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Review article

The life story of the pancreatic B cell*

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Summary. Most research on the pancreatic B cell has so far focussed on the regulation and molecular biology of insulin biosynthesis and release. The present review draws attention to some additional areas of islet research which have become accessible to investigation by recent methodological progress and which may advance our understanding of the role of the B cell in diabetes. There is now evidence to suggest that B cells arise from a pool of undifferentiated precursor cells in the fetal and newborn pancreas. These cells may contribute to islet growth and, if inappropriately stimulated, also to early islet hyperplasia. In the postnatal state, B-cell function is finely tuned by a complex set of incoming signals, one of which is the nutrient supply provided by the blood. Recent studies indicate that a disproportionately high fraction of pancreatic blood is diverted to the islets and that the islet blood flow is increased by glucose. An acute stimulus to insulin release may thus be accompanied by a process which enhances the distribution of the hormone to the target cells. Long-term adjustments of B-cell function are made by changes in B-cell number and total mass. Adaptive growth responses to an increased insulin demand occur in a number of hereditary diabetic syndromes in animals, but in some of these there is an inherited restriction on the capacity for B-cell proliferation leading to further deterioration of the glucose tolerance. Some evidence suggests that a similar mechanism may operate also in human non-insulin-dependent diabetes. A critical appraisal of this hypothesis requires, however, that human B cells should be tested for their growth characteristics. Studies of B-cell proliferation in experimental animals have shown that the B cell passes through the cell cycle at a relatively high rate but that the fraction of proliferating cells is low. Regulation of growth of the total B-cell mass seems to take place by changes in the number of B cells passing through the cell cycle rather than by changes in the rate of the cycle. The number of proliferating B cells also shows a marked decrease with age. It is at present uncertain to what extent these regulatory mechanisms apply also to the human B cell but it can be anticipated that further technical developments will elucidate this problem.

Key words: Pancreatic B cell, pancreatic islet, B-cell differentiation, islet blood flow, B-cell proliferation, B-cell cycle, hereditary diabetes.

It is generally agreed that the pancreatic B cell plays a key rôle in the aetiology of diabetes mellitus. Insufficient production of biologically active insulin is a common denominator in almost all forms of diabetes and the degree of insulin deficiency determines both the severity of the disease and the choice of therapy. This has stimulated worldwide research efforts to elucidate the function and natural history of the B cell, leading to spectacular advancements in the basic understanding of this cell. It is not surprising that most research has been concerned with the regulation and molecular biology of insulin biosynthesis and release, and that other aspects of islet histophysiology have remained relatively neglected, despite their potential importance for a full understanding of the role of the B cell in diabetes. This, no doubt, has been due to lack of suitable techniques for investigating the islet organ, with its peculiar distribution into thousands of small cell aggregates amounting to only 1% of the total pancreatic weight. The purpose of this review is to draw attention to some areas of islet research which have been made more accessible to investigation by recent technical developments and which may have important implications for our understanding of B-cell function in health and disease.

The concept of the B cell was born in 1907 when Lane, while working in Professor R. R. Bensley's laboratory, defined and described both the A and B cell of the islets [1]. Originally the B cell (or β cell as it was called by Lane) was characterized by its content of cytoplas-

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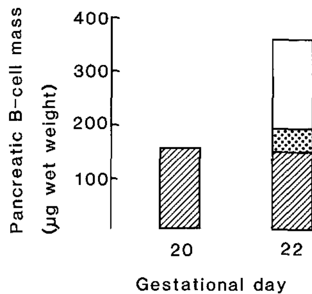

