Histol Histopathol (1996) 11: 161-170

Histology and Histopathology

Histopathological changes in the islets of Langerhans in hamsters infected with the 139H strain of scrapie: semi-thin section study

X. Ye and R.I. Carp

New York State Institute for Basic Research in Developmental Disabilities, Staten Island, New York, USA

Summary. Using histopathological analysis of semi-thin sections stained with toluidine blue, we observed profound pathological changes in the islets of Langerhans of hamsters infected with the scrapie agent (strain 139H). These included cytoplasmic vesicles, nuclear swelling, and vacuolization in the islet cells. Two types of vacuolization were seen. "Localized vacuolization" (LV) has a distinct edge and is restricted or confined within the cell. "Diffuse vacuolization" (DV) has no distinct edge and is scattered within tissues either inside or outside of cells. DV may span intracellular and extracellular regions of the islet tissues. There were abnormal structures which we termed blood vessel cores (BVCs) in the islets of 139H-infected hamsters. BVC is a hollow space filled up with blood cells. Immunocytochemical staining for insulin antibody suggested that BVC was surrounded by the B cells of the islet. In the present study, we observed that many inflammatory cells passed through the blood-tissue barriers using pathways between cell-junction in the lumen of BVC. We also observed many necklace-like hollow spaces between islet cells. They are the pockets of extracellular space. A novel concept of "the accordion effect" was described to explain a function of the extracellular space. Under normal physiological conditions, as the synthesis of insulin increase in B cells, the volume of the B cells will increase while the volume of the extracellular space will decrease. After a synchronized secretory response from the stimulated B cells, the secretory product would move from the intracellular space into the extracellular space, the volume of the B cells would be decreased and the volume of the extracellular space would be increased. Most of the secretory product might be released into the blood stream immediately, causing an insulin releasing peak in the blood stream, whereas the rest would remain in the enlarged extracellular space. As the cycle repeat, the increasing volume of the B cells will squeeze the remaining insulin into the blood stream gradually. Thus, the expandable extracellular space would serve as buffer system and a reservoir to collect and store some secretory products for future use. We refer to this concept as "the accordion effect". The concept of "the accordion effect" may also be true in other endocrine organs such as pituitary gland and adrenal gland.

Key words: Scrapie, Histopathology, Vacuolization, Islets of Langerhans, Hamster

Offprint requests to: Dr. Xuemin Ye, Division of Neurotoxicology, FDA/National Center for Toxicological Research, 3900 NCTR Drive, Jefferson, AR, 72079, USA

Introduction

Scrapie is an untreatable, slowly fatal disease of sheep and goats (Parry, 1983). The scrapie agent can be transmitted to a variety of laboratory animal species, including mice and hamsters (Chandler, 1963; Kimberlin and Walker, 1986; Kimberlin et al., 1989). Scrapie is a direct model for several human diseases, such as Creutzfeldt-Jakob disease (CJD), kuru and Gerstmann-Straussler syndrome (GSS) (Gajdusek, 1977). More recently, scrapie-like diseases have been found in several additional species: bovine spongiform encephalopathy (BSE), transmissible encephalopathy of ranch-reared mink (TME), and chronic wasting disease of mule deer and Rocky mountain elk (reviewed in Kimberlin, 1989). These diseases are characterized by long incubation periods lasting from months to decades and are always fatal. Recent studies have demonstrated that, in some scrapie strain with mouse strain or hamster strain combinations, there is an increase in body weight that starts prior to the onset of typical motor dysfunction which signals the start of clinical disease (Carp et al., 1990). For their studies in hamsters, animals were injected intra-cerebrally with scrapie strains 139H or 22CH or with homogenates of normal hamster brain (NHB) (Carp et al., 1990). Animals were then assessed periodically for body weight, insulin level and glucose tolerance throughout the incubation period. Animals injected with these two scrapie strains became obese prior to the appearance of motor changes. During the latter part of the pre-clinical and throughout the clinical

phase of disease, animals were hypoglycemic and had insulin values as much as 49 hold higher than those found in controls. At necropsy, there was marked hyperplasia and hypertrophy of the cells of the islets of Langerhans. The thyroid, adrenal glands, liver and kidneys were also enlarged (increased both size and weight). In contrast, hamsters injected with the commonly used 263K strain of hamster-adapted scrapie did not show any of the above changes. For example, total body weight of 263K-infected animals was the same as that of hamsters injected with normal hamster brain throughout the preclinical and clinical periods (Carp et al., 1990).

