functional properties of mitochondria and increases the activity of the lysosomal-vacuolar system in PT cells by augmenting intracellular autophagy. According to this view, a lysosome-mediated increase in protein degradation (catabolism) would be expected to be a significant feature of androgenic hormone action in mouse kidney PT. The testosterone-mediated effects noted here may be important in relation to certain sex differences in kidney function and metabolism, for example, organic ion transport (*14*), creatinine clearance (*15*), mevalonate metabolism (*16*), cholesterol synthesis (*16*), as well as in pathologic processes that display a male sex preference, such as idiopathic PT calcification (*17*), chloroform-induced PT necrosis (*18*), and renal carcinogenesis (*19*).

HAROLD KOENIG
ALFRED GOLDSTONE
GARY BLUME, CHUNG Y. LU
Neurology Service, Veterans Administration Lakeside Medical Center, and *Departments of Neurology* and *Biochemistry, Northwestern University Medical School, Chicago, Illinois 60611*

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Gap Junction Development Is Correlated with Insulin Content in the Pancreatic B Cell

Abstract. *The development of gap junctions between insulin-containing B cells was quantitatively analyzed in islets of Langerhans isolated from rats treated with the sulfonylurea glibenclamid for 1, 2, or 7 days. Glibenclamid treatment was associated with a marked depletion of the insulin content of B cells and with an increase in the number and size of gap junctions between these cells. A significant correlation was found between these two events.*

Morphological (*1, 2*) and physiological (*3*) studies suggest that direct intercellular communication, that is, the exchange of ions and molecules mediated by gap junctions (*4*), may participate in the complex regulatory system by which B cells adjust their level of activity in relation to need.

We showed earlier that the number and size of B cell gap junctions changed after treatment with physiological or pharmacological agents that stimulate insulin secretion (*2*). We now report that there is a significant correlation between insulin content and gap junction development in B cells that were stimulated to release insulin in vivo by the sulfonylurea glibenclamid.

Collagenase digestion (*5*) was used to isolate islets of Langerhans from the pancreases of adult female Wistar rats (250 to 350 g) that had received intraperitoneal injections of glibenclamid (0.2 mg per 100 g of body weight every 12 hours) for 1, 2, or 7 days. Normal rats that were not given injections served as controls. A portion of the islets was used for the determination of insulin content (*6*), and the rest were processed for freeze-fracturing and quantitative analysis of B cell gap junctions (*7, 8*). The identification of gap junctional structures to be evaluated was carried out as previously described (*2*).

Glibenclamid treatment resulted in a depletion ($P < .001$) of islet insulin content (Table 1). The amount of insulin measured represented 24 percent of the control value after 1 day, 14 percent after 2 days, and 46 percent after 7 days of continuous treatment.

The median number of particles per gap junction (this number represents an estimate of the average gap junction size) and the mean number of gap junctions per 100 μm^2 of membrane were calculated for the same time points (*7, 8*) (Table 1). Compared with control values, the median number of particles per gap junction increased ($P < .0005$) by 35 per-