Fig. 1. Pancreatic B-cell mass in the fetal rat pancreas on gestational days 20 and 22. The stippled part of the right bar represents the calculated growth accounted for by the formation of new B cells from pre-existing B cells. The unfilled part of the bar represents growth by mechanisms other than B-cell division, for example differentiation and proliferation of precursor cells. Data from [10, 14]

mic granules, which were soluble in alcohol but preserved in tissues fixed in chrome-sublimate and thus bestowed the cell with specific staining characteristics [1, 2]. At that time nothing was known of the hormone-producing capability of the B cell, but there was nevertheless morphological evidence that this cell type would somehow be involved in the development of diabetes [3, 4]. Today the B cell is defined not only by its histological or cytochemical staining characteristics, but also by its ability to express a complicated set of genes which provide the cell with a unique mechanism to synthesize and store insulin and to release the hormone in exact concert with the peripheral demand. The B cell may also express on its plasma membrane certain antigens which are specific for this cell type and which may lead to autoimmune reactions [5–8]. Each of these properties serves to distinguish the B cell from other cells of the body and may be specifically involved in the pathogenesis of diabetes.

Differentiation of the B cell

Knowledge of the embryonic origin of the B cell is not only of academic interest but can, as we shall see, provide us with important information on possible causes of B-cell inadequacy, or even hyperfunction, later in life. Of central interest in this context is the question as to the possible existence in the embryonic pancreas of a pool of precursor cells which are committed to develop into B cells. Such a pool should have a higher capacity for cell division than the differentiated B cell and should therefore fulfil the purpose of meeting an increased insulin demand by a rapid expansion of the B-cell mass, as is the case, for example, in late fetal and early neonatal life [9, 10]. If, on the other hand, such committed cells were stimulated to divide and differentiate at an inappropriately high rate, then a state of hyperinsulinism might ensue as in the fetus of the diabetic mother [11, 12], or, perhaps in the newborn with nesidioblastosis [13]. It is unfortunate that methods are still not available to identify directly an apparently non-differentiated precursor cell, committed to become a B cell. Indirect evidence nevertheless strongly suggests the existence of such a precursor pool, at least in the fetal pancreas. In the rat fetus the growth of the B-cell mass between gestational days 20 and 22 is considerable, with a total increase of more than 100% in 48 h (Fig. 1). However, growth that can be accounted for by the formation of new B cells from pre-existing B cells is only 20% and the remaining 80% must be accounted for by mechanisms other than B-cell division [10, 14]. Since enlargement of individual islet cells could hardly contribute to more than a fraction of the total growth, neoformation of such cells from rapidly proliferating, morphologically non-differentiated precursors appears a likely explanation. In further support of this notion, a much higher frequency of DNA-synthesizing cells has been observed in the immediate vicinity of the rapidly growing fetal rat islets than in the islets themselves [15, 16].

Recent evidence suggests that, in the early postnatal period also, differentiation of precursor cells into B cells might contribute to islet growth. It was thus reported that when streptozotocin was injected into 1–2-day-old rats, the ensuing hyperglycaemia was only transient and was completely reversed by the fourteenth postnatal day [17, 18]. These changes were accompanied by marked initial destruction and loss of B cells followed by active repair [19–21]. The latter process was characterized by the appearance of numerous insulin-positive cells throughout the exocrine parenchyma and in the duct epithelium. Budding of islets from ducts was a prominent feature. There was, however, a low mitotic index in the islets while mitoses were frequent in the non-endocrine pancreatic part including the duct epithelium. Altogether these observations suggest a rapid formation of B cells, primarily through multiplication and differentiation of precursor cells which may be located both in the acinar part and the ducts. It is still unclear whether such – so far putative – precursor cells contribute also to islet growth in the adult animal. The combined data may, nevertheless, be used to propose a pedigree of the B cell (Fig. 2).

