This was shown by failure of spleen cells, first treated with hydrocortisone, to recognize RSA as a carrier for the DNP hapten.

4) The T cells involved in helper function acquire resistance to hydrocortisone after they have been sensitized to carrier antigens. We found that injecting RSA before treatment with hydrocortisone preserved the carrier effect. This may explain why the suppression of antibody formation by adrenal steroids is much less effective when these drugs are administered after antigen stimulation (*15*).

The different susceptibilities to hydrocortisone provide evidence that both the graft-versus-host and helper functions that are dependent on the thymus are controlled by different T-cell mechanisms. It is possible that the thymus can program the differentiation of precursor lymphoid cells into two completely separate populations—a population resistant to hydrocortisone that mediates graft-versus-host and possibly other cell-mediated reactions, and a population sensitive to hydrocortisone that provides the helper function in humoral immunity. On the other hand, as suggested by Raff and Cantor (*16*), different immunologic activities may be performed by T cells as they pass through stages of differentiation. Thus, T cells at an early stage of differentiation might be active as helper cells and mediate graft-versus-host reactions only after further maturation. A third possibility is that the same T-cell type has both functions, but that the mechanisms involved in carrier interaction alone are susceptible to hydrocortisone. This possibility appears less likely because the carrier effect itself is resistant to hydrocortisone once the T cell is sensitized (Fig. 2). Moreover, hydrocortisone appears to increase the graft-versus-host potential of lymphocyte populations by destroying susceptible lymphocytes rather than by merely altering cell functions (*8*). It is unlikely that specific tolerance to RSA could have been induced by injecting RSA in complete Freund's adjuvant.

We have studied the effects of hydrocortisone on the development of lymphocytes which mediate contact-dependent lysis of target cells (*17*). Preliminary treatment of lymphocytes in vitro with hydrocortisone reduced the number of lytic lymphocytes developing after sensitization. The simultaneous presence of sensitizing antigens appeared to prevent this inhibitory effect. Hence, T cells that are needed to produce cytolysis of target cells may also differ from T cells active in the graft-versus-host reaction. In addition to different susceptibilities to hydrocortisone, T cells active in graft-versus-host reactions and T cells active in cytolysis differ in their response to antibodies against a T-cell antigen (*18*). Our conclusions pertain to peripheral T cells. Studies of the antibody response of mice to sheep red blood cells suggest that lymphocytes in the thymus may produce a carrier effect that is resistant to corticosteroids (*19*).

Therefore, at least two, and possibly three, different T cells may be distinguished. Identification of these cell types is important to our understanding of the basic processes of lymphoid cell differentiation and cooperation which are factors both in antibody production and in cell-mediated immune responses. Competition between humoral antibodies and immune lymphocytes may lead to enhanced tumor growth in man (*20*) or to allograft tolerance (*21*). Therefore, identification and separation of lymphoid cell types ultimately may provide a way of manipulating and controlling immune reactions in patients with tumors or transplanted organs.

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Pancreatic Beta-Cell Web: Its Possible Role in Insulin Secretion

Abstract. *A cortical band of fine microfilaments is consistently observed in the beta cells of the rat pancreas. Alteration of this cell web by cytochalasin B is associated with an enhancement of glucose-induced secretion of insulin by isolated islets. The microfilamentous web of the beta cell may play an important role in the emiocytosis of insulin secretory granules, by controlling their access to the cell membrane.*

In 1968, Lacy *et al.* (*1*) suggested that the integrity of the microtubular-microfilamentous system of the pancreatic beta cell is required in order that glucose exert its stimulant action on insulin release. Further investigations (*2*) again showed that both mitotic-spindle inhibitors (colchicine and vincristine) and microtubule stabilizers (D_2O and hexylene glycol) inhibit or suppress in vitro glucose-induced insulin secretion. We now report on a previously neglected component of the microtubular-microfilamentous system and its possible role in the insulin secretory process.

Our study was undertaken because, in the course of recent ultrastructural studies on the pancreatic beta cell, we became aware of the existence of a

hitherto unidentified subcellular structure which consists of a band of filamentous material located at the periphery of the beta cell. In addition cytochalasin B is useful for investigating fine microfilamentous structures in primitive contractile systems (*3*). Indeed, this agent acts rather specifically in disrupting the function of certain contractile microfilament systems of cells. We decided, therefore, to investigate the effect of cytochalasin B on both the ultrastructural appearance and secretory activity of the beta cell.

