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A Role for Crinophagy in Pancreatic Islet B-cells

Minireview based on a doctoral thesis

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INTRODUCTORY REMARKS ON LYSOSOMES

The lysosomes were first observed by Christian de Duve and his collaborators in December 1949 as "a cryptic form of latent acid phosphatase" (18). This observation lead to the suggestion that the enzyme could be located in a distinct group of subcellular granules, segregated from the rest of the cell by a limiting membrane. Further studies showed that these granules contained a number of acid hydrolases. The granules or bodies were thus supposed to have lytic properties and they were named lysosomes (18, 19). Soon after the discovery of the lysosome its role in cellular events such as intracellular digestion and phagocytosis was established. The presence of acid hydrolases, primarily acid phosphatase, as evidenced by enzyme cytochemistry, was the main criterium for morphological identification of a lysosome. In 1966 de Duve and Wattiaux (20) suggested a nomenclature for the lysosomal system, based in part on morphological criteria but mainly on the known functions of the lysosomes (Fig. 1). At that time the existence of some of the lysosomal particles was still under debate. The concept of primary lysosomes, which had not yet taken part in intracellular degradation, was thus proposed. Lysosomes which had been involved in some kind of degradation were named secondary lysosomes. Depending on whether the secondary lysosomes were supposed to have been involved in either autophagy, i.e. the degradation of endogenous cellular material, or heterophagy, i.e. the degradation of exogenous material, they were further subdivided into autolysosomes or heterolysosomes. The functional nature of secondary lysosomes was further related to their structure by Smith and Farquhar (64) who, upon suppression of prolactin secretion, observed an increase in lysosomes containing material resembling secretory granule cores in the prolactin-producing cells of the anterior pituitary gland. The fusion of secretory granules with lysosomes was considered to be a specific lysosomal function, which was designated crinophagy by de Duve (18). Thus, a third group of secondary lysosomes was distinguished, namely the crinophagic bodies. de Duve and Wattiaux included, in the lysosomal system, two groups of organelles which however do not contain active enzymes. These organelles are the phagosomes and the postlysosomes or residual bodies. The phagosomes are considered as prelysosomes as they consist of material destined to be degraded by the lysosomes. The residual bodies, on the other hand, are

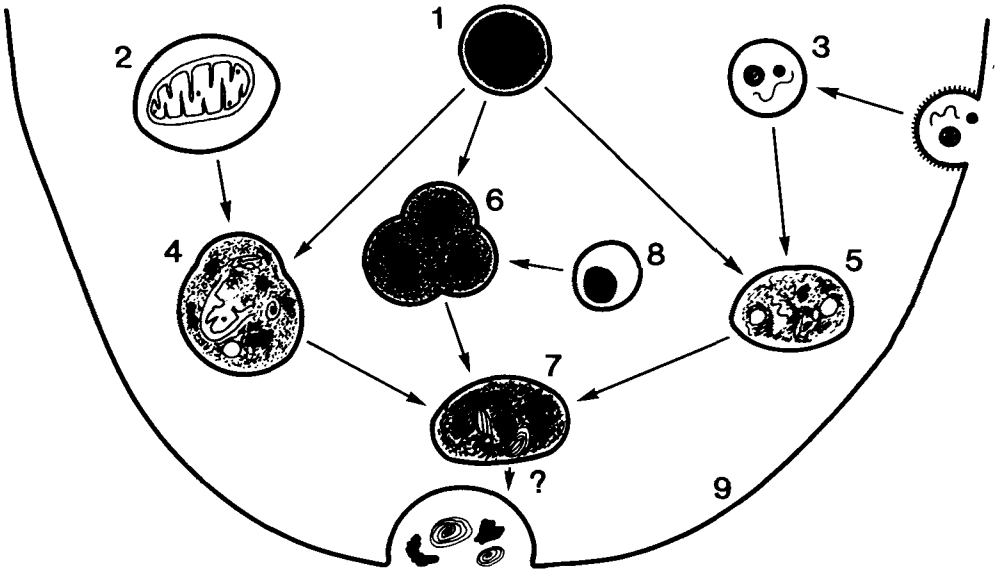

Fig. 1. The lysosomal system. (1) Primary lysosome. (2) Autophagic phagosome (prelysosome). (3) Heterophagic phagosome (prelysosome). (4) Autolysosome (secondary lysosome). (5) Heterolysosome (secondary lysosome). (6) Crinophagic body (secondary lysosome). (7) Residual body (postlysosome). (8) A secretory granule, not belonging to the lysosomal system. (9) Plasma membrane.

aged secondary lysosomes, which have lost their enzymatic activity. This nomenclature is still valid and will be used in the present review.