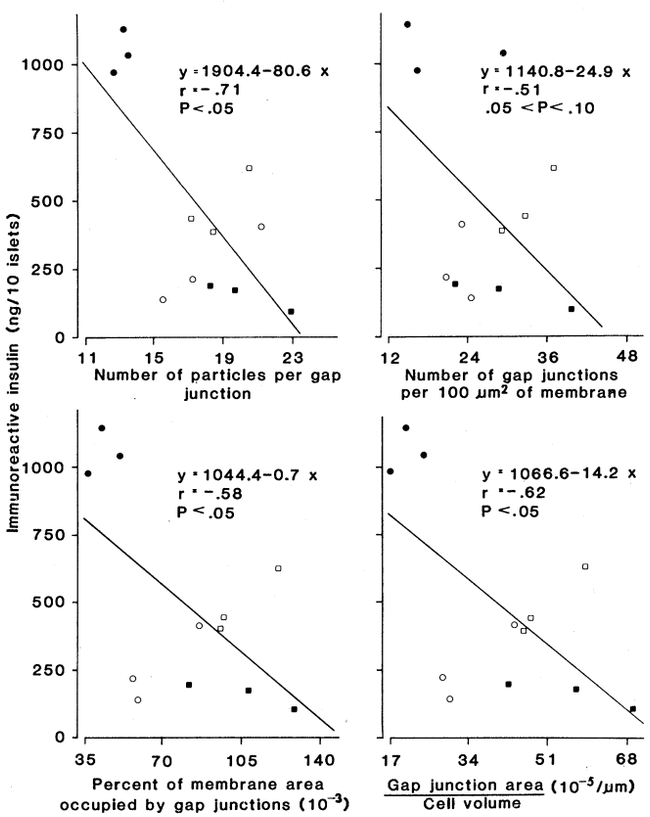

Fig. 1. For each experiment, the values of the four indices used to characterize B cell gap junctions were each plotted as a function of the insulin content of the corresponding islets. A correlation coefficient and a linear regression analysis were computed from these pairs of data. (●) Control; (○) glibenclamid, 1 day; (■) glibenclamid, 2 days; and (□) glibenclamid, 7 days.

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cent after 1 day of treatment, by 52 percent after 2 days, and by 41 percent after 7 days, whereas the mean number of gap junctions per 100 μm^2 of membrane was 30 percent (not significant), 72 percent ($P < .005$), and 92 percent ($P < .001$) higher than in controls, respectively. Using these values, we further calculated two indices for evaluating the capability of coupling mediated by gap junctions (*9*): (i) the mean percent of membrane area occupied by gap junctions was 1.6 times ($P < .02$) higher than it was in controls after 1 day of glibenclamid treatment, 2.5 times higher ($P < .001$) after 2 days, and 2.3 times higher ($P < .001$) after 7 days (Table 1); (ii) the gap junction area per unit cell volume (*10, 11*) increased 1.7 times ($P < .01$) after 1 day of glibenclamid treatment, 2.8 times ($P < .001$) after 2 days, and 2.4 times ($P < .001$) after 7 days (Table 1). To rule out that these changes were caused only by a decrease in B cell surface and volume, we calculated the number and the area of gap junctions per cell. At the three time points that we tested, these values were significantly increased (Table 2).

For each experiment and at each time point, the four indices chosen to characterize gap junction development were plotted as a function of the insulin content in the corresponding islets (Fig. 1). Linear regression analysis indicated that there were statistically significant ($P < .05$) negative correlations between the insulin content of the B cells and three of the parameters: median number of particles per gap junction, mean percent of membrane area occupied by gap junctions, and mean gap junction area per unit cell volume. Qualitatively, the insulin-rich, well-granulated B cells showed small and sparse gap junctions (Fig. 2A), whereas the insulin-depleted, poorly granulated B cells showed more numerous and larger gap junctions (Fig. 2B).

Gap junctions are found in a wide variety of tissues, including most of those specialized for secretion (*4*), but little is known about the relation between their development and the state of activity of the cells they connect. Our results demonstrate that gap junction development is correlated with the insulin content of B cells, a finding that is in agreement with previous observations of correlations between secretion and gap junction modulation in other secretory cell systems (*12*). Because the insulin content depends on the synthesis, intracellular degradation, and release of the hormone, it is not possible at present to specify which step in the complex process of in-

Table 1. Insulin content and indices characterizing the gap junctions of pancreatic B cells. All values are expressed as means ± standard errors, except the number of particles per gap junction, which are given as medians; N = number of batches of ten islets; N_j = number of gap junctions; and N_i = number of islets.

Group	Immunoreactive insulin content (nanograms per ten islets)	Number of particles per gap junction	Number of gap junctions per 100 μm^2 of membrane	Percent of membrane area occupied by gap junctions	Ratio of gap junction area to B cell volume ($10^{-5}/\mu m$)
Control	1053 ± 59 N = 15	13.2 N_j = 1530	17.3 ± 1.9 N_i = 10	0.042 ± 0.004 N_i = 10	20.5 ± 2.0 N_i = 10
Glibenclamid					
1 day	258 ± 32 N = 15	17.8 N_j = 1370	22.5 ± 2.1 N_i = 10	0.068 ± 0.008 N_i = 10	35.0 ± 4.2 N_i = 10
2 days	144 ± 17 N = 15	20.2 N_j = 1309	29.7 ± 3.2 N_i = 8	0.107 ± 0.011 N_i = 8	57.1 ± 6.0 N_i = 8
7 days	481 ± 32 N = 15	18.7 N_j = 1606	33.2 ± 2.5 N_i = 8	0.099 ± 0.008 N_i = 8	49.0 ± 3.7 N_i = 8

Table 2. Quantitative estimation of gap junction development per B cell. All values are expressed as means.