Islets of Langerhans were isolated from the rat pancreas by the method of Lacy and Kostianovsky (*4*); the islets in groups of eight were incubated for 90 minutes at 37°C in bicarbonate-buffered media (1.0 ml) containing bovine plasma albumin (5 mg/ml), glucose (0.5 or 3.0 mg/ml), dimethyl sulfoxide (10 μl/ml) and, when required, cytochalasin B (10 μg/ml). The insulin content of the media was assayed by the method of Wright *et al.* (*5*). After incubation, the islets were fixed in 2 percent sodium cacodylate–buffered glutaraldehyde, postfixed in 1 percent phosphate-buffered osmium tetroxide, dehydrated in ethanol, and embedded in an Araldite-Epon mixture. Sections were sequentially stained with uranyl acetate and lead citrate, and examined in an electron microscope (RCA EMU 3 G or Philips EM 300).

In control islets incubated in the absence of cytochalasin B, an ectoplasmic band is visible just beneath the plasma membrane (Fig. 1A). This may correspond to the cortical band seen in many different cell types and referred in light microscopy as the "cell web" (*6*). This ectoplasmic band, at low magnification (Fig. 1A), has a homogeneous, moderately dense appearance and is, generally, devoid of large cytoplasmic organelles; but it may contain free ribosomes, microtubules, some microvesicles, and profiles of endoplasmic reticulum. Occasionally the web appears continuous along the cell circumference, its thickness varying from 500 to 3000 Å. The major component of the ectoplasmic band appears to be a network of fine filaments of indefinite length measuring 50 to 70 Å in diameter (Fig. 1B). In some regions the filaments are oriented in parallel fashion; while in others they are arranged in a network of polygonal structure (Fig. 1B). A similar filamentous material forms also the core of the microvilli which protrude from the cell surface (Fig. 1, C and D).

