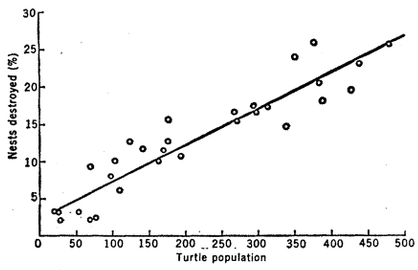

Fig. 2. Correlation of nest destruction with size of turtle population.

procedure is nonrandom, the complexity of the stochastic process is increased, and analytical and hand-simulation techniques become inapplicable. The only practical technique is a discrete computer simulation (in effect a general-purpose Monte Carlo procedure). The most powerful technique is Simscript. For this simulation, the digging of the turtles was described in extremely fine detail (Fig. 1).

We conducted a series of simulations over a range of population sizes encompassing and extending the field situation. A linear regression (Fig. 2), which fits these results with an associated probability of less than .001, indicates that nest destruction by turtles is density-dependent.

For example, we may assume that a stable turtle population contains 50 breeding females each having laid 100 eggs, and that mortality is constant, and at a level such that one female out of 95 eggs will survive to breed. Then a population with self-induced mortality (by nest destruction) of 5 percent (Fig. 2) would have 95 hatchlings per female, and the rate of population increase (P) would be 1.0, giving the same number of females in the next generation. However, if there were 300 breeding females, self-induced mortality would be 17 percent, hatchlings per female 83, and $P = 83/95 = 0.87$. The next generation would be about 261 females, that is to say population would decrease. At a population of 500 breeding females, the next generation would be only 385. In both instances decreases would continue to occur in succeeding generations, until P approaches 1.

The mechanism, therefore, tends to keep a population within certain limits. At low population density its effects are negligible; however, if a population increased greatly it would not stay at the new level due to this mechanism, which tends to restore the population to its original level.

Variation is the important attribute of mortality (*2*), low but variable mortalities having the same influence on population trends as high but relatively constant mortalities. Most factors in mortality appear to be density-dependent, and variations at high levels are potentially more important. Effects of one or a few ecological processes may account for most of the variability in trend in population numbers (*3*).

There are no nest predators on Barrier Reef cays, and the green turtle has been entirely protected by law since 1950; hence we are dealing with large natural populations of this turtle. However, predation (both human and nonhuman) is very high on many other beaches where turtles nest (*4, 5*). We anticipate that, where the species has been seriously overexploited, the population density is depressed below the level at which nest destruction by turtles would operate as a regulatory mechanism. The Barrier Reef of Australia may be the last place where natural regulation of population size by the mechanism of density-dependent nest destruction can still be observed today.

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References and Notes

1. H. R. Bustard and P. M. Greenham, *Ecology* **49**, 269 (1968).
2. R. F. Morris, *ibid.* **40**, 580 (1959).
3. L. R. Clark, P. W. Geier, R. D. Hughes, R. F. Morris, *The Ecology of Insect Populations in Theory and Practice* (Methuen, London, 1967).
4. J. R. Hendrickson, *Proc. Zool. Soc. London* **130**, 455 (1958).
5. A. Carr, *So Excellent a Fishe* (Natural History Press, New York, 1967).
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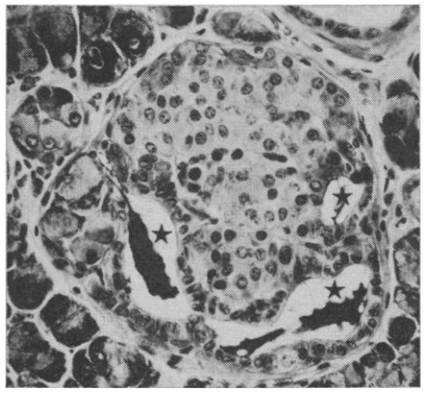
Mitotic Division in Pancreatic Beta Cells