The blood supply of the B cell

In its postnatal, mature state each pancreatic islet can be regarded as a small society or ecosystem, whose members are the different islet cells. Within the limits of each of these micro-systems B cells are born, mature, fulfill their insulin production and divide, or age and die. Their professional task, the synthesis, storage and release of insulin, is closely regulated by a complex set of incoming signals which coordinate the cells to release their stored hormone in precise relation to the needs of the body (Fig. 3). Within this system several different modes of signals can be envisaged: nutrient factors and hormones carried via the blood, as well as action potentials and, perhaps, neuropeptides transmitted via

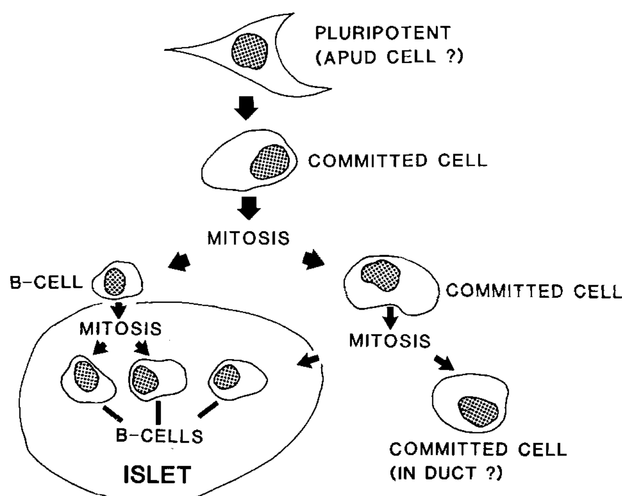

Fig. 2. Schematic view of the differentiation of the B cell. For a discussion of the APUD-cell concept reference should be made to Pearse [22]. Note that a pool of committed cells may exist in the adult pancreas intermingled with the duct epithelium

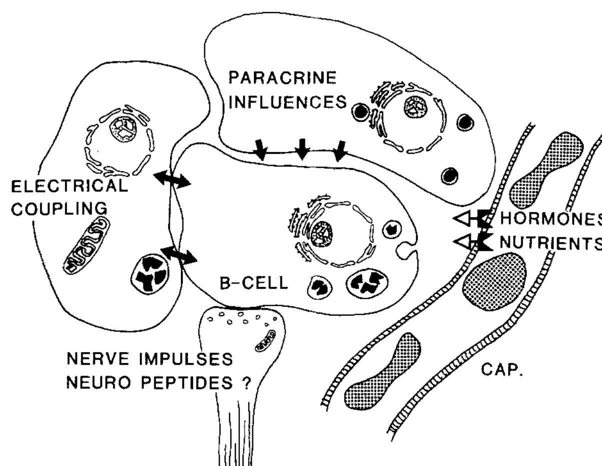

Fig. 3. Scheme of the signal input to the B cell. A capillary (cap) is shown in the right-hand side of the figure

afferent axons [23]. In addition, paracrine influences from neighbouring A and D cells in the islets seem to play a modulating role [24]. Also, the physical association between the B cells themselves may influence the control of insulin release via electrical coupling over intercellular gap junctions [25, 26].

It is conceivable that a major regulating influence is exerted on the B cell by constituents in the blood, and the islet blood flow may be regarded as the main avenue by which the cell is kept informed of the nutritional state of body. Interest in the regulation of the islet blood flow dates back several decades [27–30] but it was not until recently that reliable techniques for a more complete mapping of the structural and functional properties of this important signal system have been worked out. The rich vascular supply of the islets has been known for a long time and an example of its appearance is given in Figure 4. Details of the anatomical arrangement of the islet vasculature have been presented by Thiel [28], Ohtani and Fujita [31] and Bonner-Weir and Orci [32] and provide important information on the intra-islet microcirculation. It is evident from these descriptions that in rat islets the afferent arterioles cross into the islets through discontinuities of the mantle of non-B cells and subsequently form a glomerulus-like capillary network within the B-cell core. This arrangement makes down-stream effects from intra-islet hormones other than insulin on the B cells appear unlikely. Since the efferent capillaries pass through the non-B cells and merge into collecting venules outside the islets, a high insulin concentration in the effluent blood might have a modulating effect on glucagon release from the A cells. Such effects of insulin have previously been demonstrated in vivo [33] and in vitro [34, 35] and may explain the increased circulating glucagon levels seen in insulin deficiency [34].