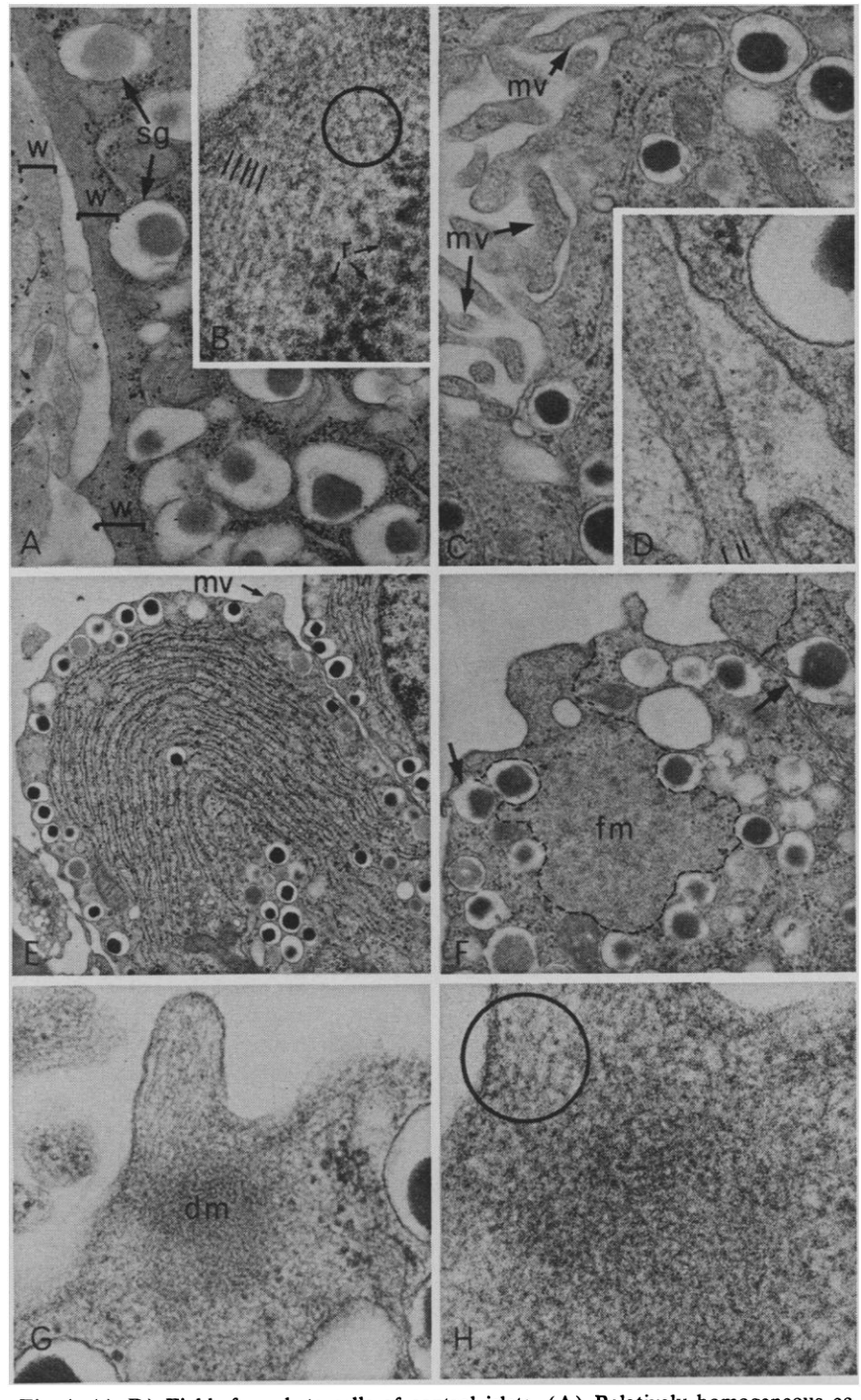

Fig. 1. (A–D) Fields from beta cells of control islets. (A) Relatively homogeneous ectoplasmic band (*w*) is present immediately beneath the plasma membrane. Several secretory granules (*sg*) are seen underlying this band (× 25,000). (B) Part of the ectoplasmic band seen at high magnification. The encircled area shows the polygonal arrangement of microfilaments. The parallel arrangement of microfilaments is emphasized by lines. Ribosomes (*r*) are seen in the lower right-hand corner (× 103,-200). (C) The ectoplasm extends into the microvillous processes (*mv*) which bathe in the intercellular space (× 24,500). (D) A microvillous process filled with filamentous network (× 66,600). (E–H) Fields from beta cells of cytochalasin B–treated islets. (E) Prominent margination of secretory granules near the plasma membrane. Only a single short microvillous process (*mv*) is seen (× 9,800). (F) A large and irregular mass of ectoplasmic material (*fm*) distributed inward from the cell periphery. The arrows point to two secretory granules probably undergoing extrusion (× 23,000). (G) A characteristic cytochalasin-induced dense mass (*dm*) is present at the base of a short microvillous process. Filamentous material is present into the microvillous process (× 60,200). (H) shows at higher magnification the dense mass of (G) in which it is difficult to resolve individual elements. Within the encircled area a polygonal structure can be seen (× 124,700).

Table 1. Effect of cytochalasin B on insulin release by isolated islets. Mean values (± S.E.) and the number of observations are shown in parentheses. Dimethyl sulfoxide (10 μl/ml) was added to each reaction mixture.

Glucose (mg/ml)	Cytochalasin B (μg/ml)	Insulin output*
0.5	Nil	19 ± 2 (10)
0.5	10.0	17 ± 3 (10)
3.0	Nil	216 ± 9 (19)
3.0	10.0	301 ± 12 (20)

* Microunits per islet per 90 minutes.

The most salient ultrastuctural changes in isolated islets exposed to cytochalasin B for 90 minutes are a shortening or disappearance of the microvillous processes (Fig. 1, E and F), a prominent margination of the beta secretory granules (Fig. 1E) toward the plasma membrane, and changes in both the organization and the spatial distribution of the cell web. The latter appeared to form large masses extending far into the cytoplasm (Fig. 1F). These consisted of more or less tightly packed filamentous or granular material and were therefore heterogeneous in their density (Fig. 1G). The normally prevailing polygonal network of the cell web is seen but rarely in these masses (Fig. 1H). These characteristic changes have been observed in other cells (*7*) after the administration of the same agent, and the question has been raised as to whether the dense masses resulted from a "contraction" of the marginal filamentous network or whether they represented an accumulation of disaggregated network material (*7*).

