accord with transpositional events taking place at different sites. The S-1 and S-2 molecules may be uniquely equipped for insertion since both DNA's contain terminal inverted repeats (*16*). Inverted repeats are often characteristic of transposable elements in prokaryotic systems [reviewed in (*17*)]. Finally, transposable elements, termed "controlling elements," have been recognized for years in maize where they have been shown to inhibit normal activities of a variety of different nuclear genes (*18*).

Common to the mtDNA of male-sterile and revertant cytoplasms are at least five *Xho*I cleavage fragments with homology to the plasmid-like DNA's. Although the significance of this finding is not clear, it may mean that the plasmid-like DNA's could have arisen from the mitochondrial genome by excision and that this particular event is associated with the origin of the S type of cytoplasmic male sterility. Studies with prokaryotic systems suggest that transposable elements are able to move from one site to another without leaving the original site (*19*). Therefore, the presence in the mtDNA of sequences homologous to the S-1 and S-2 DNA's after their excisions would be anticipated if excision events behave similarly in the two systems.

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9. The revertant stocks used were 251, 274, 285, 296, 352, 369, and 733. In all but the last strain, the male-sterile source materials from which these revertants arose can be traced to an initial cross of a *cms-Vg* version of inbred line M825 as female parent, with inbred line 0h07, carrying normal cytoplasm, as male parent; *cms-Vg* is one of a number of independently discovered male-sterile types that falls in the general category of *cms-S*. The source of revertant 733 can be traced to an original cross involving male-sterile inbred line WF9 with *cms-S*, as female parent, and inbred line M825 carrying normal cytoplasm as male parent.
10. Preparation of maize mtDNA and gel electrophoresis techniques were described in (*3, 4, 6*).
11. The endonuclease *Xho*I was purchased from New England Biolabs or Bethesda Research Laboratories, Inc. Digestions and electrophoresis were as previously described (*4*).
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13. Labeling of S-1 and S-2 DNA's (probes) was achieved by nick translation with ^{32}P-labeled deoxyribonucleoside triphosphates (Amersham) in the presence of DNA polymerase I (New England Biolabs) and DNAase I under conditions similar to those described [T. Maniatis, A. Jeffrey, D. G. Kleid, *Proc. Natl. Acad. Sci. U.S.A.* **72**, 1484 (1975)]. The labeled DNA's were purified by Sephadex G-50 chromatography with 2 × SSC (1 × SSC is 15*M* NaCl and 0.15*M* sodium citrate) and elution with 0.01 percent sodium dodecyl sulfate.
14. Hybridization mixtures contained 5 × SSC, 4 × Denhardt solution (four times each of 0.02 percent Ficoll 400, 0.02 percent polyvinylpyrrolidone, 0.02 percent bovine serum albumin), 0.02*M* tris-HCl (*p*H 7.4), 50 percent spectrograde formamide, 0.05 percent sodium dodecyl sulfate, heat-denatured, sonicated calf thymus DNA (20 μg/ml) and usually more than 10×10^6 dpm of probe per membrane. Filter membranes were incubated for 48 hours at 37°C, with occasional shaking and then washed three times with 200 ml of hybridization mixture, without carrier DNA, for 30 minutes at 37°C, washed once with 200 ml of 2 × SSC, blotted, dried, and mounted for autoradiography with two sheets of DuPont Cronex 4 x-ray film and a Kodak X-omatic regular intensifying screen. The x-ray films were developed after 2 to 4 days of exposure at −90°C.
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Testosterone-Mediated Sexual Dimorphism of Mitochondria and Lysosomes in Mouse Kidney Proximal Tubules

Abstract. *In kidney proximal tubules of male mice the mitochondria are larger and more electron-lucent, autophagic vacuoles and lysosomes (predominantly myeloid bodies) more numerous and voluminous, and exocytosed intraluminal myeloid bodies more common than in females. Males also have higher kidney activities of mitochondrial cytochrome* c *oxidase and lysosomal hydrolases, and excrete larger quantities of hydrolases and protein in the urine. Orchiectomy evokes the feminine pattern whereas testosterone administration induces the male pattern. Endogenous testosterone modulates mitochondrial structure and function and enhances the activity of the lysosomal-vacuolar system in proximal tubule cells.*