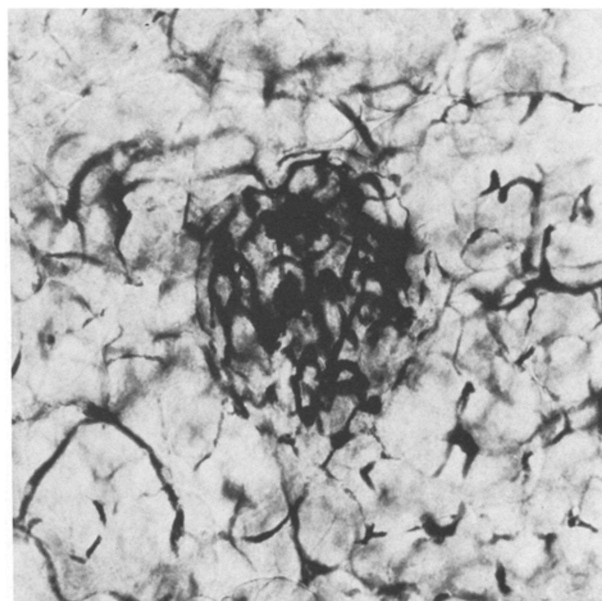

Fig. 4. Capillary network of a rat pancreatic islet (centre) after intra-arterial perfusion with a blackened film emulsion (Kodak NTB-2) diluted 1:3 with phosphate buffer pH 7.4. (Courtesy of Dr Leif Jansson, Uppsala) (× 100)

The regulation of islet blood flow has become accessible to investigation relatively recently. The prerequisite of this has been the introduction of the microsphere method combined with various techniques for the differential counting of microspheres in the endocrine and exocrine pancreas [36–38]. The application of these techniques in the rat has confirmed the previous anatomical observation of a rich vascular supply to the islets in showing that not less than 10%–15% of the total pancreatic blood flow is diverted to the islets [39, 40]. This should be viewed against the background that the

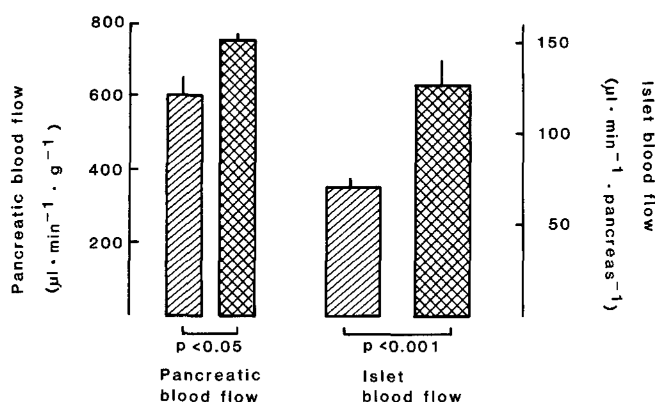

Fig. 5. The pancreatic blood flow and islet blood flow in rats which either remained non-injected (▨) or had received an intraperitoneal glucose injection 25 min before measurement (▩). The blood flow was calculated from the number of microspheres trapped in the pancreas. Data from [40] expressed as mean ± SEM

total islet mass in the adult rat is only about 1% of the whole pancreas [41]. It was furthermore found that glucose strongly stimulates islet blood flow (Fig. 5) and that this occurs within minutes after the administration of glucose [40]. These observations show that a nutrient stimulus to insulin release may be accompanied by a process which enhances the distribution of the hormone to the general circulation. The afferent loop of this mechanism is so far unknown but does not appear to be related solely to local islet factors (L. Jansson, personal communication 1984).

Long-term adaptation of the B cell to changing functional loads

As described above the peripheral demand for insulin is transmitted to the B cell through a whole spectrum of different signals. In the normal, physiological state each of these signals gives rise to an appropriate response, which precisely adjusts insulin secretion to the needs of the body. It is conceivable that two mechanisms are operative in this context, namely an immediate minute-to-minute regulation of insulin release from the individual B cells and a more long-term adaptation involving changes in total B-cell number and mass. It appears furthermore plausible that disturbances in either of these two mechanism may be of aetiological significance for the manifestations of diabetes. The regulation of the rapid insulin response has recently been the subject of several reviews [42–46]. In this article I would like to draw attention to the more long-term adaptive changes, with special regard to growth in B-cell number and mass.