While the findings of Carp et al. (1990) suggest that the 139H and 22CH strains induce a severe generalized endocrinopathy, of the tissue examined, the histopathological changes in the islets of Langerhans were the most severe (Carp et al., 1990). It is possible that the pancreatic changes could play an important role in the obesity of 139H-infected hamsters. In this study, we used semi-thin section technique to study the pathological changes of the pancreatic islets of 139H-infected hamsters. We introduce several new concepts including "localized vacuolization", "diffuse vacuolization" and "the accordion effect" in this study. We also describe the possible pathway for inflammatory cells passing through the blood-tissue barriers in the lumen of blood vessel core (BVC).

Materials and methods

1. Animals

Female, weanling LVG/LAK hamsters were obtained from Charles River (Wilmington, MA) and were maintained in a temperature- and humidity-controlled room with a 12-hour on 12-hour off light cycle. Each cage had no more than three animals. They were given food and water *ad libitum*.

2. Inocula

Two inocula were used: homogenates of NHB, and scrapie strain 139H. Each group had ten animals. The 139H scrapie strain was provided by Dr. R.H. Kimberlin (SARDAS, Edinburgh, UK) and are now maintained in our animal colony by hamster-to-hamster passage.

The origin of the 139H strain has been described in detail (Carp et al., 1990) and essentially involved a series of hamster-to-hamster passages of the well-characterized 139A mouse-adapted scrapie strain. The material used to initiate the passages in hamsters had been cloned by three limiting dilutions and a subsequent passage in Compton White mice. The hamster-to-hamster passages were performed using 1% brain homogenates, and at that concentration it yielded a mean incubation period in LVG/LAK hamsters of 134 days.

The characteristics of our passaged materials are exactly the same as those of the strains obtained from Dr. Kimberlin (Carp et al., 1990). Passages were made by the intracerebral (i.c.) injection of 40 µl of 1% brain homogenate prepared in cold (4 ºC) phosphate buffered saline (PBS) pH 7.4. Homogenization was effected by 20 strokes of a hand-operated Ten-Broeck homogenizer (Carp et al., 1990). All homogenates were stored at -70 ºC until used. The animals were inoculated 1-2 weeks after arrival at the laboratory (about 35 days old). In all experiments, researchers wear gloves and laboratory coats.

The clinical signs in 139H-injected hamsters are similar to those seen in the commonly used 263K-hamster model, but they are much less severe. The changes used to signal the start of the clinical disease include slight head bobbing and mild ataxia (Carp et al., 1990).

3. Semi-thin section study

At the end of the incubation period, animals were anesthetized with pentobarbitone sodium (Abbott Lab, North Chicago, IL, p.i., 3-4 ml/kg body weight) and perfused via the heart with normal saline (pH 7.4) at room temperature for 5 min (15 ml/min), followed by perfusion for 10-15 min (15 ml/min) with 2% paraformaldehyde (Electron Microscopy Sciences, Ft. Washington, PA) and 1% glutaraldehyde (Electron Microscopy Sciences, Ft. Washington, PA) in 0.1M cacodylate buffer solution (J.T. Baker Inc. Philipsburg, NJ) (pH 7.4). The splenic and duodenal portions of the pancreas were removed without delay and then post-fixed of immersion in the same solution.

After immersion-fixation overnight at 4 ºC, the pancreas was rinsed twice in 0.1M cacodylate buffer (pH 7.4) with 10% sucrose. The pancreas tissues were trimmed into 1 mm^3 blocks. The tissue blocks were then osmicated in 1% osmium tetroxide (Electron Microscopy Sciences, Ft. Washington, PA) in 0.1M cacodylate buffer for one hour at 4 ºC, rinsed twice in 0.1M cacodylate buffer with 10% sucrose and rinsed once in distilled water for 10 minutes. The tissue blocks were stained with 0.5% uranyl acetate overnight. The specimens were dehydrated in increasing concentrations of ethyl alcohol at 4 ºC, which was then replaced by 100% propylene oxide at 4 ºC, and then infiltrated with and embedded in unpolymerized Spurr embedding medium (Electron Microscopy Sciences, Ft. Washington, PA). After curing for several days in an oven at 60 ºC, semi-thin sections (0.5 µm) were cut on a Sorvall JB-4A Porter-Blum microtome and stained with toluidine blue. Specimens were then observed with a light microscope. Photomicrographs were taken with a Carl Zeiss Axiophot.

Results

There were profound pathological changes in the islets of Langerhans in 139H-infected hamsters. As shown in Fig. 1 to 2, cytoplasmic vesicles (Fig. 1b-d),

nuclear swelling (Fig. 1c,d) and changes in shape (Fig. 2b-2d), vacuolization (Fig. 1b-1d), cellular hypertrophy (Fig. 2b-2d) were seen in some islets cells, but not in the acinar cells of the exocrine pancreas and in the islet cells of control animals.