Group	B cell surface (μm^2)	B cell volume (μm^3)	Number of gap junctions per B cell	Gap junction area per B cell (μm^2)
Control	467	951	81	0.20
Glibenclamid				
1 day	424	821	95	0.29
2 days	397	743	118	0.42
7 days	466	946	155	0.46

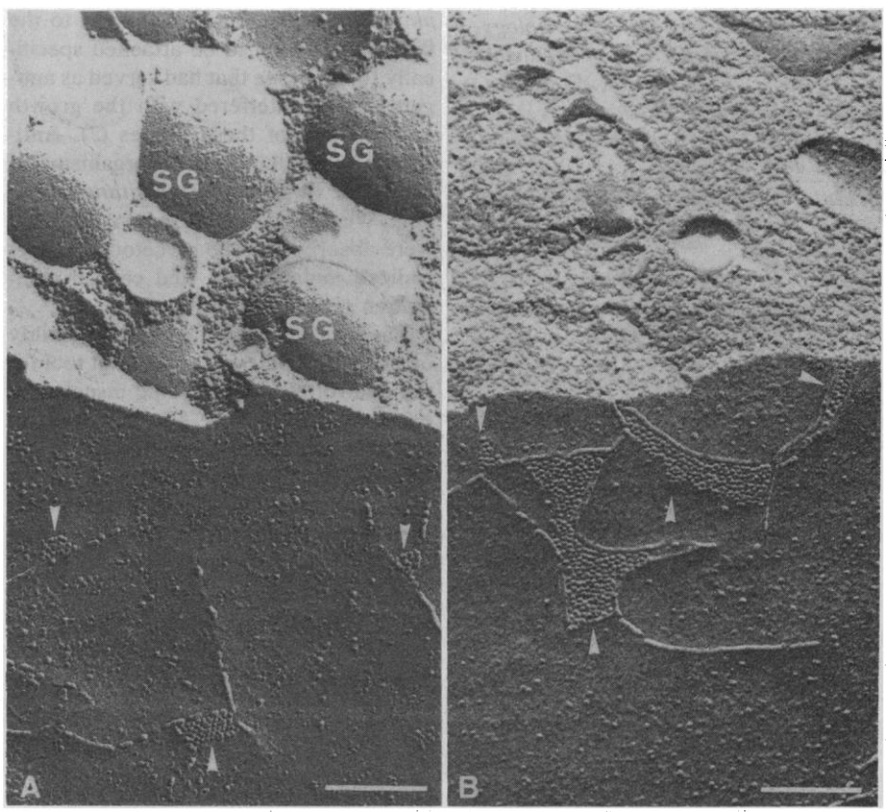

Fig. 2. Freeze-fracture replicas exposing both the plasma membrane (P-face) and the cytoplasm of a B cell in (A) control and (B) glibenclamid-treated (2 days) islets. Gap junctions (arrowheads) appear enlarged after glibenclamid treatment. Secretory granules (*SG*) can be seen only in the cytoplasm of the control B cell. Magnification, ×70,000; scale bar, 0.2 μm.

sulin secretion is correlated with the change in the gap junctions. It is also uncertain whether the increase in the number and size of gap junctions is a cause or a consequence of the modified secretory activity of the B cells. That hormones can modulate the development of gap junctions has been reported for other secretory systems (*13*).

If the gap junctions evaluated between B cells are permeable, the likely functional consequence of their increase is an enhancement of the exchange of ions and small molecules (ionic and metabolic coupling) between adjacent stimulated B cells (*9, 14*). Since there is morphological evidence that the gap junctions of glibenclamid-treated islets are permeable structures (*15*), the correlation observed between the development of gap junctions and the functional state of B cells indicates (*1-3*) that a modulation of direct intercellular communication may participate in the regulatory system by which B cells adjust their level of activity in relation to need.