Present knowledge on the relationship between the growth responses of the B cells and glucose homeostasis is based on observations made in animals, and particularly in those with hereditary disturbances in their glucose tolerance. This is not surprising since measurements of B-cell proliferation or B-cell mass in the human pancreas is a major undertaking and so far only few such studies are available (see below). There is, however, a wide range of animal models of which the most important are those hereditary diabetic syndromes which exhibit either severe insulin-dependent diabetes, resembling juvenile diabetes in man, or a milder, non-ketotic syndrome often associated with pronounced obesity. Even if not equivalent to human diabetes in every respect, these conditions may serve as important models for studies of growth reactions of the B cells in diabetes and genetic determinants of the growth response.

Of special interest in the context of B-cell growth in diabetes are the two spontaneous mutations *ob* and *db* in the mouse. These mutant genes, which both cause diabetes-obesity syndromes, have been particularly well studied by Coleman [47] who demonstrated a striking association between the phenotypic expression of mutant genes and background genome. Thus, when each of the genes was maintained on the C57BL/6J background, the animals showed a mild non-ketotic diabetes, pronounced obesity with insulin resistance and very marked B-cell hyperplasia. In contrast, when either gene was transferred to animals with the C57/KsJ background, the animals showed obesity together with severe, insulin-dependent diabetes and B-cell degeneration. Studies of B-cell proliferation in these two diabetic states show a high proliferative rate in 6J mice but a marked deficiency in DNA synthesis and mitotic rate in the B cells of KsJ mice [48, 49]. On the basis of these and other observations, Coleman suggested that the primary role of the modifying background genes is to regulate the proliferation of the B cells in periods of hyperglycaemic stress and that this may be the inherited factor that determines the glucose tolerance of the insulin-resistant and obese animals [47].

It is clear that the diabetes-obesity syndromes in mice show certain features in common with human diabetes, particularly the Type 2 (non-insulin-dependent) form associated with insulin resistance. It is generally agreed that human Type 2 diabetes is a disease which is strongly influenced by heredity but the precise pathogenesis has so far been a matter of much debate. Two factors have been postulated in this context, namely insulin resistance and a defective glucose recognition by the B cell leading to a blunted insulin response to glucose [50]. Can we now extrapolate on the observations in diabetic mice and add an inherited defect in B-cell proliferation as contributory to the manifestation of human Type 2 diabetes (Fig. 6)? Although the answer to this question is as yet only tentative, it seems worthwhile to consider the possibility for the following reasons. Firstly, quantitative studies of total B-cell volume in patients with Type 2 diabetes have consistently failed to demonstrate B-cell hyperplasia despite sustained hyperglycaemia [50–55]. In three studies there was, indeed, a 30%–65% diminution of the B-cell volume in Type 2

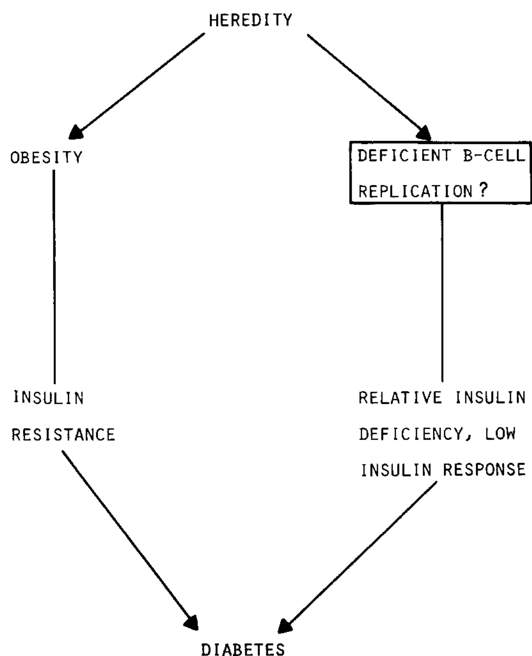

Fig. 6. Possible role of defective B-cell replication in the pathogenesis of non-insulin-dependent diabetes mellitus