The mouse kidney displays a complex response to testosterone administration which includes hypertrophy, augmented RNA and protein synthesis, and increases in β-glucuronidase and a number of other specific proteins (*1*). The testosterone-induced increase in kidney β-glucuronidase is restricted to the epithelial cells of proximal tubules (PT) (*2–4*), as is the cellular hypertrophy (*1, 5*), and large amounts of β-glucuronidase are excreted in the urine (*2–4, 6*) together with hexosaminidase (*4, 6*), β-galactosidase (*4, 6, 7*), and several other lysosomal enzymes (*6*). Testosterone administration in female mice induces an accumulation of lysosomes containing abundant layered, myelinlike membranes (cytoplasmic membranous bodies or myeloid bodies) in kidney PT cells, and enhances the exocytosis of these lysosomes into the tubule lumen (*6*). The extruded myeloid bodies and membrane-bound lysosomal enzymes are subsequently demonstrable in urine sediments. The testosterone-induced increment in urinary lysosomal enzymes reflects the hormone-mediated production and egestion of lysosomal myeloid bodies by PT cells (*6*). We now describe a testosterone-dependent sexual dimorphism in ultrastructure of PT cells involving the lysosomes and mitochondria and the tissue activities of several enzymes associated with these organelles. There is also a sex difference in the urinary excretion of lysosomal enzymes and protein.

Male and female mice of an inbred strain (A/J) were used for these experiments. Male mice were subjected to transcrotal orchiectomy under trichloroethylene inhalation anesthesia to study the effect of endogenous testosterone. Female mice received four subcutaneous injections of 1 mg of testosterone propionate (TP) in 0.05 ml of ethyl oleate

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vehicle on alternate days and were killed 24 hours after the last injection. Control females received ethyl oleate vehicle or no injections. For electron microscopic study, kidney slices were rapidly immersed in cold buffered 2.5 percent glutaraldehyde, postfixed in buffered 1 percent osmium tetroxide, dehydrated, and embedded in Araldite (*6*). In PT of the mouse, as in the rat (*8*), three sequential segments (S_1, S_2, and S_3) can be identified by their ultrastructural characteristics. Sex differences in ultrastructure are clearly evident in all three segments of PT, but are most pronounced in cells of the S_2 and S_3 segments. In female mice the mitochondria of the epithelial cells of the S_2 segment are elongate and contain an electron-opaque matrix that tends to obscure the cristae. The lysosomes have mostly a granular content, although some contain a heterogeneous matrix or vacuoles, and autophagic vacuoles are relatively infrequent. Profiles of Golgi apparatus, smooth endoplasmic reticulum, and rough endoplasmic reticulum are sparse and their cisternal spaces are narrow (Fig. 1, a and b). Extracellular membrane whorls representing exocytosed myeloid bodies are infrequent, and generally occur among microvilli and in the lumen of the S_3 segment.

In male mice the mitochondria in the S_2 segment of PT are larger and the matrix is more electron-lucent than those of female mice (Fig. 1, c and d). The lysosomes are more numerous, larger, and mostly of the myeloid body type. Intraluminal membrane whorls are relatively common and occur mostly in the S_3 segment. Autophagic vacuoles also are more frequent in the S_2 segment. These are often encircled by a stack of myelin-like membranes and generally contain degenerating mitochondria, peroxisomes, endoplasmic reticulum, or ribosomes. Attached and free ribosomes, largely as polysomal clusters, appear to be more abundant. Golgi membranes and rough endoplasmic reticulum are more prominent and their cisternae tend to be wider. The ultrastructure of the PT cells of male mice had reverted to that of female mice by 2 weeks after orchiectomy, the earliest postoperative interval at which they were examined (Fig. 1e). Conversely, TP treatment in female mice induced the male ultrastructure in PT cells by 1 week (Fig. 1f). In fact, autophagic vacuoles and myeloid bodies were generally more numerous, larger, and more complex in PT cells of TP-treated female mice than in those of male mice. The TP-treated female mice also displayed greater numbers of intraluminal membrane whorls than male mice. The ethyl oleate vehicle produced a minimal decrease in the electron opacity of the mitochondrial matrix.

