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# Differential Volumetry of A, B and D Cells in the Pancreatic Islets of Diabetic and Nondiabetic Subjects

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SAITO, K., YAGINUMA, N. and TAKAHASHI, T. *Differential Volumetry of A, B and D Cells in the Pancreatic Islets of Diabetic and Nondiabetic Subjects.* Tohoku J. exp. Med., 1979, **129** (3), 273–283 —— The percentile volumes $v_A$, $v_B$ and $v_D$ of A, B and D cells in the islets of Langerhans were histometrically estimated in the pancreases from 59 autopsy cases including maturity- and growth-onset diabetics and nondiabetics. Volumetry was performed microscopically by point counting method using a square-lattice eyepiece, then the total A, B and D cell volumes $V_A$, $V_B$ and $V_D$ were calculated from the total islet volume $V_i$ of the pancreas estimated separately. The measurement disclosed a prominent difference in $V_B$ between the three groups. Namely, it was only 0.156 and 0.388 $cm^3$ in the growth- and maturity-onset diabetics, much smaller values than 0.636 $cm^3$ in nondiabetics. The simultaneous investigation on clinical records revealed a negative correlation between $V_B$ and the maximum blood sugar level during GTT, showing that $V_B$ reflects grossly the degree of glucose tolerance of the individual. The smaller $V_B$ in diabetes was attributed to the diminution of $V_i$ because $v_A$, $v_B$ and $v_D$ were almost the same regardless of the presence or absence of diabetes. These results strongly suggested a failure of insulin production in diabetes due to quantitative deficiency of B cells. ——— pancreatic islets; A, B and D cells; volumetry; diabetes mellitus

In our foregoing paper, diabetic cases were subjected to islet morphometry, in which the results were interpreted in relation to some of the important clinical data (Saito et al. 1978b). Among several morphological indices examined, the total islet volume of the pancreas revealed special functional significance, reflecting glucose tolerance in individual cases. We have further to examine whether or not the insular cell composition undergoes a considerable change in diabetes mellitus.

It has been stated by many authors, on a morphometrical basis, that the numerical ratio of B to A cells was smaller in diabetes than in control, causing an absolute reduction of B cells in the former (Maclean and Ogilvie 1955; Gepts 1958). Practically, however, it seems rather awkward to count exactly the number of cells on islet sections. When one observes a group of B cell sections, for example, strict determination of the boundaries for individual cell sections appears

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to be unexpectedly difficult. Thus, an approach by means of cell number is abandoned in the present study, and quantitative relations between different islet cells, capillaries and hyaline masses, if present, are analyzed in terms of volumetry. This can be performed far more accurately. In addition to the practical advantage, the total volumes of A and B cells reflect changes in the hormone-producing activities more appropriately.

## Materials and Methods

The pancreases from 59 autopsy cases were submitted to the histometrical treatments (Table 1). The series consists of 26 maturity-onset diabetics (MOD), 5 growth-onset diabetics (GOD) and 28 nondiabetics (ND). Since the cases are identical to those used for islet morphometry in the foregoing study, the total islet volume $V_i$ in each case is already known (Saito et al. 1978b). The diagnosis of diabetes mellitus was made on the basis of urinalysis, fasting blood sugar level (FBS) and 50 g oral glucose tolerance test (GTT). The diabetic cases revealed varying degrees of carbohydrate intolerance, but none of them was proved to be chemical diabetic.

Table 1. *Overall examined cases*

| Group | Number of cases (M:F) | Group mean (range) | | | | |
|---|---|---|---|---|---|---|
| | | Age at death (years) | Age at diagnosis (years) | *FBS* (mg/100 ml) | $BS_{max}$ (mg/100 ml) during 50 g o-GTT | $V_i$ (cm³) |
| Nondiabetic | 28 (20:8) | 50.0 (21-81) | | 78.6 (62-103) | 134.8 (115-163) | 0.963 (0.618-1.401) |
| Diabetic | | | | | | |
| Maturity-onset | 26 (17:9) | 61.3 (42-85) | 55.1 (37-72) | 158.2 (71-324) | 290.9 (174-453) | 0.596 (0.181-1.020) |
| Growth-onset | 5 ( 2:3) | 37.6 (15-60) | 19.4 (11-23) | 181.5 (140-248) | 311.0 (291-331) | 0.255 (0.113-0.416) |

A paraffin section 2.5 $\mu$m thick was prepared from each pancreas at the caudal one-third position. The section was treated by Gomori's aldehyde-fuchsin solution for differential stain of specific granules, with modified Masson's trichrome as the counterstain. This method ensured the best discrimination between A, B and D cells, of which cytoplasm was stained red, purple and light blue, respectively (Fig. 1b). In both the diabetic and nondiabetic cases were found varying numbers of islet cells devoid of specific granules in cytoplasm; they were treated as unidentified islet cells (UI cells).