The first widely accepted hypothesis dealing with lysosomal formation was propounded by Novikoff and his coworkers (46, 47). Based on cytochemical studies of acid phosphatase they proposed that the lysosomal enzymes, after formation in the rough endoplasmic reticulum, were translocated to a specialized area of the endoplasmic reticulum, close to the Golgi apparatus, which they called GERL (Golgi-Endoplasmic Reticulum-Lysosomes). The primary lysosomes were supposed to bud off from these structures.

Studies of a number of different cell types have shown that most lysosomal enzymes are synthesized as larger proforms (33). Experiments in cell free systems have shown that the lysosomal enzymes are synthesized on membrane bound polysomes. In these systems a prepro-enzyme has also been found, the pre-sequence of which appears to be a signal peptide, that can bind to a cytosolic signal recognition particle (SRP). The SRP-enzyme complex binds to a docking protein, or SRP-receptor, on the membrane of the endoplasmic reticulum to allow the translocation of the lysosomal protein into the endoplasmic reticulum. The pre-sequence is then cleaved off during translation (22, 23, 55). These mechanisms are shared by secretory proteins, which will thus mix with the lysosomal proteins in the endoplasmic reticulum. All known lysosomal enzymes are glycoproteins and the glycosylation commences in the endoplasmic reticulum. The enzymes are then translocated to the Golgi apparatus where they aquire mannose-6-

phosphate residues (39). A receptor recognizing mannose-6-phosphate was discovered (35) during studies on the uptake of lysosomal enzymes by human fibroblasts. It has been proposed that this mannose-6-phosphate receptor is of importance not only for the pinocytotic uptake of lysosomal enzymes, but also for the intracellular segregation of such enzymes from other proteins, and for the further translocation of the enzymes to the primary lysosomes (27, 63). The exact location of this receptor in the Golgi complex is still under debate. In one immunocytochemical study on a number of different rat tissues, using an antibody against the receptor, it was found to be located exclusively in the cis-region of the Golgi apparatus (10) whereas in another study on rat liver it was found in the entire Golgi complex (24). Pulse chase experiments on human fibroblasts have shown that both lysosomal proenzymes and mature forms of these enzymes can be found in the lysosomes. Thus, it is concluded that the final conversion of the enzymes is not finished until the enzymes reach the lysosomes (25).

Our knowledge about the lysosomal membrane is limited. It is known to consist of a lipoprotein bilayer with a composition resembling that of the plasma membrane (68). The lysosomal membrane appears to contain structures with specialized functions, such as a proton pump (21, 45, 52) which maintains an acidic pH within the lysosome. Glycoproteins have been identified in the lysosomal membrane (13, 41, 42) but their role in lysosomal function remains obscure.

FACTORS INFLUENCING THE TURN-OVER OF INSULIN IN THE ISLET B-CELL

In experimental diabetes research much effort has been directed towards elucidating the mechanisms of pancreatic B-cell function. Interest has been particularly focused on insulin biosynthesis and insulin secretion. Indeed, much is now known about the regulation of these processes and their role in the maintenance of glucose homeostasis (2, 34, 43, 65, 66, 67, 69). The observation that the insulin content of islet tissue decreased during tissue culture evoked the suggestion that intracellular degradation of insulin may occur in the B-cell (53, 72). Studies on isolated islets exposed to cyproheptadine (31), and mannoheptulose (32), showed that exposure to these substances appeared to cause a loss of islet insulin. By following the fate of radioactively labelled insulin in isolated islets, incubated in vitro, Halban and Wollheim (30) were able to demonstrate that substantial amounts of insulin could be degraded within the islet B-cell. It therefore seems that the storage of insulin in the B-cell is controlled by three processes, namely insulin biosynthesis, insulin degradation and insulin secretion.