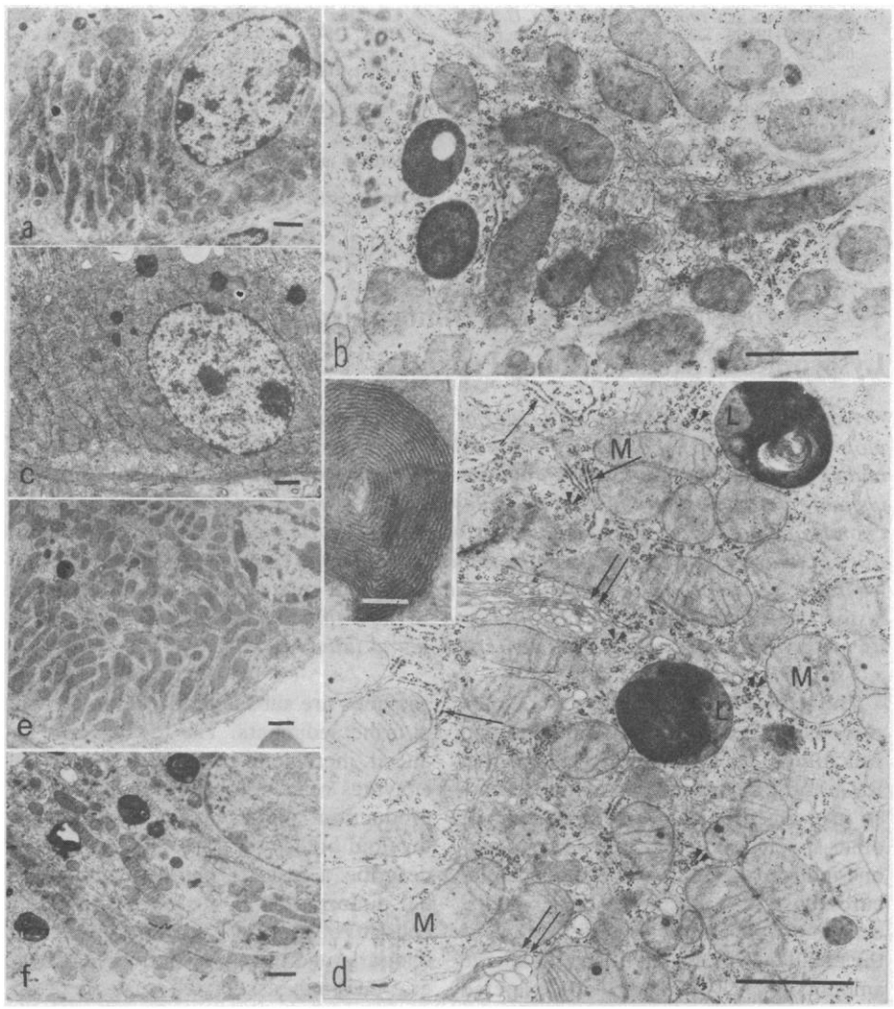

Fig. 1. Effect of sex, orchiectomy, and testosterone administration on the ultrastructure of mouse kidney PT cells. All electron micrographs are of the S_2 segment. (a) Control female (×3,400). (b) Control female (×15,000). (c) Control male (×3,400). (d) Control male (×15,000). Inset: High-power magnification of a myeloid body (×60,000). (e) Orchiectomized male (×3,400). (f) Female treated with TP (×3,400). The lower-power electron micrographs on the left reveal more obvious differences in fine structure. The PT cells of control female (a) and orchiectomized male mice (e) are similar in that the mitochondria (*M*) are electron-opaque and lysosomes (*L*) are sparse and small. The PT cells of control male (c) and TP-treated female mice (f) have larger, electron-lucent mitochondria and numerous voluminous lysosomes of the myeloid body type. These differences are more evident in the higher-power micrographs of a PT cell from control female (b) and male mouse (d). Ribosomes (arrowheads) are also more abundant and the rough endoplasmic reticulum (single arrows) and Golgi apparatus (double arrows) are better developed in the PT cell of the male. Scale bars indicate 1 μm except for the inset in (d), which is 0.1 μm.

For biochemical study mice were housed in groups of two or three in metabolic cages. Urine samples were collected under mineral oil in 0.1 ml of 10 percent sodium azide and dialyzed for three 2-hour periods in 1000 volumes of distilled water prior to enzyme assays. Tissue and urine samples were stored at −20°C until assayed for protein (*9*), β-glucuronidase (E.C. 3.2.1.31), hexosaminidase (β-*N*-acetylhexosaminidase, E.C. 3.2.1.30), β-galactosidase (E.C. 3.2.1.23), and arylsulfatase (E.C. 3.1.6.1) (*10*). Cytochrome *c* oxidase (E.C. 1.9.3.1) (*11*) was assayed in fresh tissues or tissues stored at −70°C. The results of the experiment presented in Fig. 2a are representative of three separate experiments. Compared with female mice, the kidneys in male mice were heavier, and revealed greater specific activities of cytochrome *c* oxidase, β-glucuronidase, hexosaminidase, β-galactosidase, and