According to the fundamental principles in stereology, the volume ratio between different constituents of a structure is given by their corresponding areal ratio on a two-dimensional cut surface. In the present study, the percentile volumes of A, B, D and UI cells in the islets were denoted by $v_A$, $v_B$, $v_D$ and $v_{UI}$, for which replacement, in the above context, the areal ratio of these cells on a histological section was used. Capillaries and hyaline masses as intercellular constituents were treated in the same way, and were expressed as $v_c$ and $v_h$.

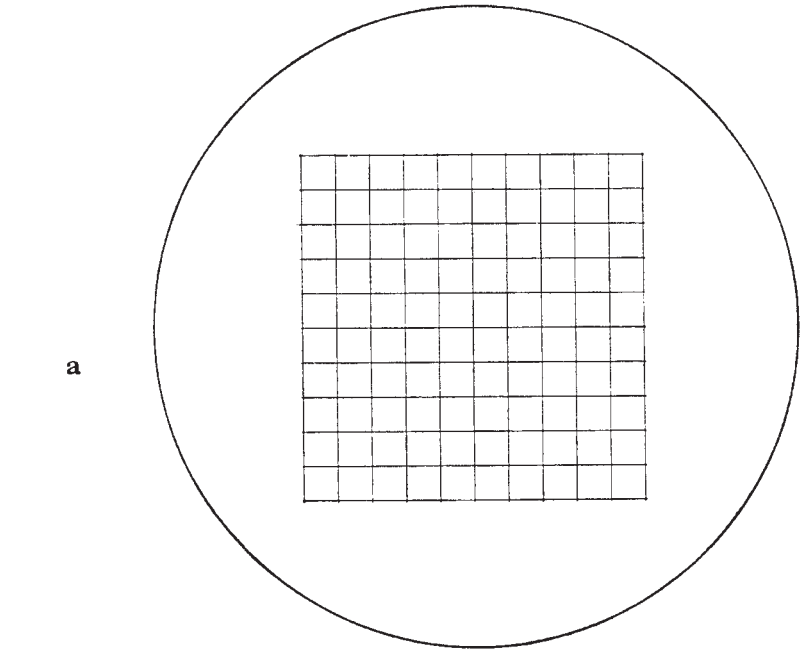
Determination of the areal ratio between different insular constituents was made by point counting. When the pattern of square lattice of an eyepiece is randomly superimposed on a microscopic picture of islet, the point at every corner of the square lattice falls in different constituents as shown in Fig. 1. Let the number of points falling in A, B, D or UI cells or intercellular components be called $N_A$, $N_B$, or the like. If a large

number of islet sections are submitted to this treatment, we obtain the percentile area, for instance, for A cells as

$$100 \times N_A/(N_A+N_B+\cdots),$$

where the denominator implies the number of whatever points falling in islet sections. This quantity was used as the equivalent of $v_A$.

Composition of an islet section appears to differ considerably from one to another on the same histological slide. In this study, point counting was performed on 50 to 150 islet