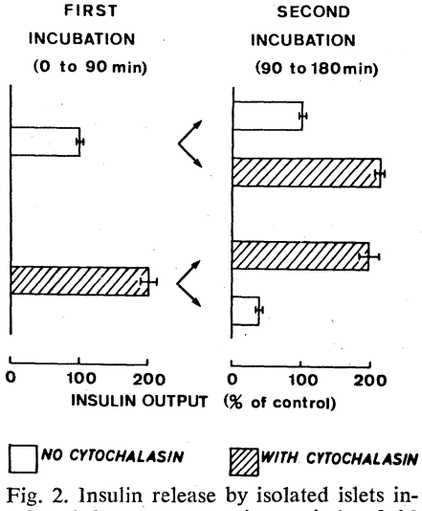

Fig. 2. Insulin release by isolated islets incubated for two successive periods of 90 minutes each in the presence of glucose (3.0 mg/ml) with or without cytochalasin B (10.0 μg/ml). Mean values (± S.E.) are shown as percentage of the control value found during the same period of incubation in islets never exposed to cytochalasin B.

Cytochalasin B exerts no significant effect on the low rate of insulin secretion observed at a nonstimulating concentration of glucose (0.5 mg/ml), whereas it enhances insulin secretion at a higher concentration of glucose (3.0 mg/ml; Table 1). Further experiments (*8*) indicate that (i) cytochalasin B also fails to affect the near-to-basal rate of insulin release observed at high glucose concentration in the absence of extracellular calcium or in the presence of D_2O (100 percent, by volume); (ii) the increase in insulin output attributable to cytochalasin B is always proportional to the increment in secretion rate above basal value induced by glucose or other insulinotropic agents; (iii) the effect of cytochalasin B on insulin release is reversible (Fig. 2); (iv) the enhancement of glucose-induced insulin release fades out at both lower (0.1 to 1.0 μg/ml) and higher (50.0 μg/ml) concentrations of cytochalasin B; (v) the enhancing action of cytochalasin B on insulin release is not due to an increase in calcium uptake by the isolated islets; and (vi) at the concentration used here (10 μl/ml), dimethyl sulfoxide has no detectable effect on glucose-induced insulin release or calcium uptake by the isolated islets.

These data indicate that the enhancing action of cytochalasin B on insulin release is not due to a passive release of insulin from damaged beta cells. Indeed, the effect of cytochalasin B is reversible, and no effect of cytochalasin B was observed whenever insulin secretion was reduced to its basal value by either decreasing the glucose concentration, omitting calcium, or replacing H_2O with D_2O in the incubation medium. It appears, therefore, that cytochalasin B facilitates the active secretion of insulin by the beta cell. It has been shown (*9*) that, under a series of experimental conditions, the amount of calcium accumulated in the beta cell is related to the rate of insulin secretion. Therefore, the failure of cytochalasin B to modify glucose-induced calcium uptake by the isolated islets suggests that cytochalasin B affects a late step in the secretory sequence, at or beyond the site of action of calcium in the beta cell.

A naive explanation for our findings would be that the microfilamentous cell web normally represents an obstacle to the emiocytosis of secretory granules, and that disorganization of this system by cytochalasin B facilitates the release of insulin in response to glucose. However, it is equally plausible that the cell web, in addition to or in combination with such a restrictive role, also gives a final and active impulse to the secretory granules and, by doing so, facilitates the contact between the plasma membrane and that of the secretory granules. In other terms, the cell web might represent, like a sphincter, both a barrier to and an effector of emiocytosis.

In conclusion, both the functional and ultrastructural findings outlined suggest that the cell web of the pancreatic beta cell of the rat plays an important role in the ultimate step of the sequence leading to emiocytosis of the insulin secretory granules. A better knowledge of the biophysical and biochemical properties of microfilaments is required to assess more precisely the role of this cell web.

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