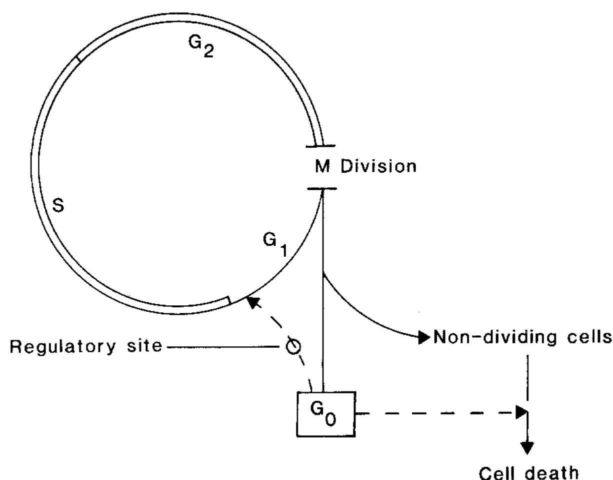

Fig. 7. The cell cycle of the pancreatic B cell. The time between two cell divisions can be subdivided into distinct phases called G_1, S and G_2. The period of DNA synthesis is confined to the S-phase. After cell division the daughter cells can progress either through a new cell cycle or enter a resting state denoted G_0. From here cells may either be recruited back into the cell cycle or die

diabetes compared with the non-diabetic normal-weight control subjects [51, 52, 55]. By contrast, the total B-cell mass in non-diabetic but obese individuals exceeded that in control subjects by more than 40% [55]. Secondly, recent evidence suggests that a sustained functional stimulation of a reduced B-cell mass may itself generate a delayed and sluggish insulin response to glucose very similar to that observed in patients with Type 2 diabetes. This phenomenon has been demonstrated in the rat and seems typically to involve a selective loss of glucose-induced insulin release [56]. Conversely, in man subjected to intense insulin therapy a blunted insulin response to glucose may revert to normal [57, 58]. Thirdly, and perhaps more controversially [59, 60], it has been suggested that cultured fibroblasts derived from diabetic patients show a diminished capacity for proliferation [61], possibly associated with raised intracellular generation of cyclic AMP [62]. If this is a general property of somatic cells from Type 2 diabetic patients, it would explain a (so far hypothetical) deficient capacity for B-cell regeneration. It would also serve as a genetic marker for individuals with an increased risk of developing Type 2 diabetes.

Mechanism of B-cell growth

A critical appraisal of the above hypothesis requires that human B cells are tested for their growth characteristics. Whereas islets or B cells are at present extremely difficult to isolate from the human pancreas, it may be anticipated that further development of methods for selective enrichment of islet cells will make such studies more feasible [63–65]. In experimental animals, methods for the study of B-cell multiplication have been applied extensively [66] and the information obtained may serve as a basis for a corresponding approach in man. The following section will summarize some of the techniques and results obtained in animals and discuss them in relation to the human situation.

Like other somatic cells, the B cell passes through a cell cycle which can be subdivided into several distinct phases (Fig. 7). Knowledge of the normal B-cell cycle is of considerable importance for a full understanding of the mechanism of B-cell proliferation, but until recently information on the B-cell cycle was fragmentary and mainly concerned with the length of the DNA-synthetic phase (the S-phase). By using isolated fetal rat islets [67], in which the progress of the B cells through the cycle had been synchronized in vitro with the aid of hydroxyurea, Swenne recently made an extensive study of the lengths of the various phases [68, 69]. With a total generation time of 14.9 h the B-cell cycle could be subdivided into a G_1 phase of 2.5 h, an S phase of 6.4 h a G_2 phase of 5.5 h and a mitosis time of 0.5 h. Glucose has previously been found to stimulate B-cell proliferation both in vivo and in vitro [68, 70], but the B-cell cycle seemed to proceed at the same rate irrespective of the glucose concentration of the culture medium [69]. This suggests that glucose stimulates B-cell proliferation by regulating the number rather than the rate at which the B cells enter the cycle. Against this background it seemed of interest to calculate the proportion of B cells which actively move through the cycle at a given moment (i. e. the proliferative compartment) and the effects of glucose on the size of this pool of cells. This became possible by