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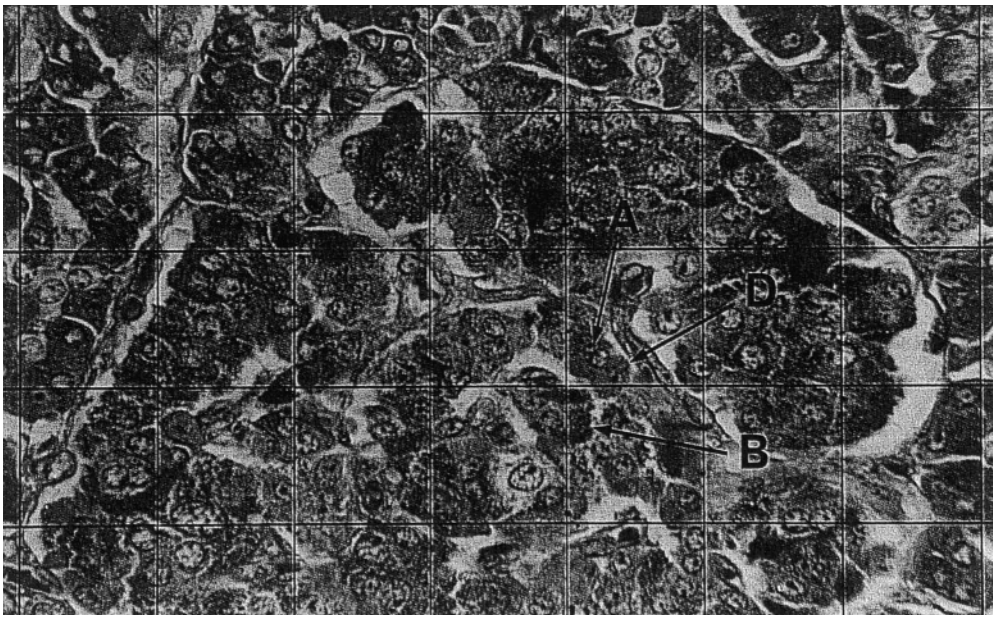


Fig. 1. a) A microscopic eyepiece with square-lattice. b) Photomicrograph illustrating the histometrical procedure. The pattern of square-lattice of the eyepiece is randomly superimposed on an islet section. By counting the number of points of corner of the square-lattice which hit A, B or D cells or intercellular constituents, relative areal ratio between them is estimated. For details see text. The histological section was treated by Gomori's aldehyde-fuchsin with modified Masson's trichrome as the counter-stain. The cytoplasms of A, B and D cells are stained red, purple and light blue, respectively. ×400.

sections, which gave fairly constant estimates of the percentile volumes. Sampling was performed under 400-fold magnification. The total number of sampling points reached 600 to 800 for each case.

The total volumes of different insular constituents retained in the pancreas were denoted by $V_A$, $V_B$, and the like. These were immediately calculated from their percentile volumes and the total islet volume $V_i$ in the pancreas.

## Results

### *Volume ratio between cellular and intercellular constituents*

Eighteen out of 28 nondiabetic cases are used hereafter as the control (ND). They are more than 40 years of age and are comparable to the maturity-onset diabetic group (MOD) in age composition.

In the first place, the percentile volume of islet cells and that of intercellular constituents are calculated, without distinguishing between A, B and D cells for the former. As shown in Table 2, volume percentage of islet cell mass is essentially the same in the two groups of diabetes as in the nondiabetic group. Insular hyalinization was noticed in 20 out of 26 cases of MOD, in 2 out of 5 cases of GOD and in 5 out of 18 nondiabetics. In MOD, the percentile volume $v_h$ of hyaline masses ranges from 0 to 12.4% of insular tissue, but its group mean is only 1.7% (Table 2).

Table 2. *Percentile volumes of insular tissue constituents*

| Group | $N$ | Cell mass | Capillary | Hyalin |
|---|---|---|---|---|
| Nondiabetic | 18 | 92.0±1.0* | 7.7±1.0 | 0.3±0.4 |
| Diabetic | | | | |
| Maturity-onset | 26 | 90.0±1.5 | 8.3±0.9 | 1.7±1.1 |
| Growth-onset | 5 | 86.7±6.5 | 13.0±6.8 | 0.3±0.7 |

* The confidence limits for group means were calculated using $t$-distribution ($\alpha$=0.05).

Statistical significance (Cochran's approximation of the Behrens-Fisher test; $\alpha$<0.05).

Cell mass: nondiabetic>maturity-onset diabetic.

Hyalin: maturity-onset diabetic>growth-onset diabetic, nondiabetic.

Table 3. *Percentile volumes of different islet cells*

| Group | $N$ | $v_A$ | $v_B$ | $v_D$ | $v_{UI}$ |
|---|---|---|---|---|---|
| Nondiabetic | 18 | 11.8±2.8* | 68.5±3.4 | 3.2±1.1 | 8.5±1.8 |
| Diabetic | | | | | |
| Maturity-onset | 26 | 11.6±1.5 | 64.7±3.1 | 3.4±1.3 | 10.3±2.5 |
| Growth-onset | 5 | 8.1±6.9 | 59.7±18.3 | 2.4±1.6 | 16.5±16.6 |

* The confidence limits for group means were calculated using $t$-distribution ($\alpha$=0.05).