Several enzymatic systems, which have insulin degrading capacity, have been demonstrated in isolated pancreatic islets (5, 37, 38, 40, 74, 75). By subcellular fractionation it has been shown that these systems are mainly present in the cytosol (5, 75) and in the microsomal fraction (37, 75). It has been suggested that some of these enzymes take part in intracellular insulin degradation whereas others are involved in the conversion of proinsulin to insulin. Since, however, insulin molecules are mainly confined by membranes, i.e. in secretory granules, within the islet B-cell, the significance of the

enzyme activities of the cytosol or the endoplasmic reticulum in intracellular insulin degradation is debatable.

B-cell lysosomes, which have a lytic capacity, provide an alternative mechanism by which intracellular insulin may be degraded. Lysosomes which have apparently fused with secretory granules have been observed in B-cells of normal rats (44), spiny mice (8, 11), Mongolian gerbils and Chinese hamsters (8). In the rat and mouse, the crinophagic function of these lysosomes has been established by immunocytochemistry (49, 58). Lysosomes, apparently containing secretory granules, and thus probably crinophagic have been observed in the B-cells of some diabetic rodents (8) and also in the B-cells of islets subjected to experimental conditions where the insulin secretion was inhibited (1, 7, 16, 31, 36, 54, 70, 73). In islet A-cells of diabetic animals lysosomes have been described which have a morphology suggesting a crinophagic function (48, 50, 51), however, these were rarely observed in the A-cells of normal animals (48, 51). Crinophagy is also known to occur in other endocrine cell types (6, 14).

Crinophagy thus appears to be rather common in islet B-cells, as in other endocrine cells, both under physiological and pathological conditions. It is, however, not known whether crinophagy is of critical importance for normal function of the islet B-cells, nor are the regulatory mechanisms of this phenomenon known. Does, for instance, glucose, which is known to directly regulate insulin biosynthesis and secretion, affect B-cell crinophagy? Nor is it known how rapidly crinophagy responds to its regulatory mechanisms.

METHODOLOGICAL STRATEGIES

Various methods are applicable to the study of the crinophagic process in pancreatic islets. The studies reviewed below involve morphological and morphometrical techniques. The combination of these techniques with biochemical measurements of insulin biosynthesis and secretion makes it possible to relate the ultrastructure of the B-cell to its function. By morphometrical analysis it is possible to get an estimate of three-dimensional variables such as volumes, numerical densities of particles etc. from two-dimensional sections of tissues. It is of crucial importance in morphometrical analysis that the particles to be measured, in this case the lysosomes, are correctly identified. In order to clearly establish the morphological characteristics of different types of lysosomes, cytochemical visualization of lysosomal enzymes, such as acid phosphatase, in electron microscopic preparations have been used (4, 26).

In most studies the lysosomes were divided into two main subclasses, namely primary and secondary lysosomes. The classification of primary lysosomes was originally based on the appearance of the azurophil granules of neutrophilic leucocytes. These granules are considered to represent a pure population of primary lysosomes. The azurophil granules are surrounded by a single limiting membrane and contain an electron-dense homogeneous material. When cytochemical staining methods for lysosomal enzymes are applied, these granules are seen to be completely filled with reaction product (3).

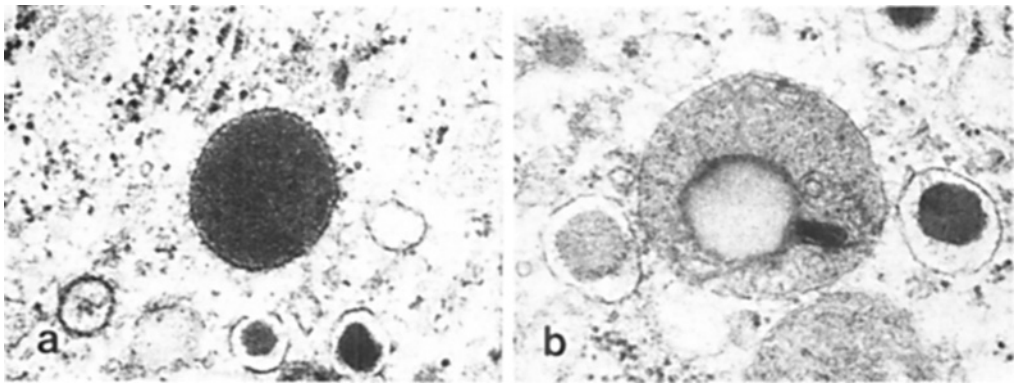

Fig. 2. Islet B-cell lysosomes. (a) Primary lysosome with a light halo beneath the membrane and a homogeneous, very finely particulate content. (b) Secondary lysosome containing some unidentifiable inclusions.

