The Role of Glucose in the In Vitro Regulation of Cell Cycle Kinetics and Proliferation of Fetal Pancreatic B-Cells

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SUMMARY

To study the cell cycle and regulation by glucose of B-cell proliferation, B-cell-rich pancreatic islets of rat fetuses (22 days gestational age) were maintained in tissue culture at various glucose concentrations. The proliferating islet cells were synchronized with hydroxyurea and their rate of progress through the cell cycle studied by pulse labeling with ^{3}H-thymidine and after exposure to colchicine.

The time for a full cell cycle was estimated to 14.9 h and could be subdivided into a G_1 phase of a duration of 2.5 h, an S phase of 6.4 h, a G_2 phase of 5.5 h, and an M phase of 0.5 h. Although glucose significantly stimulated B-cell proliferation, the progression of cells through the cell cycle was similar at different glucose concentrations. The experiments furthermore suggested that only a limited pool of islet cells was able to proliferate. Glucose seemed to stimulate B-cell proliferation by increasing the number of cells entering the cell cycle.

From the cell cycle data it was possible to calculate the rate of formation of new B-cells, which ranged from 4.2%/24 h in 2.7 mM glucose to 10.4%/24 h in 16.7 mM. When the accumulation of mitotic figures during colchicine treatment was used as an alternative method for estimation of newly formed B-cells the cell birth rates were found to be 3.1 and 6.0%/24 h at 2.7 and 16.7 mM glucose, respectively.

The notion that only a small fraction of B-cells takes part in proliferation would explain the limited regenerative capacity of this cell type. It remains to be established whether such a limitation is of significance in the development of maturity-onset diabetes.

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It is now well established that a diet rich in carbohydrates causes growth of the islet cell mass.[1–3] It has furthermore been shown that glucose stimulates DNA replication in the pancreatic B-cell in vitro.[4–6] B-cell growth may therefore be one mechanism by which the islet organ can increase its insulin output in response to a lasting hyperglycemic stimulus. This ability may indeed determine the development and course of diabetes mellitus since diabetogenic factors leading to B-cell degeneration or peripheral insulin resistance could be compensated for by multiplication of B-cells. Little is, however, known about the mechanism by which the individual B-cell responds with proliferation to glucose. An analysis of the effects of glucose on the division cycle of the B-cell should therefore increase our understanding about the regulation of the growth of the islet organ.

Experimental studies have shown that the autoradiographic labeling index of adult pancreatic islets in vitro is low[5,7] and it is conceivable that the mitotic index is even lower. In cell cycle kinetics, the counting of labeled mitotic figures after a ^{3}H-thymidine pulse[8,9] has been the method most widely used. This approach, however, requires the presence of numerous mitotic figures and, therefore, few attempts to analyze the islet cell cycle in vivo have been performed.[10,11] The present study is an attempt to overcome the technical problems by studying the cell cycle parameters after synchronization of the proliferating cells and by using a fetal rat islet preparation with a high proportion of B-cells and high proliferative capacity.[6,12]

MATERIALS AND METHODS

Preparation and culture of fetal rat islets. Fetal rat islets were prepared as previously described in detail.[13,6] Briefly, pancreatic glands of 22-day-old Sprague-Dawley rat fetuses were chopped up and digested for a short time with collagenase. The carefully washed digest was distributed in 50-mm culture dishes allowing cell attachment (NUNC, Roskilde, Denmark) and cultured in 5 ml RPMI 1640 (Flow Laboratories, Irvine, Scotland) containing 11.1 mM glucose.

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The medium was supplemented with 20 mM N-hydroxyethyl-piperazine-N'-2-ethane sulphonic acid (Hepes, Sigma, St. Louis, Missouri), antibiotics (penicillin 100 U/ml and streptomycin 0.1 mg/ml) and 10% heat-inactivated fetal calf serum (Flow). The cultures were continued for 5 days at 37°C in a humidified atmosphere of 5% CO_2 in air, with daily changes of culture medium. At the end of this period, B-cell-rich islets devoid of nonendocrine tissue could be picked with the aid of a braking pipette.