combining the above information on the length of the S-phase and the total length of the cycle (generation time) with an estimate of the labelling index in a population of unsynchronized fetal rat B cells, in which the newly-synthesized DNA had been labelled with ^{3}H-thymidine. The results of these studies indicated that the size of the proliferative compartment represented about 3% of the islet cells at a glucose concentration of 2.7 mmol/l, increasing to a maximum of about 7% at 16.7 mmol/l [69]. Based on these figures the production of new B cells per 24 h could also be calculated. This was found to be 4% at the lower and 10% at the higher glucose level [69]. Taken together these observations suggest that only a minor proportion of all B cells are able to divide but that these cells, due to a relatively rapid cell cycle, may nevertheless contribute substantially to the growth of the islet organ. It may, indeed, be calculated that these few, but actively dividing, B cells are able to double the B-cell number within a period of 10–30 days provided simultaneous loss is negligible. This plasticity of B-cell mass may be of key importance in the adjustment of insulin production in response to a chronic change in the insulin demand.

Many of the above observations were made on B cells of the fetal or newborn rat. Age related changes in the capacity for B-cell proliferation may, however, affect insulin production and contribute to a decrease in glucose tolerance with advancing age. Cell-cycle analyses of rat islets maintained in tissue culture indicate that proliferating B cells proceed through the cell cycle at similar rates irrespective of postnatal age [71]. The sensitivity to glucose in terms of DNA synthesis by the B cells is also similar, but the proliferative capacity seems to be restricted by a decreasing number of cells capable of entering the cell cycle. In 3-month-old rats the proliferative compartment of the islet B cell thus makes up only about 3% compared with about 10% in the perinatal rat. This diminution with age of B-cell proliferation may signify a gradual withdrawal of cells from the active cell cycle into an irreversible G_0 state (Fig. 7). It is conceivable, therefore, that the capacity of the islet organ to respond with proliferation to a diabetogenic stimulus decreases with age.

It is at present uncertain to what extent the mechanism of B-cell replication delineated above is valid in man. It seems, however, that we now have access to the instruments and methods necessary for a rational approach to elucidate the problem. The basic problem of stimulating human B-cell proliferation would seem to be a question of recruiting B cells from a quiescent G_0 state into the active cell cycle. Precisely what factor(s) is required for this is not known at present although we do know that the B cell can be triggered to proliferate both in man and animals. It is anticipated that the combined efforts of basic and clinical scientists will result in the formulation of methods not only for protection of B cells in case of threatening destruction, as in insulin-dependent diabetes, but also for stimulation of their renewal in response to an inappropriate functional demand as in non-insulin-dependent diabetes.

Before finishing this review it seems appropriate to recall and pay tribute to Claude Bernard and his scientific contributions. It is, indeed, hard to see how present knowledge of the B cell would have been achieved without the application of the strict rules and principles of scientific research laid down by Claude Bernard over 130 years ago. These principles, so natural and undisputable for experimental scientists today, were truly revolutionary when first presented in his famous "Introduction to the Study of Experimental Medicine" [72]. Thanks to Claude Bernard's authority and ability to communicate his ideas to colleagues and pupils, they became rapidly accepted by the scientific community. His experimental guidelines are as valid today as they were when first made public and their continued application will remain instrumental in the further expansion of knowledge on the islet B cell and on cell biology in general.

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