Because of their high electron density, primary lysosomes are sometimes referred to as dense bodies.

Thus, the primary lysosomes are identified as organelles surrounded by a single limiting membrane with a light halo just beneath the membrane and with a completely homogeneous, very finely particulate content of high electron density (Fig. 2a). By acid phosphatase cytochemistry these bodies are, invariably, completely filled with reaction product (57, 59). The primary lysosomes can be easily distinguished from the B-cell secretory granules, since the secretory granules have a much wider halo and a crystalline appearance of the granule core. Pale secretory granules are of a lower electron density than the primary lysosomes.

A secondary lysosome is identified as an organelle surrounded by a single limiting membrane, containing some kind of inclusion, i.e. secretory granule cores, membrane fragments and sometimes more or less intact subcellular structures (Fig. 2b). By acid phosphatase cytochemistry, the secondary lysosomes contain reaction product of a patchy appearance which incompletely fills the organelles (57, 59).

Immunocytochemical methods, such as the immunogold method (71), can be used to demonstrate insulin in ultrathin sections. One of the advantages with immunocytochemical methods which use colloidal gold particles as the marker, is that the distinct form of the gold particles allows the counterstaining of the sections with heavy metals and, thus, a good presentation of the ultrastructure of the tissue is possible. Furthermore, the distinct granular appearance of the gold particles makes it possible to estimate by quantitative means the background staining in the sections. Since they contain insulin crinophagic B-cell secondary lysosomes can be identified by immunocytochemistry. This makes it possible to make a quantitative estimate of crinophagy in the islet B-cell.

Crinophagy in the B-cell could alternatively be studied by the biochemical measurement of insulin degradation within the B-cell. By blocking different degradative pathways this method could be used to assess the proportion of degradation contributed by crino-

phagy.

Another way to study the degradation rate and the regulation of fusion between granules and lysosomes would be to isolate secretory granules and lysosomes and mix them in vitro. A difficulty in this context is the small amounts of tissue available.

LYSOSOMES AND PANCREATIC ISLET B-CELL FUNCTION

Relation of islet lysosomal morphology to the extracellular glucose concentration

In order to study the influence of the extracellular glucose concentration on islet lysosomal morphology, isolated pancreatic islets were maintained for one week in tissue culture at either 28, 5.5 or 3.3 mmol/l glucose (57). Islets cultured at 28 mmol/l glucose showed high rates of both insulin biosynthesis and secretion. Such islets seemed, however, unable to store more than a small amount of insulin. Islets cultured at 5.5 mmol/l glucose displayed a markedly reduced insulin secretion whereas the rate of insulin biosynthesis was not decreased to the same extent. This resulted in a substantial islet insulin content. In islets cultured at 3.3 mmol/l glucose both insulin secretion and biosynthesis were markedly decreased. The islet insulin content of these islets was decreased compared to that of islets cultured at 5.5 mmol/l glucose.

In all cultured islets different types of lysosomes could be seen and dense bodies seemed to predominate at all glucose concentrations. Secondary lysosomes, with inclusions of various kinds, were frequently seen at the two lower glucose concentrations. In the morphometrical analysis, no attempt was made to differentiate between primary and secondary lysosomes. The size distributions of the lysosomes was found to be different in each of the three experimental groups; the average lysosomal size was found to be inversely proportional to the concentration of glucose in the culture medium. However, the numerical density of lysosomes was greatest at the highest glucose concentration. Although the volume of the islet cells was increased after culture at the high glucose concentration, the number of lysosomes per cell was directly proportional to the glucose concentration. The total lysosomal volume per cell did not, however, differ between the three experimental groups.