arylsulfatase. Orchiectomy produced a decrease in kidney weight and in the specific activity of cytochrome *c* oxidase, β-glucuronidase, and the other acid hydrolases. In contrast, TP treatment in female mice induced an increase in kidney weight and in the specific activity of cytochrome *c* oxidase, β-glucu-

ronidase, and the other acid hydrolases. In another experiment TP administration in orchiectomized male mice induced similar or greater increases in these kidney enzyme activites (data not shown). The ethyl oleate vehicle produced small, statistically insignificant increases in the acid hydrolases.

Mice also exhibit a sex difference in the urinary excretion of acid hydrolases and total protein. In the experiment shown in Fig. 2b, which is typical of four separate experiments, male mice excreted 4.5 times more protein, 30 times more β-glucuronidase, and 6 to 13 times more of the other acid hydrolases than female mice. After orchiectomy the urinary excretion of these constituents in males declined to levels below those in females, whereas after TP treatment of females the excretion of these constituents exceeded that in intact males. The ethyl oleate vehicle elicited small increases in the excretion of these constituents, but except for protein, these increases fell short of statistical significance. In both sexes, irrespective of treatment, only trace amounts of cytochrome *c* oxidase, 0.0 to 0.004 percent of the kidney activity, appeared in the urine (data not shown).

Our electron microscopic study has disclosed a previously unrecognized sexual dimorphism in the fine structure of mitochondria and the lysosomal-vacuolar system in mouse kidney PT cells. In addition, ribosomes are more abundant and the rough and smooth endoplasmic reticulum and Golgi apparatus are more extensively developed in PT cells of male mice. That the sexual dimorphism of PT cells is an expression of endogenous testosterone is established by the complementary findings that orchiectomy of male mice evokes the female ultrastructure and testosterone treatment in female mice induces the male ultrastructure.