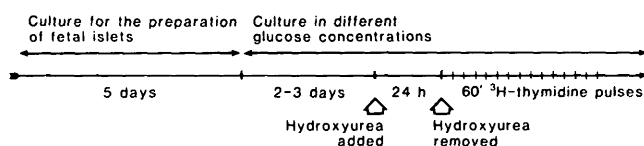
The islets were subsequently transferred to nonattachment culture dishes (Heger, Stallarholmen, Sweden) and the cultures continued in the same medium but with different glucose concentrations as described below. In the following the terms 'islet culture' and 'cultured islets' refer to these latter cultures while 'preparatory islet culture' refers to the initial culture period described above.

Synchronization of the islet cell cycle. A schematic outline of the procedure for islet cell culture is given in Figure 1. Before the islet cell cycle was synchronized, the islets were cultured for 2–3 days in RPMI 1640 containing 2.7, 5.5, or 16.7 mM glucose, with a change of medium after 2 days. Each of the glucose concentrations was subsequently maintained throughout any one experiment. Islets cultured in media containing 2.7 and 16.7 mM glucose were always run in parallel. To make the treatment as uniform as possible, batches of 100–400 islets intended for synchronization were collected in a single culture dish. To synchronize the islet cell cycle the islets were exposed to culture media containing 12.5 mM hydroxyurea (Sigma) for 24 h. This substance causes intracellular depletion of deoxyribonucleotides necessary for DNA replication and proliferating cells are collected at the G_1/S boundary.[14] The hydroxyurea was removed by carefully rinsing the islets five times in culture medium before continuation of the tissue culture. Every hour after the release of the islets from the hydroxyurea block, a batch of about 25 islets was withdrawn and incubated for 60 min in a culture medium containing 10 μCi/ml ^{3}H-thymidine (The Radiochemical Centre, Amersham, United Kingdom; specific activity 5 Ci/mmol). After the radioactive pulse the islets were rinsed in Hanks' balanced solution (HBSS; Statens Bakteriologiska Laboratorium, Stockholm, Sweden) and processed either for autoradiography or for quantitative determination of the radioactivity incorporated into islet DNA, as described below in detail.

Unsynchronized islets cultured for 3 days in 2.7, 5.5, or 16.7 mM glucose were similarly incubated for 60 min in ^{3}H-thymidine. Further controls consisted of islets synchronized for 24 h with hydroxyurea, but not released from the block prior to the ^{3}H-thymidine pulse.

In some experiments, immediately after removal of the hydroxyurea, colchicine (Sigma) was added to the cultures to give a concentration of 0.1 mM. After histologic processing the accumulation of mitotic figures was determined.

FIGURE 1. Schematic outline of the experimental procedure.



Quantitative estimation of DNA synthesis. To determine quantitatively the rate of DNA synthesis the group of islets, which had been exposed to 60-min pulses of ^{3}H-thymidine, were sonicated in 250 μl distilled water. Two 50-μl aliquots of the homogenates were precipitated in 500 μl ice-cold 5% trichloroacetic acid (TCA) and the precipitates were collected by filtration through a glass fiber disc (Whatman GF/A 2.5 cm; Labora, Stockholm, Sweden). Any remaining free isotope was removed by washing with distilled water. The filters were dried and the radioactivity in the precipitates determined by liquid scintillation counting. Another 50-μl duplicate of the homogenates was assayed for DNA content[15,16] and the incorporation of ^{3}H-thymidine expressed as cpm/μg islet DNA.

Autoradiography. Islets intended for autoradiography were fixed in Bouin's solution after the ^{3}H-thymidine pulse. They were then dehydrated, embedded in paraffin, and sectioned at 7 μm. After removal of the paraffin the slides were dipped in Ilford K2 emulsion (diluted 1:1 in distilled water) and exposed at 4°C for 1 wk. They were then developed in a Kodak D-19 developer for 5 min and fixed for 10 min in Kodak F-24. The slides were dried overnight, counterstained with hematoxylin, and mounted.

To determine the labeling index (LI), labeled islet cell nuclei were counted using an oil immersion lens (final magnification 1250×) and expressed as a percentage of the total number of nuclei scored. In each experiment, 500–2000 nuclei in at least 10 islet sections were scored. The accumulated mitotic index (MI) was determined in a corresponding way by counting the number of mitotic figures in islet sections stained with hematoxylin and eosin.