Relation between insulin biosynthesis and secretion and the morphology of islet lysosomes

To study whether glucose directly affects islet lysosomes, or if glucose acts indirectly via effects on insulin biosynthesis and secretion, isolated islets were maintained for one week in tissue culture at either 3.3 or 28 mmol/l glucose (9). At both glucose concentrations experimental conditions producing either low or high rates of insulin secretion were examined. Differences in intracellular insulin degradation between the low and the high insulin secretory conditions were estimated by measuring islet insulin biosynthesis and the total amount of intracellular and extracellular insulin in the islet cultures.

The intracellular degradation of insulin was significantly enhanced in the low-secretory conditions, irrespective of the glucose concentration. By morphometric analysis the islet lysosomes were subdivided into primary and secondary lysosomes. In all experi-

mental groups the diameter and average volume of the primary lysosomes were smaller than those of the secondary lysosomes. However, at both glucose concentrations, primary lysosomes occured with higher frequency in islets with a high insulin secretion, whereas the lysosomal population was mainly composed of secondary lysosomes in islets with a low rate of secretion.

The crinophagic activity of islet B-cells as estimated by morphometry of the lysosomes

When sections of islets, which had been maintained in tissue culture for one week, were immunostained for insulin, clusters of gold particles were found over the secretory granules of the B-cells (59). A number of secondary lysosomes were also found to be positive for insulin. In most of these lysosomes, the gold particles were concentrated over structures similar to secretory granule cores. Morphometry showed that there was an increase in both the volume and numerical density of the primary lysosomes with increasing glucose concentration of the culture medium. The insulin-containing secondary lysosomes were intermediate in size to primary and secondary lysosomes in all three experimental groups. The proportion of insulin-containing, secondary lysosomes was highest at 5.5 and lowest at 3.3 mmol/l glucose.

Studies of the dose-response relationships of both the glucose-stimulated insulin biosynthesis and the insulin secretion of the pancreatic islets showed that the rate of biosynthesis attained a half maximal value at a glucose concentration of 7.0 mmol/l, whereas for secretion the corresponding value was reached at a glucose concentration of 14.5 mmol/l. Thus, at 3.3 mmol/l glucose both the insulin biosynthesis and secretion were low. However, at 5.5 mmol/l glucose the insulin biosynthesis was stimulated whereas the secretion was low. At 28 mmol/l glucose both insulin biosynthesis and secretion were markedly stimulated.

The time course of lysosomal alterations in the B-cell and their relation to changes in insulin biosynthesis and secretion after abrupt changes in glucose concentration

The morphology of islets in situ was compared to that of isolated islets incubated in vitro. Under the in vitro conditions the islets were initially exposed to 28 or 3.3 mmol/l glucose for 24 hours. The glucose concentration was subsequently changed to 3.3 or 28 mmol/l glucose, respectively, and the islets were incubated for up to another 24 hours (61).

The B-cell lysosomal population in vivo was predominantly composed of secondary lysosomes, which frequently contained secretory granule cores. After the initial 24-hour period at 3.3 mmol/l glucose both the volume density and the average volume of the secondary B-cell lysosomes were increased compared to islet B-cells in vivo. The mean diameter of the primary B-cell lysosomes was decreased after 24 hours at either 28 or 3.3 mmol/l glucose. No other change in the lysosomal population was observed after the initial 24-hour period at 28 mmol/l glucose.

The rapid change in glucose from 28 to 3.3 mmol/l resulted in alterations in insulin biosynthesis and secretion, which correlated with an accumulation of insulin within the B-cells. Lysosomal transformations were observed 24 hours after the change; the

size and number of the secondary lysosomes increased. The change from 3.3 to 28 mmol/l glucose resulted in a parallel increase in insulin biosynthesis and secretion without a change in islet insulin content. Six hours after the change, a decrease in the volume of the secondary lysosomes was observed. Twenty-four hours after the change in glucose concentration the lysosomal population was changed from one predominantly comprising secondary lysosomes to one predominantly composed of primary lysosomes.

When isolated islets were pulse-chase labelled with radioactive leucine and subsequently processed (49) it was found that autoradiographic labelling of the secretory granules increased during the first three hours of the chase period. It then decreased during the subsequent 21 hours. In contrast the autoradiographic labelling of secondary lysosomes (multigranular bodies) remained constant during the first three hours and then was increased at 24 hours. This suggests a transfer of labelled protein from the secretory granules to the lysosomes.