Our biochemical observations show that the sexual dimorphism of PT cells is accompanied by an enzymatic dimorphism of the mouse kidney. Thus, the higher kidney activity of cytochrome *c* oxidase, an inner mitochondrial membrane enzyme (*11*), in male mice is correlated with the larger, electron-lucent mitochondria, and the greater kidney acid hydrolase activities are correlated with the expansive lysosomal-vacuolar system in the PT cells of male mice. Further, the greater urinary excretion of lysosomal hydrolases and protein in males parallels the increased frequency of exocytosed lysosomal membrane whorls appearing in PT lumens of male mice. The lack of cytochrome *c* oxidase in the urine is consistent with the absence of mitochondria, whether isolated or in exfoliated cells, from the tubule lumens and urine sediments (*6*). Sex differences have previously been reported for kidney β-glucuronidase (*2, 12*), β-galactosidase and hexosaminidase (*7, 12*), and the urinary excretion of these hydrolases (*2, 7*) and protein (*13*). That this enzymatic dimorphism is also dependent on endogenous testosterone is demonstrated by the marked decrease in kidney enzymes and the urinary excretion of hydrolases and protein following orchiectomy, and the even greater increase in kidney enzymes, lysosomal enzymuria, and proteinuria induced in female mice and orchiectomized male mice by testosterone administration.

Our findings are consistent with the hypothesis that testosterone, in addition to stimulating RNA and protein synthesis (*1*), modulates the structural and