*Percentile volumes of different islet cells*

As listed in Table 3, the mean volume percentage $\bar{v}_B$ of B cells is 64.7% in MOD and 59.7% in GOD. Although these are slightly smaller than the value of 68.5% in the nondiabetic group, the difference is not significant statistically. The percentile volume $v_{UI}$ of unidentified cells is slightly larger in GOD on account of severe degranulation of islet cells frequently found in this group. From an overall viewpoint, however, the mutual ratio between $v_A$, $v_B$, $v_D$ and $v_{UI}$ does not differ remarkably in the three groups. Islet cell composition appears to be uninfluenced by the presence or absence of diabetes.

*Total volumes of A, B and D cells; volume ratio of B to A cells*

The mean of total B cell volume $V_B$ is 0.636 cm$^3$ in ND, whereas it is only 0.388 cm$^3$ and 0.156 cm$^3$ in MOD and GOD, respectively (Table 4). Difference between any two of the values is significant. It should be noted that $V_A$ and $V_D$ are also reduced in the diabetic groups along with $V_B$.

On the other hand, the volume ratio $V_B/V_A$ is 7.2, 6.3 and 9.5 in ND, MOD and GOD, respectively (Table 4). Statistical test proves no significant difference between any two of the values.

TABLE 4. *Total volumes of A,B and D cells, and volume ratio of B to A cells*

| Group | $N$ | $V_A$ (cm$^3$) | $V_B$ (cm$^3$) | $V_D$ (cm$^3$) | $V_B/V_A$ |
|---|---|---|---|---|---|
| Nondiabetic | 18 | 0.109±0.028* | 0.636±0.078 | 0.028±0.008 | 7.2±1.8 |
| Diabetic | | | | | |
| Maturity-onset | 26 | 0.068±0.013 | 0.388±0.065 | 0.020±0.009 | 6.3±1.0 |
| Growth-onset | 5 | 0.026±0.034 | 0.156±0.114 | 0.007±0.008 | 9.5±6.7 |

* The confidence limits for group means were calculated using $t$-distribution ($\alpha$=0.05).

Statistical significance ($t$-distribution).

$V_A$: nondiabetic>maturity-onset diabetic>growth-onset diabetic ($\alpha<0.01$; $\alpha<0.01$).

$V_B$: nondiabetic>maturity-onset diabetic>growth-onset diabetic ($\alpha<0.001$; $\alpha<0.01$).

$V_D$: nondiabetic>growth-onset diabetic ($\alpha<0.02$).

*Relation to the laboratory data*

Data of glucose tolerance test (GTT) were available in 17 nondiabetic and 26 diabetic cases in the present series. In these cases, the maximum blood sugar level $BS_{max}$ during GTT rises conspicuously along with decreasing total B cell volume $V_B$ (Fig. 2). Mathematically, the relation is expressed as a negative correlation on logarithmic coordinates:

$$\log_{10} BS_{max} = -0.859 \log_{10} V_B + 2.013$$

$$(r = -0.726;\ \alpha < 0.01)$$

which is rewritten in Cartesian scales as:

$$BS_{max} = 103.1 V_B^{-0.859}$$

The correlation lends support to the assumption that, no matter whether the case is diabetic or not, $V_B$ reflects grossly the glucose tolerance in individual cases. In Fig. 2, in addition, the nondiabetic and diabetic cases are mixing in the range of $V_B$ from 0.4 to 0.6 cm³. $BS_{max}$ does not correlate with the ratio $V_B/V_A$ at all (Fig. 3).

In the diabetic group the level of fasting blood sugar (*FBS*) also increases with decreasing $V_B$ (Fig. 4). The negative correlation is significant at 1% level. A similar relation is also noticed between *FBS* and $V_A$, namely, the higher the level of *FBS*, the smaller is the value of $V_A$. Thus, in severe diabetics with high *FBS*,

