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

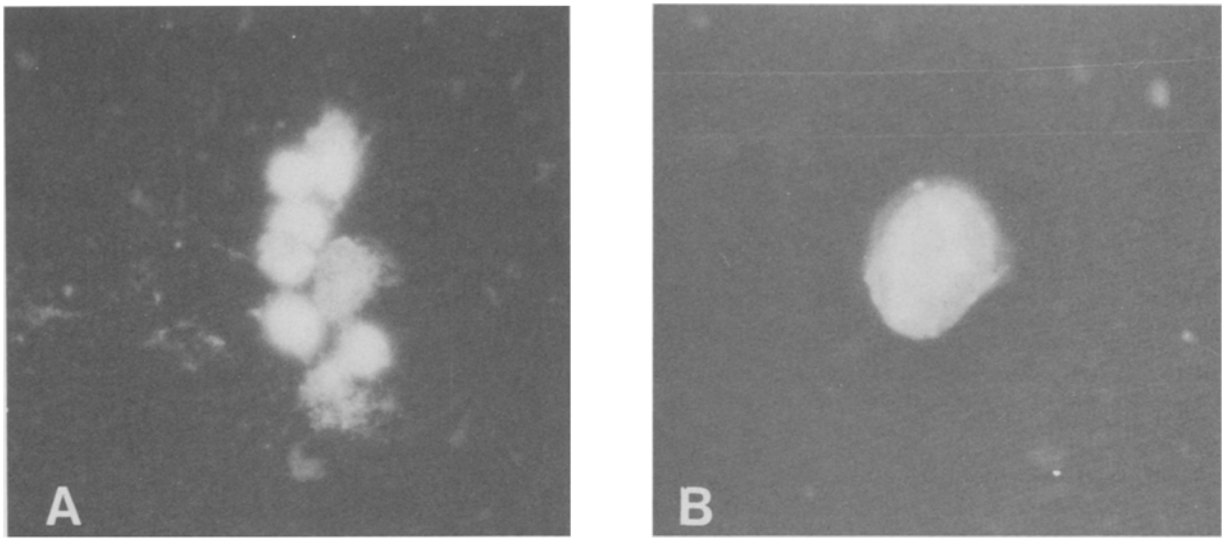

Figure 1. *A* Normal sized insulin-immunoreactive cells in primary culture of adult rat pancreatic islets. × 600. *B* Giant insulin-positive cell in the same culture. × 600. This culture was three weeks old at the time of staining. See methods for description of immunohistochemical techniques used to obtain this data.

were separated by centrifugation and isolated using a micropipette.

The islets were resuspended in 1.0 ml of Krebs Ringer bicarbonate (KRB) containing 2.5 mg trypsin (TRTPCK, Worthington) and agitated by gently sucking into and expelling from a pasteur pipette for approximately 2 min. The disrupted islets were washed twice with KRB containing 1% bovine serum albumin (RIA grade, Sigma), interspersed with low speed centrifugation. All solutions were sterile filtered, and procedures were performed under sterile conditions.

Dissociated pancreatic islet cells were cultured on collagen-coated 35 mm Petri dishes containing Dulbecco's Modified Eagle Medium (Gibco) with 10% fetal bovine serum (Gibco), 5 mM glucose, 50 µg/ml streptomycin and 50 U/ml penicillin G. Cultures were fed twice weekly with 1 ml of fresh medium and incubated at 37 °C in a 5% CO_2 atmosphere. For electrophysiological experiments, culture medium was replaced with a balanced salt solution containing 142 mM NaCl, 5.6 mM KCl, 1 mM $CaCl_2$, 10 mM $MgCl_2$, 5 mM glucose and 5 mM Hepes buffer (pH 7.4). Extracellular patch clamp records were obtained using a List EPC-5 amplifier and patch electrodes filled with the normal bathing solution. Patch clamp currents were filtered at 500 Hz and digitized by a Data Precision 6000 wave form analyzer. Patch recordings were obtained in the cell attached configuration after formation of an electrode/membrane seal of about 10^9 Ω. In this mode, membrane patch voltage is the sum of the cell resting potential and an applied command voltage, V_c which could be varied from 0 mV to ± 100 mV[5]. Experiments were performed at 23–25 °C.

After patch recordings were completed, the balanced salt solution was washed off with phosphate buffered saline (PBS) and the cells fixed in a 0.5% parabenzoquinone solution[6] for 10 min at 22 °C. The cells were subsequently washed with PBS and incubated overnight at 4 °C with a 1:300 dilution of guinea pig anti-insulin serum. The bound antibodies were localized using a fluorescence labeled rabbit anti-guinea pig IgG conjugate at 1:200 (Miles). The cells were then viewed under a Zeiss Universal Microscope with epifluorescence and the positive cells photographed.

Results. The fluorescence micrograph in figure 1A shows the typical appearance of dissociated pancreatic islet cells after three weeks in culture. The majority of cells were spherical in shape and were 12–14 µm in diameter. This population of cells closely resembles cell profiles noted in previous studies on the rat islet in culture[3,7]. Many of these cells stained intensely when exposed to the insulin antiserum, indicating the presence of insulin, and hence of β-cells in these cultures.

In cultures ranging in age from six days to three weeks the unexpected appearance of a novel cell population was noted. As shown in figure 1B, these cells were characterized by their large size (40–50 µm) and by their nonspherical shape. A number of these giant cells were also stained intensely with the insulin antiserum (fig. 1B).