Stathmokinetic experiments. Islets intended for stathmokinetic experiments (i.e., determinations of cell birth rates by estimations of the rates of accumulation of mitotic figures) were cultured for 3 days in medium containing 2.7, 5.5, or 16.7 mM glucose. Colchicine was then added to the medium to give a final concentration of 0.1 mM and the cultures continued. After 2, 4, and 6 h, batches of islets were removed and immediately fixed in Bouin's solution. They were subsequently processed for histology and stained with hematoxylin and eosin. The accumulated number of mitotic figures was scored in at least 500 cells in at least 10 islet sections and expressed as a percentage of the total number of nuclei scored. For each glucose concentration the average mitotic indices of the different time points were used to construct a least square regression line. The slopes of the fitted lines were then used to express the islet cell birth rates, or the number of new islet cells formed per hour.

Calculations of islet cell proliferation. When the labeling index of the unsynchronized islets (LI), the duration of the DNA synthetic phase (S phase duration; S_t) and the duration of a full cell cycle (generation time; GT) had been determined graphically (see below) the following formulas were applied. These formulas should preferably be used in situations when steady state is maintained (i.e., the number of cells lost and produced are equal). However, since the formation of new cells is less than 10%/24 h (see RESULTS) and the exact cell loss unknown this mode of calculation provides proliferative parameters which can be assumed to be valid approximations.

If the fraction of the entire cell population which is progressing through the cell cycle at a given moment is designated as the proliferative compartment (PC; i.e., the

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fraction of actively proliferating cells), it is given by the following formula:

$$PC = \frac{GT}{S_t} \cdot LI \quad (1)$$

Since the GT is known, the cell birth rate (CBR; i.e., the production of new cells per 24 h) expressed as a fraction of the total islet cell population, can be calculated as

$$CBR = PC \cdot \frac{24}{GT} \quad (2)$$

assuming that every full cell cycle added one cell to the total population. If PC in (2) is substituted for the right side of (1), the formula

$$CBR = \frac{24}{S_t} \cdot LI \quad (3)$$

is obtained, permitting the calculation of the CBR without a previous estimate of GT.

RESULTS

Experiments with unsynchronized islets. The labeling index of unsynchronized islets was stimulated by glucose in a dose-dependent manner (Table 1). A 2.5-fold increase was observed from 2.7 to 16.7 mM glucose. Furthermore, the increase was more pronounced between 5.5 and 16.7 mM than between 2.7 and 5.5 mM glucose. When the DNA synthesis was measured as radioactivity incorporated into TCA-precipitable islet material a similar result was obtained, although the stimulation caused by 16.7 mM glucose was only 1.7-fold.

Cell cycle experiments. When islets were incubated with ^{3}H-thymidine during the hydroxyurea block labeled nuclei were found only very rarely in the autoradiographs. This finding was confirmed by the very low incorporation of isotope into islet DNA, when measured by liquid scintillation (Table 1).

When islets, which had been first cultured and exposed to hydroxyurea in medium containing 5.5 mM glucose, were subsequently pulse labeled with ^{3}H-thymidine, the DNA synthesis was low during the first hour. A wave of DNA synthesis then occurred and lasted for about 9 h (Figures 2 and 3). The DNA synthetic waves reached peak values more than twofold higher than those observed in unsynchronized islets cultured in the same glucose concentration (Table 1). The peak obtained in the autoradiographic experiments (Figure 2) was wider and more plateau-like than the one generated in the biochemical determinations of DNA synthesis (Figure 3). A subjective assessment of the degree of autoradiographic labeling furthermore suggested that the nuclei at the beginning and end of the plateau were not as heavily labeled as those scored in its middle parts. As seen in Figure 3 the ^{3}H-thymidine incorporation decreases to reach a nadir after 13–15 h. Subsequently, the DNA synthesis increased again and reached a new plateau 22–24 h after release from the hydroxyurea block, at a level only slightly above the one of the unsynchronized islets.