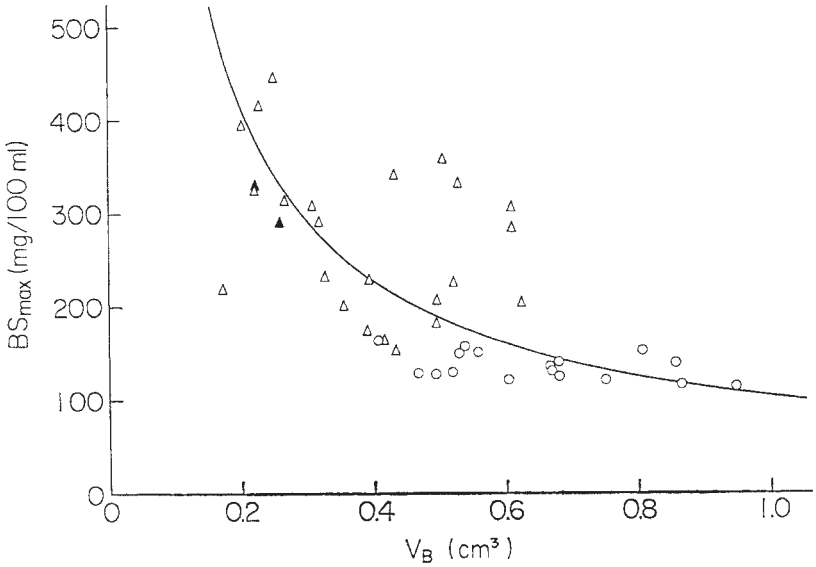


Fig. 2. The maximum blood sugar level $BS_{max}$ during GTT increases along with decreasing total B cell volume $V_B$. The regression equation is: $BS_{max}=103.1\ V_B^{-0.859}$. ○, nondiabetic; △, maturity-onset diabetic; ▲, growth-onset diabetic. Note also an obvious overlap of the nondiabetic and diabetic groups in the range of $V_B$ from 0.4 to 0.6 cm³.

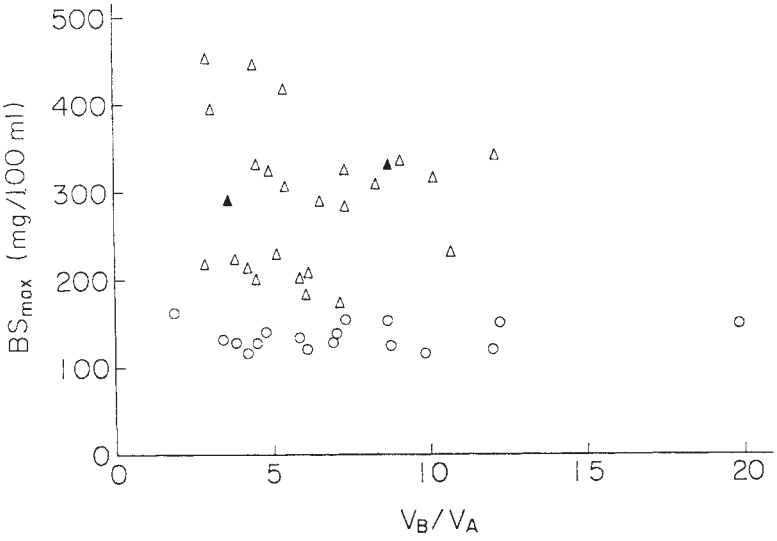


Fig. 3. $BS_{max}$ does not correlate with the volume ratio $V_B/V_A$ at all. The same symbols as used in Fig. 2.

the volume not only of B cells but of A cells is remarkably small.

Further, $V_B$ reduces definitely when the age at diagnosis of diabetes becomes younger (Fig. 5). Likewise, $V_A$ decreases with descending age at diagnosis (Fig. 6). Here again, we find no correlation between the ratio $V_B/V_A$ and the age at diagnosis.