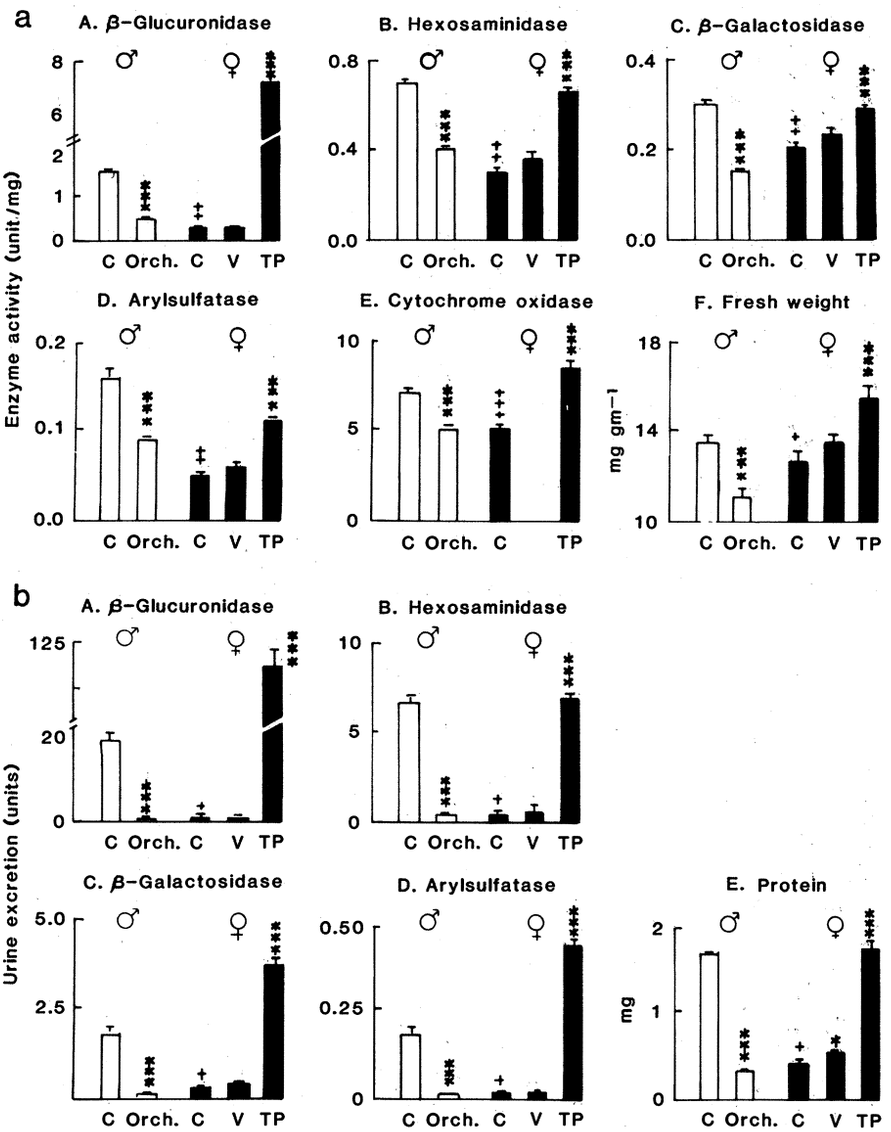

Fig. 2. Effect of sex, orchiectomy, and testosterone administration on (a) mouse kidney fresh weight, cytochrome *c* oxidase, and acid hydrolases, and (b) the urinary excretion of acid hydrolases and protein. Male A/J mice (open bars) were not operated on (*C*) or they were orchiectomized (*Orch.*) 63 days before they were killed. Female A/J mice (solid bars) received four subcutaneous injections of testosterone propionate (*TP*) (1 mg in 0.05 ml of ethyl oleaste) or ethyl oleaste vehicle (*V*) in 7 days and were killed 1 day later with female controls (*C*) that received no injections. Urine samples were collected from mice one or two 24-hour periods before they were killed. (a) Fresh weight is expressed as milligrams per gram of body weight. The enzymes are measured in terms of absorbance at 550 nm per minute for cytochrome *c* oxidase, and as micromoles per hour for acid hydrolases. The data are expressed as means ± standard error ($N = 7$ or 8): ***, $P < .001$ (treated compared to controls); +, $P < .05$; ++, $P < .01$; +++, $P < .001$ (female controls compared to male controls). (b) Urinary excretion is expressed as units of enzyme or milligrams of protein excreted per mouse in 24 hours. Results are means ± standard error ($N = 6$ for males, 4 for females): *, $P < .05$; ***, $P < .001$ (treated compared to controls); +, $P < .001$ (female controls compared to male controls).

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*).

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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-

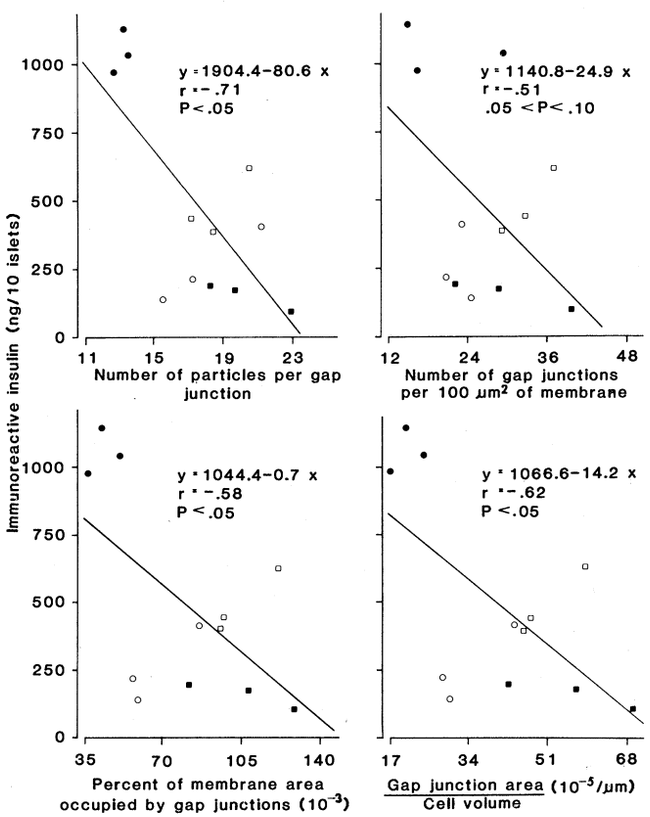

Fig. 1. For each experiment, the values of the four indices used to characterize B cell gap junctions were each plotted as a function of the insulin content of the corresponding islets. A correlation coefficient and a linear regression analysis were computed from these pairs of data. (●) Control; (○)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

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