 12 h has not been determined. The five treatments for a particular analysis are the five time periods (2, 4, 6, 12 and 24 h). There are five replications for each of the five treatments (time periods) in every experiment including the control.

The ANOVA (analysis of variance)[11], procedure for a one-way classification has been used to determine whether or not there are significant differences at 5% between the means of the five treatments (time period) for a particular tissue. If the ANOVA procedure has yielded a significant F-value and the means display an increasing trend, the treatment means for that particular tissue were graphed. Also, the treatment means from the controls experiment was plotted on the same graph for the sake of comparison. Each mean is accompanied by standard error bars representing ± one standard error. The standard error (SE) of a mean was calculated using the formula: $SE = s^2/r$, where s^2 is the mean square error from the appropriate ANOVA table and r is the number of replications per treatment.

Results. The pancreas was the only tissue showing a retention of (^{125}I)F_5 at 24 h (fig. 1). The activity in the pancreas following the control injection, free (^{125}I)Na remained above the activity in the experimental (^{125}I)F_5 tissue for the first 12 h. This was followed by a rapid decline in activity to nil at 24 h. The activity in the experimental pancreatic tissue was below the control at 2 h yet, it showed a slow but steady increase. At 24 h, this activity was above the control, indicating the retention of (^{125}I)F_5 in the pancreas. Autoradiograms showed no labeled material in either the control tissue or in the exocrine pancreatic acini of the experimental tissue. Labeled material was found only in the capillary network of the endocrine pancreas, the islets of Langerhans (fig. 2). No other fraction of intraglandular colloid studied thus far could be located in this organ.

In parallel with the autoradiographic processing, pancreatic extracts were prepared from each organ sample after the F_5 injection. The biological activity of the extracts were measured and identified chromatographically indicating that the radioactivity originated from intraglandular colloid fraction F_5. A morphometric analysis of the particles was performed in the endocrine pancreas from 12 rats in which intraglandular colloid fraction F_5 was injected. The size of the area sample, 7145 μm^2 contained an average of 110 particles in the capillary

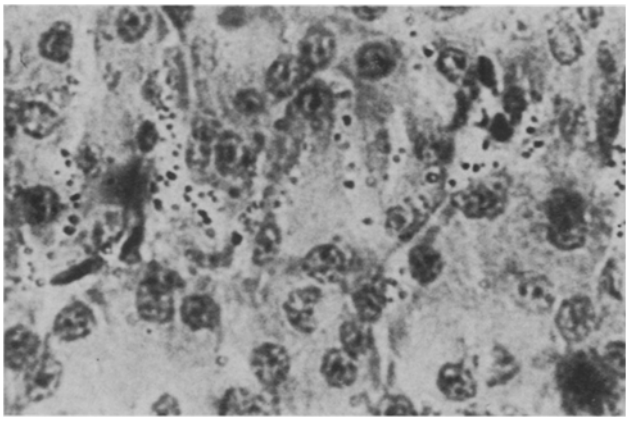

Figure 2. A light microscopic-autoradiograph of an islet of Langerhans from the rat pancreas 24 h following an i.v. injection of intraglandular colloid (^{125}I)F_5. The tissue was fixed in formalin (10%), paraffin embedded, sectioned at 5 μ, slide mounted, stained with hematoxylin-eosin, coated with Eastman Kodak NTB^2 emulsion, exposed for four weeks at 4 °C and photographically developed. In routine hematoxylin-eosin preparations, the islet appears as a spheroidal mass of pale staining cells, arranged in the form of irregular anastomosing cords. Between the cell masses are anastomosing vascular networks containing variably sized radiolabeled material (×1135).

bed and in direct contact with certain pancreatic cells. No attempt, at this time, was made to identify the various pancreatic cells. The average measurement of the granules was 12,700 Å.

Discussion. The bovine pituitary gland is provided with a novel system for the passage of intermediate lobe materials by way of its holocrine secretion, colloid, into the venous circulation[7]. This is the most efficient entrance of intermediate lobe materials into circulation since the gland is poorly vascularized. Indeed, intraglandular colloid becomes a unique transport medium since there is no major blood flow between the median eminence and the intermediate lobe, and there is no portal system like that involving the anterior lobe into which hypothalamic releasing factors are carried to the gland. Although the bovine intermediate lobe is provided with neurosensory and neurosecretory nerve fibers which terminate on neurosensory and neurosecretory cells[14]; these are very few compared to the number of intermediate lobe cells, and are most likely not involved in transport from the lobe. The venous transport of intraglandular colloid fraction F_5 and its retention in the pancreas, strongly suggests that intraglandular colloid is not a waste product but plays an important role in the transport of intermediate lobe materials.

Continuing studies of other intraglandular colloid fractions show that each have specific anatomical loci. The only reported systemic role of any intermediate lobe product in higher vertebrates is that for 'corticotrophinlike intermediate lobe peptide', or CLIP[15,16]. The continuing investigation of intraglandular colloid F_5 is now directed towards determining the presence of CLIP and determining whether this fraction can potentiate insulin production in the β cells of the endocrine pancreas.

Although not reported heretofore, recent ongoing studies show that peptides α MSH, ACTH and β LPH synthesized by cells in the intermediate lobe are present in bovine intraglandular colloid. These findings were discovered when intraglandular colloid was subjected to a series of radioimmunoassays (RIA) including α MSH-RIA, ACTH-RIA and β LPH-RIA.

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Rat pancreatic islet cells in primary culture: occurrence of giant cells amenable to patch clamping

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Summary. Neonatal and adult rat pancreatic islet cells were maintained in dissociated cell culture for up to three weeks. The unexpected occurrence of giant (40–50 µm) cells was noted, some of which reacted positively to an insulin antiserum, indicating the presence of insulin. The giant cells were amenable to study using the extracellular patch clamp technique, which was used to demonstrate a population of membrane channels gating outwardly directed current in these cells.
Key words. Pancreas; islet; patch clamp; cell culture; channels.

Primary cell cultures derived from the mammalian pancreas have proved useful in the study of electrical and humoral factors believed to control the secretory activity of the pancreas in vivo. The majority of these studies have been carried out with the exocrine part of the pancreas[1,2]. Endocrine cells derived from the islet of Langerhans have also been successfully maintained in primary cell culture[3].

Cultures of this type afford an excellent opportunity to examine in detail the cellular mechanisms by which insulin release is controlled in the β-cell population of the pancreatic islet tissue. Electrophysiological investigation of these mechanisms is however hampered by the very small size of the β-cells (12–14 µm in diameter). In this paper culture conditions are described in which the formation of giant insulin-containing cells has occurred. The electrical properties of these cells can be readily examined using the extracellular patch clamp, a method which resolves the activity of single ionic channels in biological membranes[4].

Methods. Preparation of dispersed cultures of islet cells. Male Wistar rats were anesthetized with i.p. sodium pentothal [50 mg/kg]. The pancreas was disrupted by injecting 10 ml Hanks solution into the common bile duct, after clamping near the hilus of the liver. The pancreas was removed, placed in a small vessel and chopped to a fine consistency with scissors. The pieces were decanted and digested with collagenase (Worthington Biochemical Corporation) at 37 °C with intermittent vigorous agitation. The reaction was stopped by diluting with 50 ml ice cold Hank's solution in a graduent cylinder. The islets