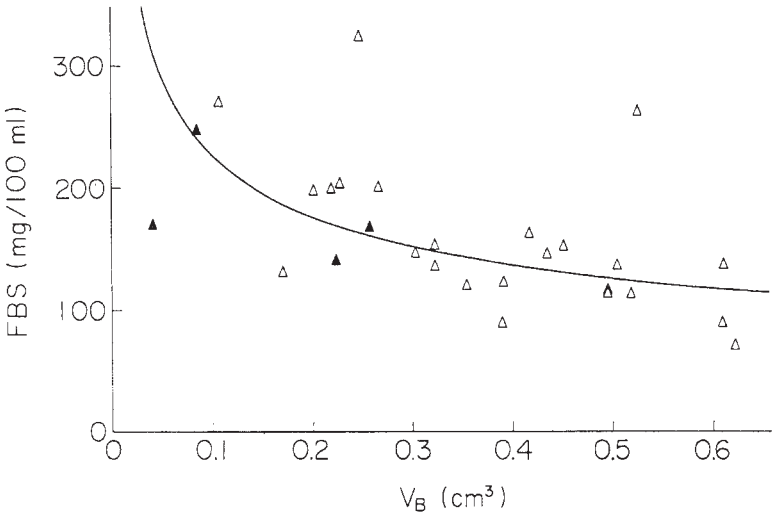


Fig. 4. In the diabetic group, the higher the level of fasting blood sugar *FBS*, the smaller is the value of $V_B$. The regression equation is $FBS=98.2V_B^{-0.366}$, rewritten from the linear regression: $\log_{10} FBS=-0.366 \log_{10} V_B+1.992$ ($r=-0.535$, $\alpha<0.01$). △, maturity-onset diabetic; ▲, growth-onset diabetic.

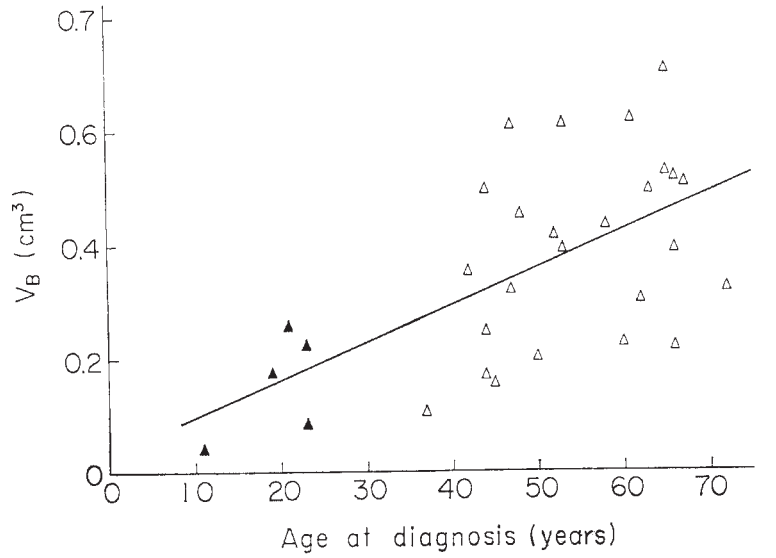


Fig. 5. The total B cell volume $V_B$ reduces definitely as the age at diagnosis of diabetes descends. The regression equation is: $V_B=0.0066x+0.0302$ ($x$: age in years at diagnosis; $r=0.608$, $\alpha<0.01$). △, maturity-onset diabetic; ▲, growth-onset diabetic.

*Quantitative changes with aging*

In the 28 nondiabetic cases the total B cell volume $V_B$ does not decrease with aging (Fig. 7), nor does the total A cell volume $V_A$. However, when the ratio

$V_B/V_A$ is examined, it tends to reduce with aging (Fig. 8), though not significant statistically ($0.05<\alpha<0.10$). Decrease in $V_B/V_A$ in higher ages is not noticed in the diabetic cases.

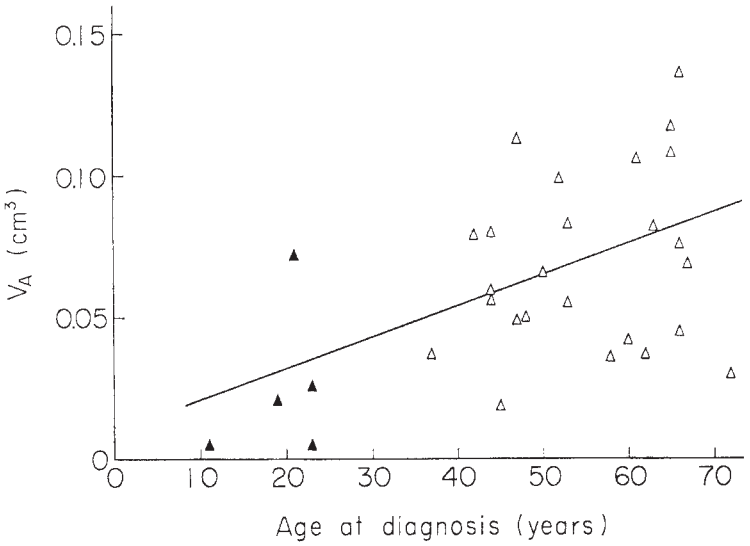


Fig. 6. The total A cell volume $V_A$ also diminishes with descending age at diagnosis. The regression equation is: $V_A=0.0011x+0.0101$ ($x$: age in years at diagnosis; $r=0.506$, $\alpha<0.01$).