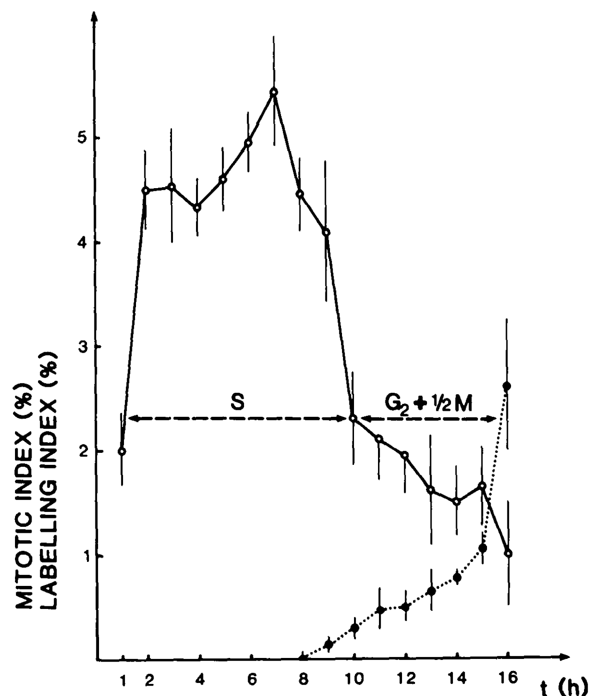
To estimate the approximate length of the DNA synthetic wave (average S_t) the height of its plateau was calculated. In the autoradiographic series (Figure 2), the plateau lasted between the 2nd and 9th hour after release and the average height was 4.6%. In the biochemical determinations (Figure 3), the two highest incorporation values occurred at the 5th and 7th hours, the average being estimated to 1737 cpm/μg

TABLE 1
Incorporation of tritiated thymidine into fetal rat islets cultured for 3 days in medium RPMI 1640 containing 2.7, 5.5, or 16.7 mM glucose and labeled for 60 min with ^{3}H-thymidine

	Unsynchronized islets		Hydroxyurea block
Glucose concentration (mM)	Labeling index (%)	Thymidine incorporation (cpm/μg DNA)	Thymidine incorporation (cpm/μg DNA
2.7	1.13 ± 0.04 (5)	523 ± 108 (6)	100 ± 15 (14)
5.5	1.70 ± 0.10* (7)	643 ± 70 (15)	89 ± 11 (11)
16.7	2.78 ± 0.09*‡ (5)	888 ± 55† (6)	78 ± 15 (11)

Thymidine incorporation was assessed as autoradiographic labeling index (left column) and as radioactivity incorporated into TCA-precipitable material (middle column). The thymidine incorporation into DNA after exposure for 24 h to 12.5 mM hydroxyurea (right column) was measured as radioactivity incorporated into TCA-precipitable material. The values are given as means ± SEM for the number of observations shown in parentheses. Probability of chance difference between values obtained at 2.7 mM glucose and those obtained at either 5.5 or 16.7 mM glucose: * $P < 0.001$; † $P < 0.05$. Probability of chance difference between values obtained at 5.5 and at 16.7 mM glucose: ‡ $P < 0.001$.

FIGURE 2. DNA synthesis in fetal rat islets cultured in medium RPMI 1640 containing 5.5 mM glucose after synchronization with hydroxyurea. Cultures were continued in medium containing 0.1 mM colchicine and batches of islets were withdrawn with 1-h intervals and pulsed with ^{3}H-thymidine for 60 min. After processing the islets for autoradiography, the labeling index (○—○) and the accumulated mitotic index (●······●) were determined. The values are given as means ± SEM for three observations. The S phase duration (S) and the duration of the G_2 phase and half the mitosis (G_2 ± ½M) are indicated in the figure and the measurements described in detail in MATERIALS AND METHODS.



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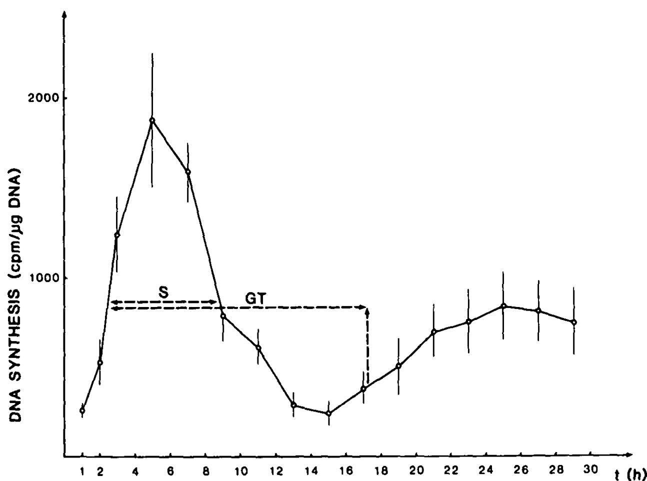