P. MEDA
Institute of Histology and Embryology, Geneva University School of Medicine, 1211 Geneva 4, Switzerland
P. HALBAN
Institute of Clinical Biochemistry, Geneva University School of Medicine
A. PERRELET
Institute of Histology and Embryology, Geneva University School of Medicine
A. E. RENOLD
Institute of Clinical Biochemistry, Geneva University School of Medicine
L. ORCI
Institute of Histology and Embryology, Geneva University School of Medicine

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6. For each experiment, five batches of ten isolated islets were washed three times with cold Krebs-Ringer bicarbonate buffer containing 0.5 percent bovine serum albumin and were subsequently dispersed into 1 ml of acid ethanol (ethanol, water, and HCl, 140:57:3 by volume) at 4°C. Rat insulin was used as standard for radioimmunoassay of acid ethanol extracts with guinea pig antibody to porcine insulin.
7. For freeze-fracture, batches of 150 to 200 islets were centrifuged into pellets and fixed for 60 minutes in 0.1*M* phosphate-buffered glutaraldehyde (2 percent). After being rinsed several times in phosphate buffer, the pellets were infiltrated for 60 minutes in 30 percent phosphate-buffered glycerol and frozen in Freon 22 that had been cooled with liquid nitrogen. The pellets were subsequently fractured and shadowed in a Balzers BAF 301 apparatus. The replicas obtained were washed in a sodium hypochlorite solution, rinsed in distilled water, and mounted on copper grids. They were examined with a Philips 301 electron microscope whose magnifications were calibrated with a grid containing 2160 lines per millimeter. For each experimental condition, eight to ten islets were randomly selected in at least three replicas, each obtained from a different experiment. The B cells and their gap junctions were identified in the fractured islets by previously discussed criteria (*2*). Gap junctions found on randomly selected P-fracture faces were photographed at a fixed magnification of ×24,400. The number of their constitutive particles was counted on negatives enlarged 4.5 times by projection on a screen. Gap junction areas were estimated by multiplying this number by 10^{-4} μm^2, the average area of a single gap junction particle (*2*). The areas of B cell plasma membranes were measured on ×23,000 enlarged micrographs with a graphics tablet (Tektronix 4953) connected with an IMSAI 8080 microcomputer system (*8*). Gap junction size is expressed in medians because of the highly asymmetric distribution of gap junctions (*2*). Accordingly, statistical comparisons were done using either Student's unpaired *t*-test or the median test.
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Specific Antibodies: A Potential Insecticide

Abstract. *When tsetse flies are fed on human blood, the hemolymph of the flies contains human albumin. If the flies then ingest antibodies to human albumin, they die within a short time. The albumin fraction in their hemolymph disappears and osmoregulation is severely disturbed.*

Many insects are able to absorb orally administered antibodies. Antibodies to muscles or nerves of the flesh fly *Sarcophaga falculata* Pand., when fed to the flies, were found to be attached specifically to the tissue that had served as antigen (*1*) and interfered with the growth and function of these tissues (*2*). Antibodies to symbiotic microorganisms of the tsetse fly *Glossina morsitans morsitans* Westwood, when fed to the flies, were absorbed by the mycetocytes, specialized midgut cells, and caused elimination of the symbionts (*3*).

These results suggested the possibility of using antibodies as a potential tool for the control of blood-feeding insects, for example, tsetse flies. Albumin was selected as an antigen for the following reasons. (i) Albumin is a constituent of the diet of tsetse flies that is essential for their reproduction (*4, 5*). (ii) Tsetse flies are able to absorb undigested albumin from the gut into the hemolymph (*6*). Flies fed on human blood contain human albumin in their hemolymph. Human albumin disappears from the hemolymph and is replaced by bovine albumin when the flies are fed on bovine blood. (iii) Albumin is an easily available antigen.

Table 1. Ionic concentrations and osmotic pressure in the hemolymph of experimental and control flies (about 20 flies in each group).

	Experimental flies	Control flies
Sodium (m*M*)	360	150.7
Potassium (m*M*)	80	3.32
Osmotic pressure (mosmole)	620	345

An antiserum was produced by injecting human albumin intramuscularly into rabbits. Injections were repeated at intervals until an antibody titer of 1:40 was achieved (as indicated by Ouchterlony tests). After the antiserum had been withdrawn from the rabbit, the albumin was removed by affinity chromatography with Affigel Blue (Bio-Rad). This albumin-free antiserum was fed to tsetse flies, *G. m. morsitans*, through a membrane feeding system (*7*), with the use of a silicone rubber membrane (*8*). All of the flies that had previously been fed on human blood, and therefore contained a human albumin fraction in their hemolymph, died within 2 hours after becoming engorged with the antiserum. Their metabolism seemed to be severely disturbed, since the transport of the meal from the crop to the midgut was suppressed and no primary excretion could be observed. Sodium dodecyl sulfate–polyacrylamide gel electrophoresis showed that the albumin fraction was eliminated from the flies' hemolymph, apparently as a result of precipitation by

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