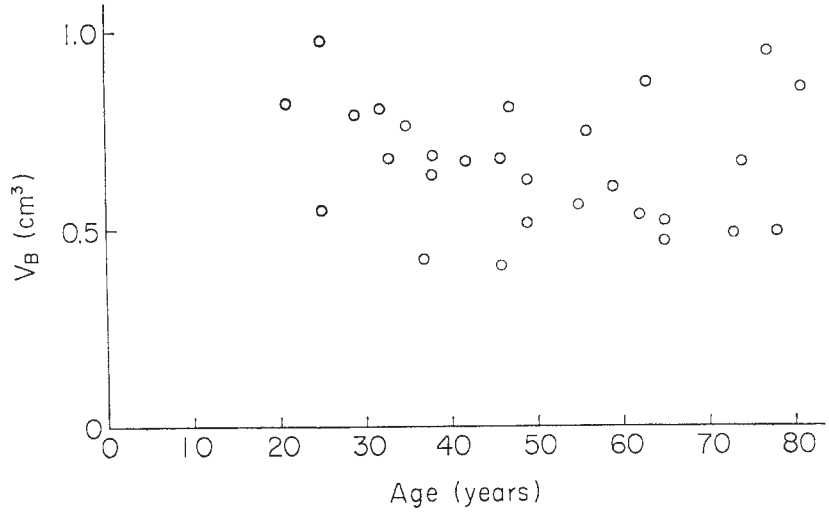


Fig. 7. In the nondiabetics, no senile decrease is noted in the total B cell volume $V_B$.

## Discussion

We employed in the foregoing study a geometrical model, in which the pancreatic islets were approximated by spheres of different radii $r$ randomly dispersed in the space (Saito et al. 1978a, b). On this basis, we calculated various quantities such as the mean and distribution pattern of $r$, or the number and volume of islets in a unit volume. After all these modelings and computations, however, the impediment of glucose metabolism in diabetes could not be related to any of the morphological parameters but one, that is the total islet volume $V_i$

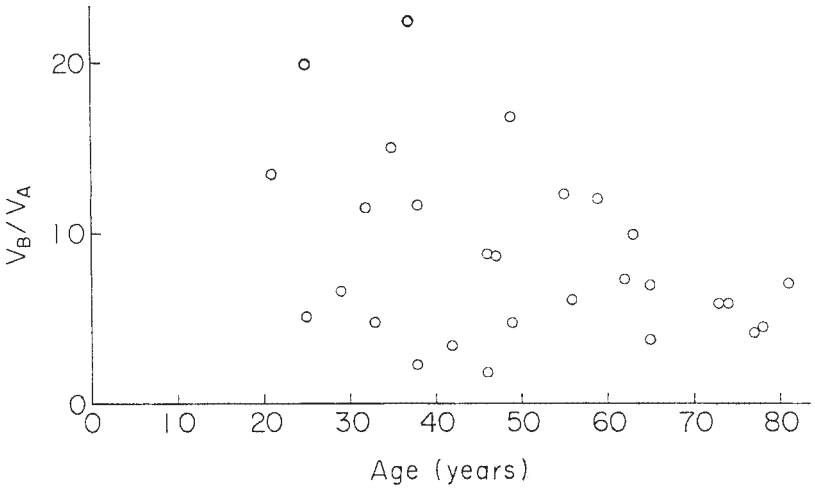


Fig. 8. The volume ratio $V_B/V_A$ shows a slight downward trend with aging in the non-diabetics

in the pancreas. This quantity showed an obvious difference between ND, MOD and GOD decreasing in this order. Besides, investigation on clinical records disclosed that the smaller the value of $V_i$, the more pronounced the laboratory indications of glucose intolerance, such as the level of fasting blood sugar ($FBS$) or the maximum blood sugar ($BS_{max}$) during GTT. Thus, the glucose tolerance of an individual appears to depend largely upon the volume of islets his pancreas retains.

A study of this kind requires a cell population analysis as the second step, because there may be a difference in islet cell composition when diabetic and nondiabetic pancreases are brought to comparison. Maclean and Ogilvie (1955) stated that the number of B cells was obviously small in the diabetic insular tissue. According to their counting, the numerical B/A cell ratio was 3 in the control, whereas it was only 1.5 in the diabetics. Gepts (1958) presented a similar report. For the sake of practical advantage in histometry, the authors adopted volume analysis instead of counting the number of cells. It is worth attention that, disagreeing with the former numerical studies, the present volumetry does not show a selective reduction of B cells in diabetes in any respect. The percentile volume $v_B$ as well as $v_A$ is almost the same in the nondiabetic and the two diabetic groups (Table 3).