FIGURE 3. DNA synthesis in fetal rat islets, cultured in medium RPMI 1640 containing 5.5 mM glucose, after synchronization of the islet cell cycle with hydroxyurea. Cultures were continued in the same medium and batches of islets were withdrawn with 2-h intervals and pulsed with ^{3}H-thymidine for 60 min. DNA synthesis was subsequently determined as radioactivity incorporated into TCA-precipitable material. Values are given as means ± SEM for 5–8 observations. The S phase duration (S) and the generation time (GT) are indicated in the figure and the measurements described in detail in MATERIALS AND METHODS.

DNA. The average S phase duration was then estimated as the distance between the ascending and the descending arm of the first DNA synthetic wave at a height of 50% of the average plateau height. This yielded an S phase duration of 8.8 h in the autoradiographic series of experiments and 6.4 h in the biochemically analyzed series. The mean generation time (GT) was determined as the distance along the abscissa from the 50% plateau level of the ascending arm of the first DNA synthetic wave to the corresponding position on the ascending arm of the second wave (Figure 3; the average thymidine incorporation during the 23rd to 29th hours was 788 cpm/μg DNA). This measurement yielded a generation time of 14.9 h. Note that in these experiments the baseline incorporation of isotope (Table 1, right column) was disregarded, since it had little influence on the subsequent calculations.

In the autoradiographic series of experiments, as expected, a second wave of DNA synthesis did not occur, since colchicine had been added to the medium at the time of release from the hydroxyurea block (Figure 2). The first accumulated mitotic figures appeared during the 8th hour after release from the block and a slow accumulation ensued for several hours. The mitotic index subsequently doubled between the 15th and 16th hour after release. To estimate the G_2 phase plus half the duration of the mitosis (G_2 + ½M), the horizontal distance between the descending arm of the first DNA synthetic wave and the ascending arm of the graph describing the accumulation of mitotic figures was measured at the estimated 50% plateau level of the first DNA synthetic wave (Figure 2). Thus, a duration of G_2 + ½M of 5.8 h could be recorded. Note that the accumulated mitotic index did not increase beyond the 16th hour. From the 15th hour and onwards, mitotic figures with few and fragmented chromosomes and islet cells with distorted nuclear morphology were indeed observed. These observations would indicate a deficient accumulation of mitotic figures after prolonged exposure to colchicine which could have caused some overestimation of the duration of the G_2 + ½M phases.

When the cell cycle kinetics of synchronized cells maintained in either 2.7 or 16.7 mM glucose were compared (Figure 4), the durations of the various cell cycle events appeared to be similar. Thus, the peak of the first DNA synthetic wave occurred between the 5th and 7th hours after release from the hydroxyurea block in both groups. The lowest rates of DNA synthesis were recorded during the 15th hour after release and subsequently the second DNA synthetic wave followed. At all time points, the thymidine incorporation values were highest in islets cultured at 16.7 mM glucose, reaching significantly elevated levels during the 5th and 7th hours after release.

Stathmokinetics. In the stathmokinetic series of experiments, the islets were exposed to colchicine for up to 6 h. When the accumulated mitotic indices were scored fragmented mitotic figures or other disturbances of islet nuclear morphology were not observed, presumably because of the limited exposure time to the drug.

The accumulation of mitotic figures (i.e., the cell birth rate, CBR) in islets cultured at different glucose concentrations and treated with colchicine is depicted in Figure 5. The slopes of all three groups differed from zero ($P < 0.01$)