Abstract. *Successful expansion of the islet cell mass occurs in genetically diabetic mice (C57 Bl/Ks-dbdb) following a period of dietary restriction, in the absence of a population of precursor cells. Differentiated cells that synthesize insulin retain the capability of undergoing mitotic division.*

The pancreatic diverticulum develops as an evagination of the primitive gut, and, in the subsequent morphogenesis of the embryo, is transformed into the ducts and acinar cells of the exocrine pancreas (*1*). The belief that the forerunners of the islets of Langerhans also originate in epithelial cells of the primitive ducts (*1*) has recently been questioned. Noting that mesodermal cells accumulate in juxtaposition to the evaginating pancreatic diverticulum, Wessels (*2*) was unable to determine whether the initial cells possessing beta granules were ductal (endodermal) or mesodermal in origin. The frequent observation of proliferating ductal elements in the pancreases of diabetic experimental animals has been interpreted as evidence of postnatal beta cell neogenesis (*3*). However, convincing ultrastructural evidence of transformation of duct cell to islet cell is lacking. The conversion of pancreatic acinar cells into islet cells was originally suggested (*4*) as a physiologic means of varying the relative exocrine and endocrine functional capacities. This hypothesis has few modern advocates (*5*) and has suffered from the absence of acceptable ultrastructural evidence of transition forms between the two cell types. Mitotic division among islet cells has not been stressed as a significant cause of postnatal beta cell proliferation because mitoses are not frequently observed among islets; furthermore, it is rarely, if ever, possible to determine with the light microscope the cell type undergoing mitosis. One may question, as workers using other systems have (*6*), whether cells that synthesize a protein such as insulin are too differentiated to undergo cell division.

We find that insulin-producing cells do divide and suggest that mitotic division among differentiated beta cells may be an important mode of their postnatal proliferation.

Diabetic mutants (dbdb) of C57 Bl/Ks mice, if allowed free access to food, die after 5 to 7 months with evidence of marked hyperglycemia and decreasing concentrations of insulin in the serum (*7*). Their islets of Langerhans reveal decreased numbers of beta cells and numerous small ducts (Fig. 1). The latter have been interpreted as evidence of an unsuccessful stimulation of beta cell neogenesis (*7*). When food is made available for only 8 hours per day on Monday, Wednesday, and Friday of



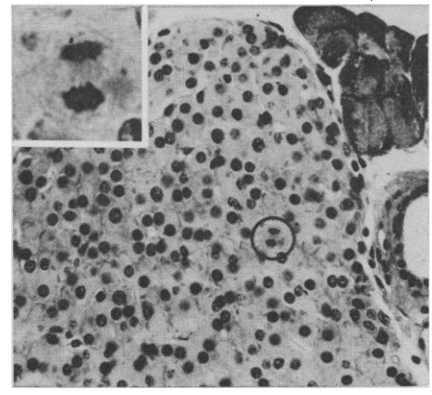


Fig. 1 (left). Islet from unmodified diabetic mutant mouse. Proliferating ducts (★) are at the periphery. The remainder of the islet includes a mixture of alpha and beta cells (hematoxylin and eosin; × 230). Fig. 2 (right). Enlarged islet from diabetic mutant after dietary manipulation. Circled mitosis is enlarged in the inset. Majority of islet consists of beta cells. Proliferating ducts are absent (hematoxylin and eosin; × 240, inset, × 1000).

each week, for 4 to 8 weeks (starting at 6 weeks of age), the syndrome is no longer lethal. When the mice are again given free access to food, concentrations of glucose in the blood remain normal or moderately increased, and concentrations of serum immunoreactive insulin remain elevated indefinitely (*8*). The pancreatic islets of these animals are prominently enlarged by increased numbers of beta cells. Mitotic figures are common and proliferating ducts are far less frequent than in the control mice (Fig. 2).