Regulation of the crinophagic activity of the B-cell lysosomes in vivo

Two different approaches have been taken to study the in vivo regulation of B-cell lysosomes (60). A local inbred colony of C57BL/6 mice, originally obtained from the Jackson Laboratory, Bar Harbor, ME, USA, was used for these studies. This strain carries the autosomal recessive ob-gene, and the homozygous animals develop a typical syndrome characterized by obesity, hyperphagia, moderate hyperglycemia and hyperinsulinemia (15). This syndrome, thus, has similarities with human type II diabetes.

The first approach was to study the B-cell lysosomes of the obese hyperglycemic mice and compare them to those of their lean siblings. The second approach was to use a transplantation model, by which normal islets could be transplanted under the kidney capsule of various recipients and could thereby be subjected to different functional demands in vivo. By using an inbred mouse strain rejection of the graft was avoided. Three different groups of mice were used as islet recipients: Lean mice made diabetic with a single injection of alloxan, obese hyperglycemic mice and normal lean mice serving as controls.

Ultrastructural examination of islets transplanted under the kidney capsule showed that the grafts were vascularized by two weeks after the implantation. Islets transplanted into normal mice were ultrastructurally similar to the endogenous islets of normal mice, exhibiting well granulated B-cells and a lysosomal population mainly composed of secondary lysosomes, occasionally containing secretory granules. B-cells of islets transplanted into either alloxan-diabetic or obese hyperglycemic mice were markedly degranulated and had a lysosomal population predominantly composed of primary lysosomes.

Although experiments in vitro indicated that both insulin biosynthesis and secretion would be markedly stimulated in the obese mice at the prevailing serum glucose concentrations, the ultrastructural appearance of the endogenous obese mouse islets was similar to that of the endogenous lean mouse islets. Despite the high functional activity of obese mouse islet B-cells, secondary lysosomes, some of which contained secretory granule cores, formed the major proportion of their lysosomal population.

Table 1. Summary of the findings in vivo and in vitro.

	Islets transplanted into normal mice	Islets transplanted into alloxan-diabetic mice	Islets transplanted into obese hyperglycemic mice	Endogenous islets of normal lean mice	Endogenous islets of obese hyperglycemic mice
Serum glucose concentration (mmol/l)	8 - 10	28 - 37	14	8 - 10	14
Insulin biosynthesis (in vitro)	high	high	high	high	high
Insulin secretion (in vitro)	low	high	high	low	high
Granule content	high	low	low	high	high
Crinophagic activity	high	low	low	high	high

Table 1 summarizes the ultrastructural and morphometrical findings in relation to the in vitro determinations of the dose-response of glucose-stimulated insulin biosynthesis and secretion by islets isolated from either normal lean or obese hyperglycemic mice.

GENERAL DISCUSSION

When morphological and morphometrical methods are applied to the study of the islet B-cell lysosomes, a snapshot of a very dynamic system is obtained. The picture we get of the lysosomal population would depend on three ongoing processes. The first of these would be the formation of primary lysosomes. There are reasons to believe that the formation of lysosomes in islet B-cells will increase when protein biosynthesis is stimulated, as is the case when the extracellular glucose concentration is raised. Some lysosomal enzymes have been shown to increase in the islets at high glucose concentrations (12, 62). The second of these processes would be the transformation of primary lysosomes to secondary ones by fusion with autophagosomes, heterophagosomes or secretory granules. The third process would be the degradation and dissipation of the secondary lysosomes. It is unknown whether the secondary lysosomes of the B-cells are completely degraded within the cells, there are indications that they may be extruded from the cell by exocytosis (73). However, they are apparently disposed of, since no accumulation of residual bodies is observed in normal islet tissue.

The number of primary lysosomes observed in morphometric analysis would thus depend on their rate of formation and the rate by which they are consumed in transformation to secondary lysosomes. The size and number of secondary lysosomes, on the other hand, would be dependent on their rate of creation by transformation and their degradation rate.

