The growth and development of the endocrine pancreas has been studied for many years, but questions remain concerning the regulation of the mass of insulin-producing β-cells both in the normal growing pancreas and during the pathogenesis of diabetes. The homeostatic control of β-cell mass in both normal and pathophysiological conditions is based on the balance of cell proliferation, cell growth, and cell death. To gain insight into the relative contribution of each of these dynamic processes, we first mathematically analyzed the data available on the components involved in the maintenance of β-cell mass, including rates of replication, β-cell volume, and the β-cell mass itself, at various ages in normal Sprague-Dawley rats. Then these data were combined in a simple mass balance equation to construct a mathematical model of the dynamics of the β-cell mass in the normal growing rat pancreas. Such a model has allowed us to infer the contributions of fluxes that cannot be measured, i.e., neogenesis and cell death, to the known mass of β-cells. Another important contribution of this model is to raise unanswered questions concerning the control of the balance of cell death and cell renewal in the endocrine pancreas. Diabetes 44:249-256, 1995

The growth and development of the endocrine pancreas has been studied for many years. Nonetheless, many questions remain concerning the regulation of the mass of insulin-producing β-cells both in the normal growing pancreas and during the pathogenesis of diabetes. New pancreatic cells (both islet and exocrine) are formed by differentiation of embryonic ductal cells, a process termed neogenesis, or by replication of the preexisting differentiated cells. It is generally accepted that until late gestation, most β-cells are the result of neogenesis and that after birth, most β-cells are formed by replication. Even though the replication rate of β-cells in the adult rodent is about 3% per day, it is often ignored that the β-cell mass continues to grow well into adulthood. This low replication rate has led many to assume that the β-cell mass does not turn over significantly and that one is born with all the β-cells one will ever have. Hellerstrom et al. (1) pointed out, however, that with a β-cell birthrate of just under 3% new cells per day, the β-cell mass would double in 1 month if there were negligible cell death. Likewise, if the rate of β-cell death approaches the replication rate of 3%, then complete replacement of the β-cell population could occur. Since the β-cell mass does not continue to double on a monthly basis throughout life, the β-cell, like most other cell types, must have a finite life span. Cell deletion is needed as a means of removing old or defective cells and as a response to reduction of demand. Apoptosis, or cell death, is receiving increased acceptance as the widespread physiological mechanism of cell deletion; it is considered the counterbalance of mitosis (2).

Experimental paradigms have shown that the mass of pancreatic β-cells is dynamic and can be regulated in an effort to maintain euglycemia (3). For example, a 96-h glucose infusion results in a 50% increase in β-cell mass through both enhanced β-cell replication and hypertrophy of β-cells (4). After pregnancy, there is an involution of β-cell mass due to a reduction of β-cell replication and of individual cell size, as well as an increase in apoptosis (5,6). There is also evidence from autopsies of human pancreas to suggest a compensatory growth of the β-cell mass in obesity (7). The mechanisms to achieve such a dynamic state could theoretically include changes in new cell formation, including replication and differentiation of new islets (neogenesis), changes in individual cell size/volume, and changes in cell loss or death rates. It appears likely that homeostatic control of β-cell mass in both normal and pathophysiological conditions is due to the balance of cell proliferation, cell growth, and cell death.

Even so, the relative contribution of each mechanism to the dynamic changes during the normal growth and development of the endocrine pancreas is not understood. The partially pancreatectomized rat model (8) demonstrated that neogenesis can occur in the adult, but there is little, if any, evidence of neogenesis occurring after the perinatal period in the absence of a major external stimulus. Although techniques for the quantification of replication rate are now well established, the lack of straightforward techniques for identifying the age of cells has hampered the estimation of rates of neogenesis and cell death. As a result, our knowledge of these rates and their effects on the life span of the β-cell is minimal.

To gain insight into the relative contribution of each of the
dynamic processes that determine β-cell growth, we mathematically analyzed the data available on the components involved in the maintenance of β-cell mass, including rates of replication, β-cell volume, and the β-cell mass itself, at various ages in normal Sprague-Dawley rats. Data from published reports of several laboratories were combined with our published and unpublished data, giving an increased power of analysis. We then used this information along with a simple mass balance equation to construct a mathematical model of the dynamics of β-cell mass in the normal growing rat pancreas. This model has allowed us to infer the contributions of the fluxes that cannot be measured, i.e., neogenesis and cell death, to the known mass of β-cells. These predictions have been examined in light of the available data and the assumptions that have been incorporated into the model calculations.