The diets of diabetic mutant mice (obtained from the Jackson Laboratory, Bar Harbor, Maine) were restricted, as described above, and the animals were killed 2 to 4 weeks after being again given free access to a commercial laboratory chow. Pancreatic fragments were fixed in buffered glutaraldehyde or osmium tetroxide (or both), dehydrated, and embedded in Epon. Thin sections of islets containing cells undergoing mitotic division were examined with an RCA-EMU 3G electron microscope.

Dividing cells were found within the interior as well as at the periphery of the pancreatic islets. Seventy-five percent of these cells were beta cells, as judged by the presence of characteristic beta secretory granules (Fig. 3). Each of the cells possessed mitochondria, cisternae of the granular endoplasmic reticulum, and numerous ribosomes. These cells might simultaneously have been synthesizing insulin and undergoing division, or the beta granules may have been remnants of synthetic activity that preceded the process of cell division. The remainder of the cells undergoing mitotic division were ductal in origin and possessed no beta secretory granules. At no time in this study or in a study of the unmodified mutant mice were instances of transformation of duct cell to islet cell observed.

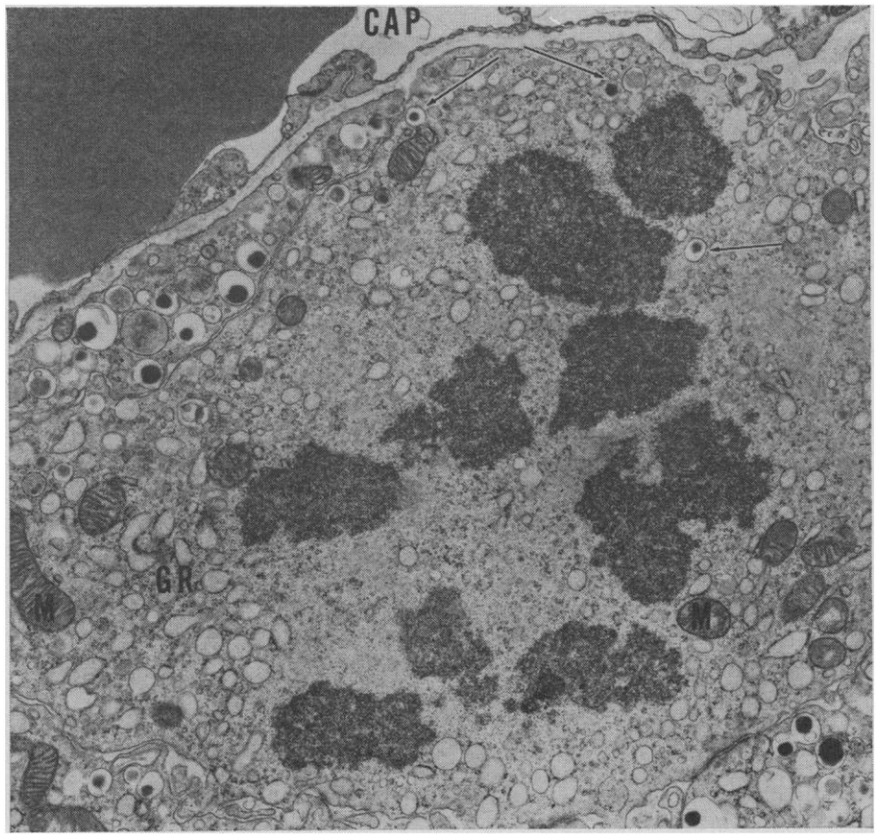


Fig. 3. Electron micrograph of beta cell in mitosis. Chromosomes occupy central portion of cell. Secretory granules (arrows), granular endoplasmic reticulum (*GR*), and mitochondria (*M*) are present; *CAP*, capillary (× 8800).

The occurrence of mitoses among cells containing beta granules and the absence of a population of undifferentiated ductal cells progressively being transformed into islet cells suggest that the increase in cell numbers is the result of divisions in the population of preexisting beta cells and not attributable to differentiation of precursor ductal cells. These findings agree with those of Logothetopoulos and Bell (*9*) who observed by light microscopy a four- to sixfold increase in the number of islet cells incorporating H^3-thymidine after the administration of antibody to insulin. In three other animals with experimental diabetes—the Egyptian sand rat (*10*), Swiss-Hauschka mice, and Wellesley hybrid mice (*11*)—the number of beta cells also increases markedly, without any noteworthy proliferation of ducts.