Grossly speaking, the almost constant B cell share makes its total volume $V_B$ ($=v_B \cdot V_i$) be proportional to the total islet volume $V_i$. On this ground we can translate the volume reduction of the islets in diabetes immediately into that of B cells. The total B cell volume $V_B$ is 0.636 $cm^3$ in ND, whereas it is only 0.388 $cm^3$ in MOD and 0.156 $cm^3$ in GOD (Table 4). It may be reasonable to assume that in diabetes mellitus the amount of insulin production is reduced to the degree, to which the volume of B cells is diminished.

The functional significance of the quantity $V_B$ may become more apparent when it is subjected to correlative analysis with some laboratory data. In the 43

cases of the present series, including diabetics and nondiabetics, the maximum blood sugar level $BS_{max}$ during GTT rises with decreasing $V_B$ (Fig. 2). A similar relation is also confirmed between the level of fasting blood sugar ($FBS$) and $V_B$ (Fig. 4). Thus, glucose intolerance as indicated by the elevated $BS_{max}$ or $FBS$ is closely related to a quantitative reduction of B cells. These relationships may be generalized into an assumption that the degree of glucose tolerance or intolerance of an individual is determined, though grossly, by the volume of B cells retained in his pancreas. At the same time, the close clinico-pathological interrelation impresses how effectively a volumetric analysis can be applied to endocrine organs in dealing with their secretory behaviors.

Here, the quantitative behavior of the islets in mild diabetics should be noted, because hyperinsulinemia in such cases has been one of the most controversial problems since the report of Yalow and Berson (1960). This was interpreted as a reactive hyperfunction of B cells against some insulin antagonism. However, our volumetry does not substantiate this hypothesis. Mild diabetics of the present series reveal no particular sign of B cell hyperplasia, but they exhibit a diminution of $V_B$ quite parallel to their somewhat elevated level of $BS_{max}$ or $FBS$ (Figs. 2, 4). This is in accord with the results of recent studies on blood glucose-insulin interrelation (Perley and Kipnis 1966; Cerasi and Luft 1967; Seltzer et al. 1967; Goto et al. 1971; Fujita et al. 1975).

In addition, to be clarified from this viewpoint is the functional character of the cases which belong to an intermediate category between the diabetic and non-diabetic. There are some nondiabetics with smaller $V_B$ and at the same time with somewhat higher $BS_{max}$ (Fig. 2). They mix with the diabetics in the range of $V_B$ from 0.4 to 0.6 $cm^3$. The obvious overlap of the diabetic and nondiabetic groups in this range appears to provide a feasible definition of prediabetes on a morphological basis. The nondiabetics with $V_B$ of the above range may be influenced easily, when disposed to extrainsular diabetogenic factors, finally manifesting the diabetic syndrome.

In this connection, possible senile changes in islet cell composition must be examined, because it has been assumed that the aging itself implies a potential impairment of glucose tolerance (Silverstone et al. 1957; Pozefsky et al. 1965; Crockford et al. 1966). Seifert (1954), examining the numerical ratio of B to A cells, reported a remarkable decline after the sixth decade of life in the nondiabetics. In our study, $V_B$ does not change with advancing age in the nondiabetics, giving no support to the assumption of relative glucose intolerance in higher ages. The volume ratio $V_B/V_A$ shows a slight downward trend with aging. However, the influence of this quantity on glucose tolerance seems to be quite doubtful (Fig. 3).

Unger (1976), from his experiences in evaluating serum glucagon by radioimmunoassay, emphasizes a possible contribution of this hormone to pathogenesis of diabetes. In the present morphometry, however, the total A cell volume $V_A$ in the diabetics is definitely smaller than in the control. The volume

ratio $V_B/V_A$ does not decrease in the former (Table 4). It appears quite unlikely that the pancreatic A cells, in a state of evident hypoplasia, should play some important role in diabetogenesis. Felig et al. (1976), through their examinations of blood insulin-glucagon-somatostatin interrelations, demonstrated the primacy of insulin deficiency rather than insulin-glucagon imbalance in the pathophysiology of diabetes.

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