β-CELL REPLICATION RATE

β-cell replication rate has been estimated from in vivo data of accumulated mitotic figures in β-cells of colchicine-treated rats (4,9,10), 5-bromo-2'-deoxyuridine (BrdU)-incorporating β-cells (11), or tritiated thymidine-incorporated immunostained β-cells (12).

β-cell replication is significantly higher during late gestation and the neonatal period than after weaning, with little change in replication rates occurring beyond 30-40 days of age. The data were fitted (Fig. 1) with an exponential decay to a constant, because this appeared to provide an appropriate representation of the observations. Thus, β-cell replication rate can be described as follows:

\[
\text{REP} = 16.10 e^{-0.065 \times \text{age}} + 2.31
\]

where REP is the β-cell replication rate in percent of the β-cell mass per day, and age is given in days after birth. Our assumption that the β-cell replication rate fell exponentially to a constant level as a function of age provided a reasonable fit with the available data, although it fell short of some of the very high replication rates observed by Kaung (12). Beyond 100 days of age, data at only two time points were available, and they did not clearly indicate a specific trend. More data points will be needed to rule out conclusively that the rate falls to zero.

β-CELL VOLUME

β-cell volume has been determined by a number of different techniques, at both the light- and electron-microscopic levels. While our own light-microscopic data have been useful for comparisons of β-cell volume within the same study, theoretically they must be overestimations (11). In fact, these estimates were significantly larger than those we obtained from ultrastructural analysis (4). Thus, in the present analysis, we used only data generated from ultrastructural analysis. In most cases, the cross-sectional areas of individual β-cells were measured from micrographs. Since densely packed cells are irregular polyhedra and their mean tangent diameter has been estimated at about 8% larger than a sphere of equal volume (13), the β-cell volume calculated as the volume of a sphere is reasonable. The data from Pipeleers' group (14-16) were obtained from single, sorted β-cells that assume a spherical shape; their data used in our analysis were the averages of the mean values of the separate populations of high- and low-responding β-cells.

Using the available data (Fig. 2), no particular functional form for the relationship between β-cell volume and age was suggested; in fact, β-cell volume seemed constant at a mean of 1,020 μm³. Because no data on animals younger than 21 days were in the literature, the values at 2 and 9 days were determined (LS, unpublished data) using our previous technique (4). These new values (511 and 508 μm³, respectively) suggest that the newly formed β-cell is smaller than the more
mature β-cell. Without data on the average cell size between the 9- and 21-day observations, we made the simplest assumptions possible, i.e., that β-cells from animals <9 days old were 510 μm³, that those from animals older than 21 days were 1,020 μm³, and that there was a linear growth in the mean cell size between 9 and 21 days. Additional studies are in progress to more clearly define the relationship between β-cell size and animal age.

Nonetheless, these estimates of mean cell volume allow us to calculate the number of cells per milligram of tissue. If the specific gravity or density of the tissue is assumed to be 1 mg/mm³ and the volume of a single β-cell is 1,020 μm³ (1.02 × 10⁻³ mm³/cell), there are 9.8 × 10⁶ cells per milligram of β-cell mass.

β-CELL MASS

β-cell mass has been determined morphometrically by linear scanning (17,18) or by point counting (4,11,19–23). Data available for normal Sprague-Dawley rats are plotted in Fig. 3. Several interesting features of these data made it impossible to fit the data with any simple mathematical representation. Although at first glance the data from late fetal to almost 100 days of age appear linear (Fig. 3A), the deviation from this line between 5 and 20 days is not likely due to random error (Fig. 3B). This plateau in β-cell growth is defined by four time points determined in a single study (17) and is confirmed by data from two other laboratories (19,23). Although the data available for >100 days of age are quite limited, these data appear to suggest a slowing of growth of the β-cell mass in the adult animal.

Since no particular function could define all these characteristics, we chose to fit the data and predict the mass and its rate of change (Fig. 3) using the optimal segments (OPSEG) smoothing method (24). This technique minimizes the segment-to-segment difference in slope for a given estimated error (difference between the smoothed and observed points).