Thus, in the diabetic mutant to the C57 Bl/Ks mouse, the presence of proliferating ducts cannot be correlated with a subsequent increase in the number of beta cells, and transformation of duct cells to islet cells in all probability does not occur. On the contrary, in the mutants with a restricted diet, differen-

tiated beta cells frequently divided and the capacity to synthesize insulin was increased in the relative absence of proliferating ducts.

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References and Notes

1. W. L. Hard, *Amer. J. Anat.* **75**, 369 (1944); S. A. Bencosme, *ibid.* **96**, 103 (1955); B. L. Munger, *ibid.* **103**, 275 (1958); P. Robb, *Quart. J. Exp. Physiol.* **46**, 335 (1961); J. L. Conklin, *Amer. J. Anat.* **111**, 181 (1962).
2. N. K. Wessells, in *Epithelial-Mesenchymal Interactions*, R. Fleischmajer and R. E. Billingham, Eds. (Williams and Wilkins, Baltimore, 1968).
3. S. S. Lazarus and B. W. Volk, *The Pancreas in Human and Experimental Diabetes* (Grune and Stratton, New York, 1962), pp. 66–76.
4. E. Laguesse, *J. Anat. Physiol. Paris* **T.21**, 475 (1895), cited by R. R. Bensley, *Harvey Lectures Ser.* **10**, 251 (1915).
5. L. Herman, T. Sato, P. J. Fitzgerald, *Electron Microscopic Anatomy*, S. M. Kurtz, Ed. (Academic Press, New York, 1964); R. Pictet, L. Orci, A. E. Gonet, C. Rouiller, A. E. Renold, *Diabetologia* **3**, 188 (1967).
6. J. Abbott and H. Holtzer, *J. Cell Biol.* **28**, 473 (1966).
7. K. P. Hummel, M. M. Dickie, D. L. Coleman, *Science* **153**, 1127 (1966); D. L. Coleman and K. P. Hummel, *Diabetologia* **3**, 238 (1967).
8. D. L. Coleman, personal communication.
9. J. Logothetopoulos and E. G. Bell, *Diabetes* **15**, 205 (1966).
10. A. A. Like and E. Miki, *Diabetologia* **3**, 143 (1967).
11. A. A. Like and E. E. Jones, *ibid.*, p. 179.
12. Supported by PHS grants AM 09584, T1-AM05077, and AM 12538.

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Esterase Heterogeneity: Dynamics of a Polymorphism

Abstract. *In the freshwater fish* Catostomus clarkii, *the frequency of alleles for polymorphic serum esterase varies with latitude. The activity of the allele more frequent in southern populations increases as temperature increases from 0° to 37°C, whereas activity of the allele more frequent in northern populations increases as temperature decreases. Coefficients of selection used to calculate allelic equilibrium within populations are derived from the activity profiles of the genotypes.*

Genetic heterogeneity or phenotypic variation of serum esterase (or both) has been demonstrated in many animal populations (*1*). Variation in serum esterase exists in populations of the Gila mountain sucker *Catostomus clarkii* in past and present tributaries of the Colorado River system, downstream from the Grand Canyon (*2*). This variation is due to the segregation of two codominant alleles, *Es-I*^{a} and *Es-I*^{b}, at a single locus and is manifest as three electrophoretic phenotypes—a fast migrating band of esterase activity, a slow band, and a double band in the heterozygote. There is a cline in the frequency of the *Es-I*^{a} allele which is positively correlated with latitude of the sampled population. The frequencies of the *Es-I*^{a} allele in populations of *C. clarkii* from southern Arizona, central Arizona, southwestern Utah, and northern Nevada are 1.00, 0.84 to 0.92, 0.46 to 0.60, and 0.17, respectively. The maintenance of the cline is dependent upon a spatial gradient in selection associated with changes in the relative fitness of the two homozygotes (*2*). Although temperature was suspected as the selective force in the maintenance of heterogeneity, this was not supported by available evidence. I find temperature is the component of selection, which, according to the enzymatic characteristics of the *Es-I* alleles, must operate through single locus heterosis.