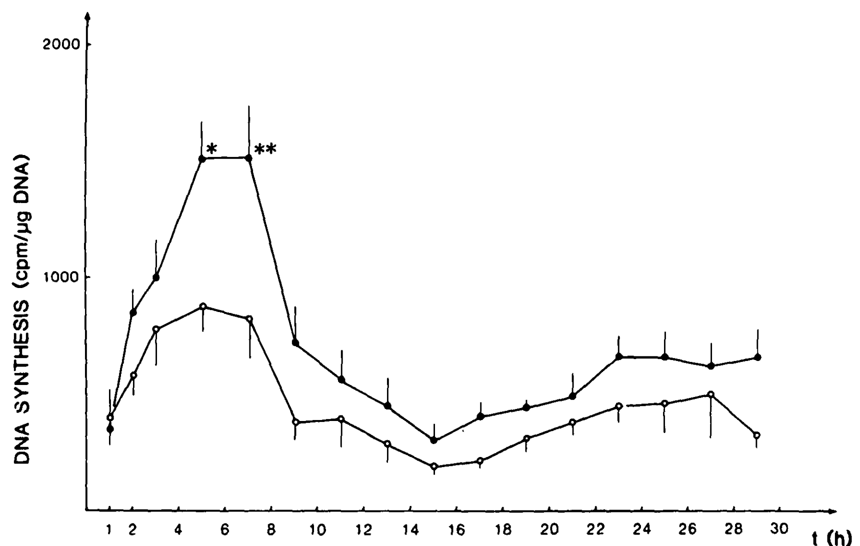

FIGURE 4. DNA synthesis in fetal rat islets, cultured in medium RPMI 1640 containing either 2.7 (○—○) or 16.7 (●—●) mM glucose, after synchronization of the islet cell cycle. Cultures were continued in the same media and batches of islets withdrawn with 2-h intervals and pulsed with ^{3}H-thymidine for 60 min. The DNA synthesis was subsequently determined as radioactivity incorporated into TCA-precipitable material. The values are given as means ± SEM for 5–8 observations. Probability of chance difference between values obtained at 2.7 and 16.7 mM glucose: * $P < 0.05$, ** $P < 0.01$.

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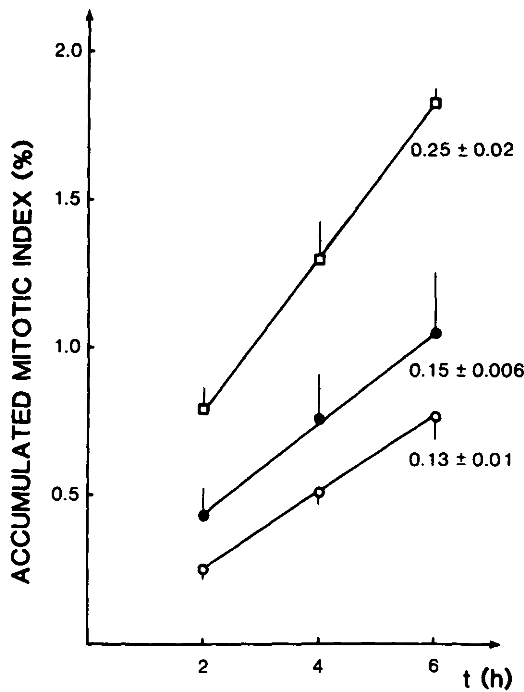

FIGURE 5. Accumulation of mitotic figures in cultured fetal rat islets. After 3 days of culture in medium RPMI 1640 containing 2.7 (○), 5.5 (●) or 16.7 (□) mM glucose colchicine was added to the cultures to a concentration of 0.1 mM. Batches of islets were withdrawn and fixed after 2, 4, and 6 h, and the accumulated mitotic index was determined in hematoxylin-eosin stained sections. For each time point and glucose concentration the values are given as means ± SEM for 3-4 observations. The mean mitotic indices of each glucose concentration were used to construct least square regression lines of which the slopes ± SEM are given in the figure.

indicating a significant accumulation of mitotic figures. Islets cultured in 2.7 and 5.5 mM glucose showed similar CBR values, as evidenced by the nearly parallel slopes of their respective regression lines. Culture of islets in 16.7 mM glucose stimulated the accumulation of mitotic figures compared with either of the lower glucose concentrations ($P < 0.01$).

Calculations of islet cell proliferation. The experimental values obtained for S_t, GT, and LI were used to calculate values for PC and CBR at different glucose concentrations (Table 2). As can be seen in Figure 4, the second wave of DNA synthesis in 2.7 mM glucose was low, and the GT could therefore not be measured in this experiment. Since for all the glucose concentrations tested the DNA synthetic peaks and valleys coincided the values for S_t and GT obtained at 5.5 mM glucose (Figure 3) could nevertheless be used as approximations irrespective of the glucose concentration. Of the two series of experiments performed at 5.5 mM glucose, the parameters obtained by biochemical determinations of DNA synthesis were used since both S_t and GT could be measured in this particular series. Since CBR could be calculated without an estimate of GT (eq. 3), it was thus not affected by the above approximations.