It has been shown that insulin is indeed taken up by the B-cell lysosomes (49, 58, 59). There is no direct evidence, however, that this insulin is then degraded. It is of interest in this context that subcellular fractions containing lysosomes from liver have

been shown to degrade insulin (28). If the B-cell lysosomes provide the only route for intracellular degradation of insulin their capacity must be considerable. It has been shown that mouse islet B-cells contain, on average, 13 000 secretory granules (17) and up to 30 % of the islet insulin content may be degraded within 24 hours (29, 30). The number of lysosomes per cell has been found to vary between 25 and 70 (57). Each lysosome appears to be able to fuse with many secretory granules, as judged by the appearance of multigranular bodies. However, until the rate of degradation within the lysosomes has been determined, it is impossible to assess the degradative capacity of the lysosomal system of the B-cell.

One crucial point is the identification of the different kinds of lysosomes. As far as primary lysosomes are concerned, this has been a matter of controversy in the literature. The dense body has been previously speculated to be a primary lysosome by Meda (44), whereas other authors have suggested that the multivesicular bodies represent primary lysosomes in the B-cell (49). The classification of dense bodies as primary lysosomes in the B-cells seems, however, to be justified. These lysosomes were consistently found to be smaller than the secondary lysosomes (9, 59, 60, 61) which seems plausible for a newly formed particle. Also, insulin containing secondary lysosomes were intermediate in size to the dense bodies and insulin-negative lysosomes (59). This would be the expected result from the fusion of a small primary lysosome and a small secretory granule. The dense bodies, furthermore, increased in number in situations in which there was a high rate of protein biosynthesis and a low rate of transformation to secondary lysosomes (9, 59, 61).

Immunocytochemistry has shown that a high proportion of the secondary lysosomes are involved in crinophagy in the B-cell (59). This proportion is probably an underestimate since one prerequisite for the recognition of a crinophagic body by this method is that the immunoreactivity of the insulin has not yet been destroyed by the degradation. Autophagy also exists in the islet B-cells, as evidenced by secondary lysosomes which contain structures of apparent endogenous origin, e.g. mitochondria. Autophagy appears to be of greater importance in situations in which the metabolic rate of the islets is low, as when they are exposed to a low glucose concentration. However, from the data of Schnell et al. (59) it seems reasonable to conclude that the major proportion of the alterations observed in the lysosomal population of the B-cell, reflect changes in the crinophagic activity.

The results indicate that a high crinophagic activity is related to a hign secretory granule content of islet B-cells. The simplest explanation for this would be that when the granule content is high the number of contacts between lysosomes and secretory granules increases. The likelihood of fusion between the organelles may depend on the characteristics of their membranes. Sawano et al. (56) have shown that a population of, probably older, secretory granules in the B-cells appears to easily fuse with endocytotic vesicles. This could imply that their membranes have been modified in some way during maturation. An increase in the "stickyness" of the secretory granule membrane

may be of importance for the exocytotic process, but it may also increase the possibility of a fusion between secretory granules and lysosomes. It could be speculated that glucose might be a factor which affects the "stickyness" by glycosylation of membrane proteins.

During long-term exposure of normal islets to high glucose concentrations the B-cells show a marked degranulation (1, 9, 57, 59, 60). This would indicate that in the long run the rate of insulin biosynthesis is lower than the rate of insulin secretion. To retain the ability to respond to sudden stimulation of insulin secretion, it is important for the B-cell to maintain a store of insulin. This could explain why the insulin biosynthesis is more markedly stimulated at intermediate glucose concentrations than the insulin secretion. However, if a proper stimulus did not occur, there would be an inappropriate accumulation of insulin within the B-cell. Crinophagy could thus be a mechanism by which this accumulation would be prevented.

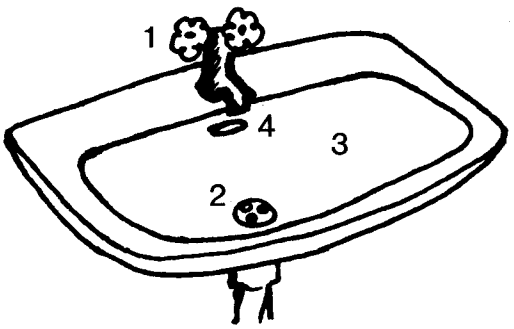

Fig. 3. The role of crinophagy in the B-cell. (1) Insulin biosynthesis. (2) Insulin secretion. (3) Insulin content. (4) Crinophagy.

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