My results were obtained with frozen serums and with fresh serums, and no differences were noted. All individual samples were surveyed with horizontal starch-gel electrophoresis (*2*, *3*), and serums from individuals of the same *Es-I* phenotype were pooled. Serums were vigorously shaken with two volumes of methylene chloride and centrifuged (27,000*g*) at 0°C to remove lipids. Two milliliters of serum standardized to 1.0 percent total protein concentration with a hand refractometer were added to a column (3.2 by 60.0 cm) of G-200 dextran equilibrated with degassed 0.2*M* sodium phosphate buffer. An ascending flow rate of 25 to 30 ml/hour was maintained, and the effluent was collected in 5.0-ml fractions.

Esterase activity was measured colorimetrically on fractionated serums. Individual fractions were adjusted to *p*H 7.0 with tris(hydroxymethyl)aminomethane and maleic acid buffer (*2*). Each fraction was brought to the desired temperature and mixed with an equal amount of substrate solution (1.0 percent α-naphthyl acetate in acetone) at the same temperature. After a 1-hour incubation at the desired temperature (± 0.05°C) with constant agitation, 1.0 ml of 0.2 percent Fast Blue RR was added, and the usual brown precipitate was formed. After 3 minutes, 1.0 ml of 50 percent trichloroacetic acid was added and the colored precipitate was extracted with 5.0 ml of ethyl acetate. Activity, determined at 430 nm, is expressed in arbitrary units of optical density (Fig. 1).

There are significant differences among the three genotypes (Fig. 1) at various reaction temperatures. At 37°C, activity of *Es-I*^{a} was more than ten times that of *Es-I*^{b}, the observed activity of the latter genotype being nearly zero. As might be expected, the activity of the heterozygote was intermediate between the two homozygotes.

Esterase activity observed at 0°C dif-

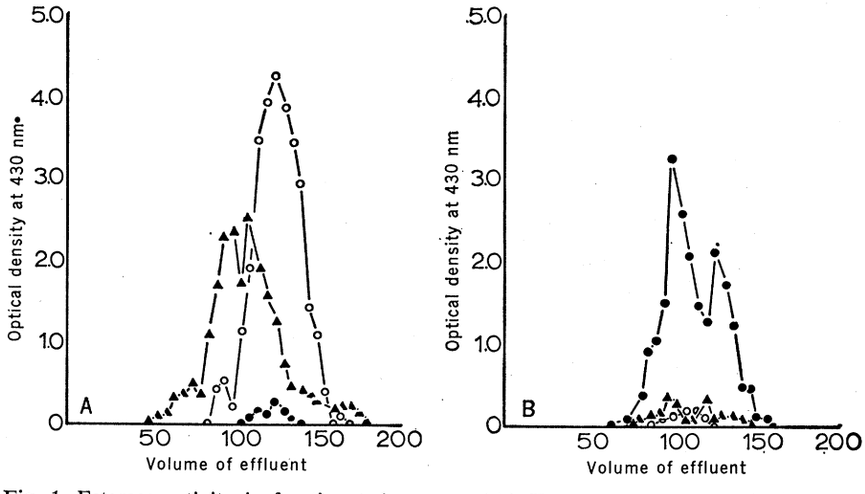

Fig. 1. Esterase activity in fractionated serums. (A) Temperature 37°C; (B) temperature 0°C; ○—○, *Es-I*$^{a/a}$; ▲—▲, *Es-I*$^{a/b}$; ●—●, *Es-I*$^{b/b}$.