As the values for S_t and GT used were the same at all the glucose concentrations PC and CBR varied in direct proportion to the labeling index. It can be seen in Table 2 that the proliferative compartments comprised only a small fraction of the islet cells, with a maximal value of approximately 6% in islets cultured in 16.7 mM glucose. With a proliferative compartment of this size approximately 10 new cells were formed per hundred islet cells and 24 h. By comparison the cell birth rates per 24 h obtained in the stathmokinetic experiments (Table 2) were somewhat lower than the corresponding values calculated from the cell cycle data, reaching 6% in islets cultured in 16.7 mM glucose. Likewise, in these latter experiments the stimulation of cell birth rate by glucose was less pronounced.

DISCUSSION

Islet cell synchronization. The method for islet isolation presently used provides a suitable means for the study of the B-cell cycle since a large number of islets with a high capacity for proliferation can be isolated and maintained in tissue culture.[13,6] Moreover, the high proportion of B-cells (>90%) in these islets[13,17] makes it likely that the results are representative of this cell type. The use of a pure islet preparation also circumvents the problem of an admixture of nonislet cells with a capacity for transforming into B-cells, and thereby increasing the B-cell number without cell divisions. Furthermore, the in vitro technique avoids the effects of diurnal variations in proliferation which are likely to occur in vivo.

In the present investigation, it was possible to synchronize the proliferating islet cells with hydroxyurea as evidenced by the very low thymidine incorporation during the hydroxyurea block and the wave of DNA synthesis occurring after release from the block. The second wave of DNA synthesis was lower and longer than the first one suggesting a partial loss of synchrony. This observation may reflect not

TABLE 2
Calculated cell cycle parameters of fetal rat islets cultured in medium RPMI 1640 containing 2.7, 5.5, or 16.7 mM glucose

Glucose concentration (mM)	Cell cycle analysis					Stathmokinetics	
	S_t (h)	GT (h)	LI (%)	PC (%)	CBR (%/24 h)	slope (%/h)	CBR (%/24 h)
2.7	6.4	14.9	1.13 ± 0.04	2.6	4.2	0.13 ± 0.01	3.1
5.5			1.70 ± 0.10*	4.0	6.4	0.15 ± 0.006	3.6
16.7			2.78 ± 0.09*†	6.5	10.4	0.25 ± 0.02*‡	6.0

Using the experimentally obtained values for S phase duration (S_t), generation time (GT) and labeling index (LI), the fraction of actively proliferating islet cells (proliferative compartment, PC) and the production of new cells (cell birth rate, CBR) were calculated and expressed as a percentage of the total islet cell population (for details, see METHODS). Alternatively the cell birth rates were calculated from the slopes of the fitted lines in the stathmokinetic experiments (Figure 4). The values are given as means ± SEM. Probability of chance difference between values obtained at 2.7 mM glucose and those obtained at either 5.5 or 16.7 mM glucose: * $P < 0.001$. Probability of chance difference between values obtained at 5.5 and 16.7 mM glucose: † $P < 0.001$; ‡ $P < 0.01$.

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only small variations between individual cells in the duration of the cell cycle phases but also the fact that cells blocked within the S phase will, upon release, finish DNA synthesis earlier than the cells collected at the G_1/S boundary. This latter mechanism may be the more significant one, since according to the present observations, the S phase comprised over 40% of the B-cell cycle and a considerable number of cells would therefore be trapped within this phase. The existence of such cells was indicated in the morphologic series of experiments by an early appearance of mitotic figures while most of the synchronized cells still were in late S phase. Moreover, proliferating cells may stop progressing through the cell cycle between the DNA synthetic peaks and hitherto nonproliferating cells may enter the cell cycle at any time during the interval studied. A comparison of the areas under the first and second DNA synthetic waves nevertheless suggests that a majority of the cells that took part in the first peak also proceeded into a second cycle.