With the estimated number of cells per milligram of tissue (see above) and the curve fit of the mass data at different ages, cell numbers and their rates of change can be calculated for each day.

THE MASS BALANCE EQUATION: THE MATHEMATICAL MODEL

The generic mass balance equation states that the rate of entry of new cells minus the rate of loss of cells will equal the rate of change of the number of cells in the mass. In the case of the β-cell mass, there are two processes that could contribute new cells: replication of preexisting β-cells and formation of new islets by differentiation of ductal precursors (neogenesis). There are data on the rate of replication of β-cells but none on neogenesis, so we considered these two processes separately. Cell death occurs by either apoptosis or necrosis. Because there are no data on either process, their contribution was considered together as the cell death rate. On this basis, the mass balance equation is

\[ \text{REP} + \text{NEO} - \text{DEATH} = \frac{d(\beta\text{-cell mass})}{dt} \]  

(2)

where REP is the rate of replication of the β-cell, NEO is the rate of islet neogenesis, DEATH is the total rate of β-cell death, and \( \frac{d(\beta\text{-cell mass})}{dt} \) is the rate of change of the β-cell mass.

FIG. 3. β-cell mass as a function of age in normal rats. Morphometric data on immunostained sections were from Swenne et al. (19) (●), from our laboratory (4,11,20-23,42, and L.S., unpublished data) (▲), and from McEvoy (17,18) (♦). The solid line is the curve fit determined by the OPSEG routine (24). Values observed on the same day were averaged for submission to the OPSEG program. Two passes through the smoothing program and splicing of the smoothed segments were required because of the variability in the ages observed in the different studies and the need to predict the β-cell mass and its rate of change daily.

ESTIMATION OF THE RATES OF NEOGENESIS AND CELL DEATH

We can rearrange Eq. 2 so that the unknown rates of neogenesis and cell death are expressed in terms of the known quantities of the rates of β-cell accumulation and replication as follows:

\[ \text{NEO} - \text{DEATH} = \frac{d(\beta\text{-cell mass})}{dt} - \text{REP} \]  

(3)

When this expression is positive, i.e., the rate of increase in cell number is greater than the replication rate, there must be a greater contribution of neogenesis. When replication contributes more cells than can be accounted for by the rate of increase in cell number, then there must be greater cell death (Fig. 4). However, because we have no information that allows us to determine the separate rates of neogenesis or
cell death, the predictions of neogenesis and cell death will only reflect the lower limits of the true rates of neogenesis and cell death.

Quite surprisingly, our analysis as seen in Fig. 4 predicted a "wave of β-cell death" in the neonatal period ending shortly before weaning; this prediction was unexpected and not easily accepted by us. To evaluate the likelihood of this occurrence, we analyzed numerous slides of control neonatal rat pancreas from birth to 4 weeks of age from our previous studies, looking for any evidence of apoptotic bodies. Since apoptosis is thought to be very rapid and morphological characteristics are evident for <3 h (maybe for <1 h) (25), the detection of any apoptotic bodies would suggest a significant occurrence of apoptosis. In most slides of 9- to 16-day pancreas, we found a number of apoptotic bodies in islets, in exocrine tissue, and even in ductules; fewer or no apoptotic bodies were seen in pancreas from 0- to 8- or 17- to 26-day-old rats. At 9 days of age, apoptotic bodies in β-cells were found at the ultrastructural level (Fig. 5). Interestingly, Kaung (12) calculated a loss of β-cells in the neonatal period; however, she found no evidence of cell death and thus hypothesized that dedifferentiation or transdifferentiation may occur. In our study, apoptotic bodies are seen in all the pancreatic components. We speculate that during this neonatal period there is a remodeling of the pancreas that is not specific for islets. We can find a similar suggestion in the well-documented discordance of growth of pancreatic weight and body weight during the neonatal period.
period (Table 1). Considerable cell death was seen as a part of normal development in both the central nervous system (50%) (26) and the kidney (3%) (25), leading to the suggestion that the extent of normal cell death in developing animals has been greatly underestimated (25). It is unclear whether such cell death is a reflection of tissue remodeling or of competition for a limited supply of growth or survival factors.