The large size of the giant islet cells noted above greatly facilitated the use of the patch clamp technique to study electrical events in pancreatic cells. The results of a recording of this kind are shown in figure 2. On depolarizing the membrane patch from a holding potential of $V_c = 0$ mV, brief, rectangular jumps in current were seen. These currents resemble events attributed in other cells to the opening and closing of single ionic channels[4]. On further depolarization of the membrane patch, the amplitude of these events increased, indicating that these outward currents were probably generated by a conductance change to an ion with a negative Nernst potential, such as Cl^- or K^+. The occurrence of these outward events showed no tendency to decrease during prolonged depolarization of the membrane patch. Outwardly directed single channel currents believed to reflect K^+ movement have previously been detected in cultured rat islet cells[8].

Histograms of the amplitudes and durations of the outward current jumps were constructed. As indicated in figure 2, B, the current amplitudes were distributed in a unimodal fashion. The event durations showed an approximately exponential distribution. These results are consistent with the notion that the observed currents were generated by a single population of membrane channels which close according to a first order process[9].

Discussion. β-cells derived from perinatal rats proliferate extensively when maintained in dissociated cell culture[7], or when cultured as whole islets[3]. However, a marked increase in the size of individual insulin-positive cells during cell proliferation was not reported in those studies. The present results show that, when adult rat islet cells are cultured under appropriate conditions, abnormally large insulin-positive cells are formed.

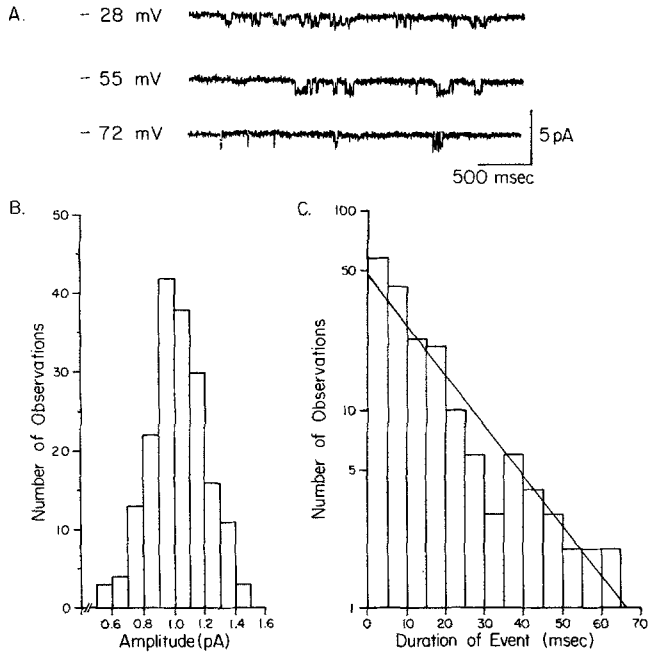

Figure 2. *A* Extracellular patch clamp records obtained from a giant cell in a 3-week-old culture of dissociated rat pancreatic islet. The recording was made in the cell attached configuration. The membrane patch was depolarized by applying the negative command voltages (V_c) indicated to the left of each trace. The depolarizations evoked outwardly directed single channel currents (downward deflections). *B* Histogram of the amplitudes of single channel currents recorded in this patch at $V_c = -55$ mV. Current amplitudes are unimodally distributed with an average value of 1.0 ± 0.83 pA (183 events). *C* Histogram of the duration of 185 single channel currents recorded in this patch at $V_c = -55$ mV. Note logarithmic scale of y-axis. The straight line was fitted to the data using least squares regression (correlation coefficient −0.960) and indicates a mean channel duration of 17.3 msec.

It is at present unclear whether these giant cells result from the fusion of normal islet cells, or represent the amitotic growth of single precursor cells.

Glucose induced release of insulin from adult β-cells in vivo is known to be accompanied by slow wave depolarization and action potential activity in the β-cell membrane[10]. The ionic basis of these events and their relation to insulin release have previously been studied using intracellular recording techniques[11,12]. Studies of this kind may be greatly facilitated if conducted using the giant insulin-containing cells reported above, since stable penetration with low resistance microelectrodes should be possible. Further, the present results show that these giant cells are also amenable to study using the extracellular patch clamp technique. This method allows identification of the types of ionic channel present in islet cell membranes. In addition, the modulation of these channels by secretagogues and by inhibitors of hormone release can also be investigated.

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Estimation of serotonin and its action on oviducts and uteri of some oviparous and viviparous insects

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Summary. Serotonin was not found in the oviducts of *Blabera gigantea, Clitumnus extradentatus* nor in the uterus of *Glossina,* but it is present in the uterus of *Blabera.* It is also found in the central nervous system of all three insects. In vitro experiments confirm these data by showing that serotonin increases the contractions of the uterus of *Blabera,* but has no effect on the uterus of *Glossina.*

Key words. Central nervous system; insects; oviduct; serotonin; uterus.

In insects, serotonin (5-hydroxytryptamine) has been found in the central nervous system (CNS) (see Evans[1]) and the heart and gut[2–4]. It has been shown to stimulate several visceral organs: the semi-isolated heart[5,6], hindgut preparations[2,7,8] from cockroaches and locusts, and the oviduct from the horsefly *Tabanus sulcifrons* and *proximis*[9].

The purpose of this preliminary study was to investigate the presence of serotonin in the uteri of two viviparous insects, the tsetse fly *Glossina fuscipes* and the cockroach *Blabera gigantea* and to record the effects of this compound on isolated uteri. To establish a comparison, serotonin was also measured in the oviducts of *Blabera* and *Clitumnus extradentatus* (an oviparous insect) and in the central nervous systems of the 3 insects studied.

Methods. The content of serotonin in the tissues was estimated by the method of Reinhard et al.[10] using HPLC with electrochemical detection. The tissues were homogenized in 100 µl 0.1 M $HClO_4$ containing 0.2 mM ascorbic acid. The homogenates were centrifuged for 15 min at 30,000 × g; 20 or 50 µl of the supernatant was then injected into the HPLC. Experiments