An important point is whether the synchronizing procedure interferes with the cell cycle kinetics after removal of the blocking agent. Hydroxyurea does not inhibit RNA and protein biosynthesis and the cells may grow in size during the hydroxyurea block. The subsequent G_1 phase may thereby be shortened since the minimal cellular size necessary for cell division can be reached at an earlier stage.[18] Whereas this is true for some cell types maintained in vitro, differences in generation time were not observed in the present study although high glucose concentrations markedly stimulate growth in size by the B-cell.[19]

The present analysis of the B-cell cycle allows an estimation of the duration of both the GT and the S phases and also, with some approximation, the G_1 + ½M phases. Assuming a mitotic duration of 30 min[20,21] and that the G_1 duration equals the generation time minus the durations of the other phases, a complete mapping of the B-cell cycle can be achieved. This results in a generation time of 14.9 h, a duration of G_1 of 2.5 h, S of 6.4 h and G_2 of 5.5 h. In a previous analysis of the B-cell cycle Bunnag[10] reported a GT of 10.6 h subdivided into a G_1 phase of 2.6 h, S of 5.8 h, G_2 of 1.8 h, and M of 0.4 h. Although this study was performed in vivo and on mice the results on the whole correspond to the present findings, the only difference being the longer G_2 phase in the present work. Furthermore, the finding of Bunnag[10] that the S phase comprises a considerable part of the generation time is confirmed, a notion of importance for the subsequent calculations of proliferative compartments and cell birth rates.

The calculations of the proliferative cell compartments give the number of B-cells progressing through the cell cycle at any given moment. It cannot, however, be inferred from this figure whether the proliferating cells originate from a common small pool or whether cells enter the cycle at random and undergo divisions more infrequently. The present investigation nevertheless supports the view that only a minor part of the islet cells take part in proliferation. Thus, there was a very low rate of thymidine incorporation after the first DNA synthetic peak. If proliferation of the islet cells were due to cells entering the cycle randomly this nadir would not have occurred, since cells would be expected to initiate DNA synthesis at all times during the interval studied independently of the previous block. Furthermore, the appearance of a second wave of DNA synthesis indicates that cells taking part in the first wave directly proceeded into a second cell division cycle. This notion is supported by the findings of Bunnag[10] who also demonstrated a low nadir and a prominent second wave when determining the fraction of labeled mitotic figures after a ^{3}H-thymidine pulse in vivo. Altogether the present data suggest that proliferation of B-cells occurs in a common small pool of cells which may comprise some 10% of the total B-cell population.

Regulation of islet cell proliferation. When adult rats are injected with ^{3}H-thymidine, labeled islet cells are retained over long periods of time indicating that the islet cell population is not undergoing continuous renewal.[22,23] Islet cell proliferation nevertheless expands the islet mass[24,25] and the B-cells increase in number also in the adult rat, although at a slow rate.[26] In the present investigation the cell birth rates at a physiologic glucose concentration (5.5 mM) were estimated as approximately 4% and 6% per 24 h, using a stathmokinetic technique and cell cycle analysis, respectively. The difference between the two estimates may reflect partly an overestimate of the labeling index due to the fact that the 1-h pulse period was not negligibly short in comparison to the S phase duration. Moreover, loss of mitotic cells ('mitotic detachment') from the surface of the islets may have retarded the accumulation of mitotic figures in the stathmokinetic experiments.[27] The cell birth rates presently observed in fetal rat islets nevertheless appear sufficient to cause a rapid increase in islet cell number. If cell division is to continue in the adult rat without considerable simultaneous cell loss the cell birth rate must decrease markedly when the animal approaches maturity.

The present results suggest that glucose controls B-cell proliferation by regulating the number of cells which enter the cell division cycle. The cell cycle then proceeds in a uniform way irrespective of the glucose concentration. Although glucose-stimulated growth of the B-cell mass represents a mechanism to meet an increased insulin demand, this mechanism appears deficient in cases of maturity-onset diabetes.[28] In this context, the present hypothesis suggesting that only a limited fraction of the islet cells takes part in proliferation is to be noted. It may be that the majority of the islet cells have entered an irreversible G_0 phase. The growth capacity of the islet organ would then be dependent on not only the growth stimulus itself, but also on the number of cells capable of cell division. If the number of such cells is low, a strong stimulus for B-cell division, such as hyperglycemia, may not be sufficient to cause an expansion of the islet cell mass capable of controlling glucose homeostasis.

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