Another interesting aspect of the predictions in Fig. 4 is that neogenesis is significant in the postweaning stage. With our scanning of neonatal pancreas sections mentioned above, we often found ducts with a number of small buds that immunostained for islet hormones near the time of weaning (Fig. 6A). Islet neogenesis has been suggested to occur in the normal postnatal rodent (27), but it was not clearly shown. However, it has been shown after a number of experimental manipulations, such as dietary treatment with soybean trypsin inhibitor (28), overexpression of interferon-γ in the β-cells of transgenic mice (29), and cellophane wrapping of the head of the pancreas (30). A further layer of complexity may be that during normal growth, new lobes of pancreas (exocrine and endocrine) are added, as seen in some experimental situations (3). Such a continual addition of pancreatic lobes is suggested by our observation in retired breeders (6 months old) of lobes with high BrdU incorporation (Fig. 6B) when the rest of the pancreas has almost no BrdU incorporation. If new lobes of pancreas continue to form in adult animals, then the observation of marked differences between lobes in the occurrence of atrophic islets and insulitis in new-onset insulin-dependent diabetes mellitus (31,32) may have new interpretations. A difference in age of pancreatic lobes could conceivably affect the course of the autoimmunity and the pancreatic response to it, but a clear understanding of this possibility must await the definition of markers for the age of pancreatic cells.

Our approach for estimating neogenesis and cell death required several assumptions that could potentially affect the model predictions. Our use of an exponential fall to describe the replication rate as a function of time (Fig. 1) does not completely describe the dynamics observed by Kaung (12). The curve fit in the prenatal period appears poor because of the highly variable observations made in the different studies. Note, however, that this lack of fit has little effect on the predictions of net neogenesis or cell death shown in Fig. 4. During the prenatal period, neogenesis remains the dominant mechanism contributing to new cell formation. If the replication rate were higher than that estimated with our fit, then neogenesis would fall more rapidly and vice versa. Also, the higher the replication rate during the immediate postnatal period, the greater the subsequent rate of cell death. The prediction that the wave of cell death occurs, however, remains the same.

For the data depicted in Fig. 2, we assumed a specific

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functional form between β-cell volume and age; however, data on which to base this assumption were few. The data strongly suggest that the average cell size remained constant at ~1,020 μm³ in the adult rat but was only half that at early neonatal ages. While it is unlikely that these cells have a linear growth curve from 9 to 21 days of age, linearity is the simplest assumption one can make. We can determine the upper and lower limits of the effects of this assumption by using a constant volume set at either 510 or 1,020 μm³ to generate the model predictions. These alterations in the model calculations do not appear to affect the basic dynamic pattern of neogenesis or cell death. There continues to be a wave of cell death from 2 to 18 days of age, followed by a new wave of neogenesis starting from 19 days. However, the β-cell volume has a significant effect on the amplitude of these waves. If the cell size were underestimated in our assumption, then both the number of new cells formed before 2 days and the number of cells dying between 2 and 18 days would be less than predicted. Likewise, if the cell size were overestimated, then a larger number of cells must have contributed to neogenesis and cell death to account for the known rate of mass growth.

Thus, the assumptions incorporated into the estimation of β-cell replication rates and β-cell volume have only minor effects on the basic dynamic patterns predicted for neogenesis and cell death. This suggests that the dynamics are dictated by the apparent slowing of the rate of growth of the β-cell mass between 5 and 20 days of age (Fig. 3). When we fitted the β-cell mass data before 100 days with a simple linear model, the resultant predictions were that neogenesis fell to a low basal rate at 10 days and cell death increased gradually to levels approaching the replication rate: there was no "wave of cell death" before weaning. However, as discussed above, the apparent plateau of β-cell growth before weaning is likely to be real because it is consistent with the growth of the whole pancreas relative to the body and is shown by several data points. In addition, we observed apoptotic bodies during this time period, which is consistent with increased cell death.

ESTIMATION OF THE LIFE SPAN OF THE β-CELL
Since the above model provides estimates of the total rate of appearance of new cells and the disappearance of dying cells on any given day, we can theoretically estimate the average age of the cells present each day, as well as the age of the oldest cells on that day. This calculation requires additional knowledge regarding the rate of a given cell and the population of cells that die. While it is reasonable to assume that the age of a new cell formed by neogenesis is counted from the day it contributes to the β-cell mass, how does one count the age of a cell formed through replication? Are both daughter cells considered new cells, or should their age be based on the age of their parent cell? Which cells die? Do only the oldest cells die, or once a cell reaches a certain age does it enter a pool of cells that are eligible for cell death? Are all cells, regardless of their age, equally eligible for cell death? Does the particular location of a cell within an islet (central versus more peripheral) or within a lobe influence its likelihood for cell death? Does one population of β-cells have a rapid turnover while another has an extremely slow turnover? In the absence of clear answers to these questions, we estimated the cell age using assumptions that most simplified the calculation, i.e., that the oldest cells die first and that after replication, one daughter cell retains the age of the parent cell and one is considered new. The estimated life span of the β-cell is highly dependent on the replication rate in the adult. The graph was generated using Eq. 1, but because the data on replication from 50 to 100 days were between 1 and 4%, the life span of the rat β-cell is likely to be >1 month but <3 months.

Figure 7 illustrates the average age of the cells on a given day and the age of the oldest cell on each day. Although we do not yet know the most appropriate way to estimate cell age, we can determine the effects of our other assumptions on the life span estimates that were made. We found that changing cell volume did not affect the estimated life span of the β-cells in the adult rat but that the functional form of the replication rate had a significant impact. The higher the replication rate, the greater the turnover of cells must be to maintain the β-cell mass, resulting in a shorter estimated life span. If the replication rate approached a constant level of 4% per day in the adult, the oldest β-cells would be only 26 days old. If, however, the steady-state replication rate were 3%, the life span would extend to 35 days; if only 2%, the life span would be 52 days; if only 1%, 103 days. Thus, the estimated life span is highly dependent on the replication rate of the β-cell in the adult animal. Because the data on replication from 50 to 100 days (Fig. 1) are between 1 and 4%, the life span of the rat β-cell is likely to be >1 month but <3 months.

FURTHER CONSIDERATIONS
A mathematical model was constructed using the data presently available that relate to the dynamics of β-cell mass in the normal growing rat pancreas. The novel predictions resulting from this model were that a period of β-cell death or loss occurs during postnatal development and is followed by a new wave of neogenesis in the immediate postweanling period. Although complete validation of the model predictions will require extensive new experiments, we have been
able to provide some evidence in support of its predictions. These predictions concerning neogenesis, cell death, and estimated life span should generate new studies and eventually new understanding of the life history of a pancreatic β-cell. Furthermore, this model should be useful in estimating the mechanisms normally involved in the dynamics of the β-cell mass in experimental and pathological conditions.

Perhaps the most important contribution of this model is to raise a number of unanswered questions concerning the control of the balance of cell death and cell renewal in the endocrine pancreas. There are questions related to whether there is a limitation to the capacity for growth, whether the life span of β-cells can be altered, and which cells undergo cell death. Experimental evidence over the years has suggested a limitation (1,83), but the nature and extent of such a limitation are unclear. In most systems, the number of replications a differentiated cell can undergo is finite (34), but is that true for precursor cells? Is there a population of β-cells that selectively replicates? Brele et al. (35) have shown that nondividing β-cells can be recruited into the cell cycle and that most of the daughter cells will immediately reenter the cycle. Does this population change with age or with location within the islet? While presumably both environmental and genetic parameters are involved, the parameters that affect the differentiation, replication, and life span of the β-cells have not been defined yet. One of the few studies that have examined such questions found that the proliferative response to prolonged hyperglycemia may be genetically determined. The proliferative rate of islet cells of C57 BL/6J mice was about twice that of the closely related C57 BL/KsJ mice, irrespective of age or glucose concentration (36). As of yet, the genes involved in this difference are totally unknown.

ACKNOWLEDGMENTS

This work was supported by National Institutes of Health Grants DK-35449, DK-44523, and DK-38636. D.F. is a scholar of the Alberta Heritage Foundation for Medical Research and a scientist of the Medical Research Council of Canada. L.S. is a recipient of a postdoctoral fellowship from the Juvenile Diabetes International Foundation.

We thank Dr. Gordon C. Weir for helpful discussions, C.J. Cahill for expert technical assistance, and R.E. Champagnie for secretarial assistance.

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