We also observed cellular atrophy, necrosis, hypertrophy, elongation, changes in cell shape and in cell orientation in the islet of 139H-infected hamsters (Fig. 3a, small arrow). As shown in Figs. 2 and 3, two types of vacuolization were seen. "Localized vacuolization" (LV) has a distinct edge and is restricted to and confined within the cell (Figs. 2c and 3b). "Diffuse vacuolization" (DV) has no distinct edge, and can be seen scattered within tissues either inside or

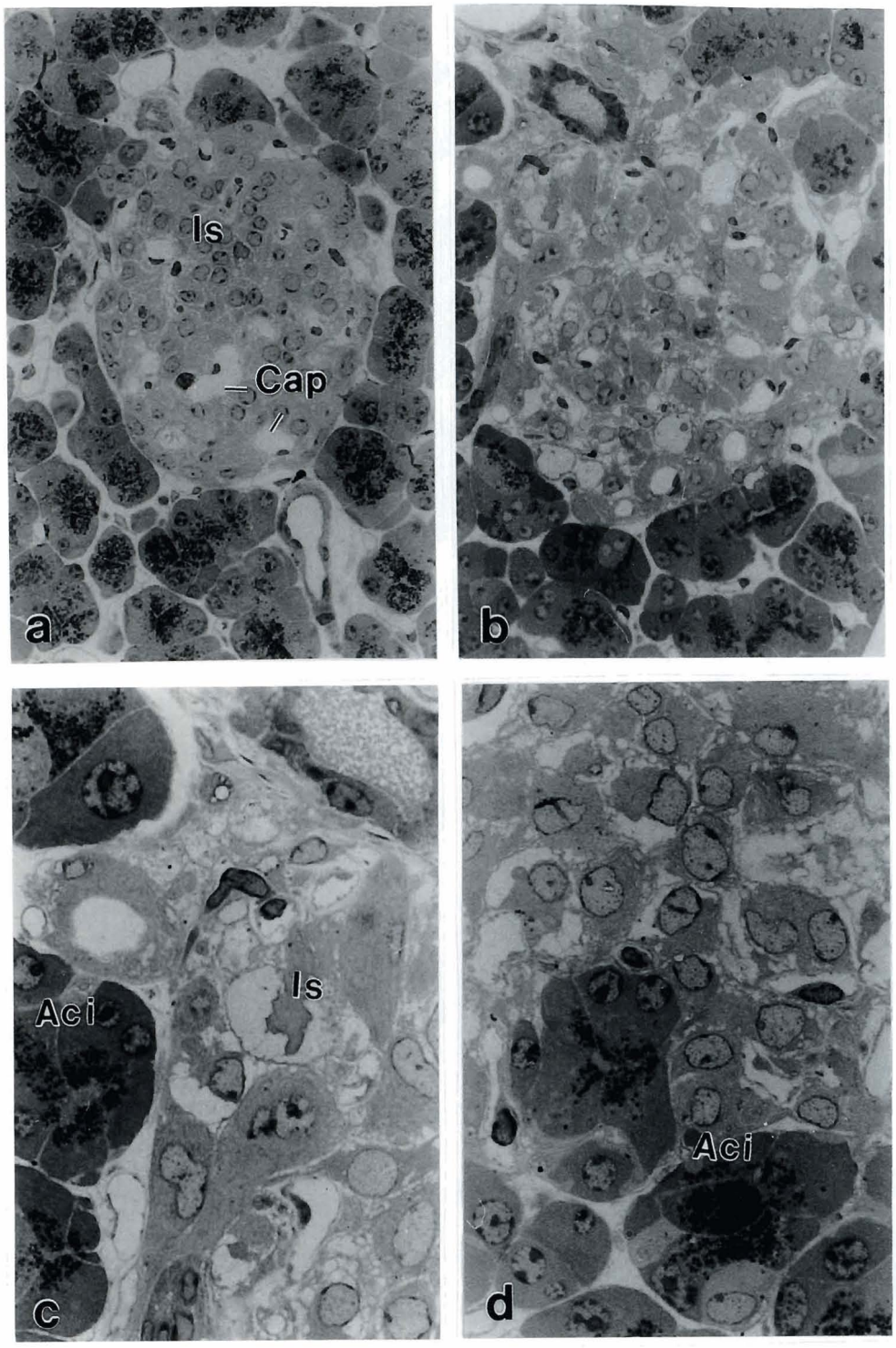

Fig. 1. Comparison of islets of Langerhans in control and 139H-infected hamsters. **a.** Pancreatic islets of a control hamster. **b.** Pancreatic islets of 139H-infected hamsters. **c** and **d.** Pancreatic islets of a 139H-infected hamster. Aci: acini, is: islet. Cap: capillary. a and b, x 328; c and d, x 820

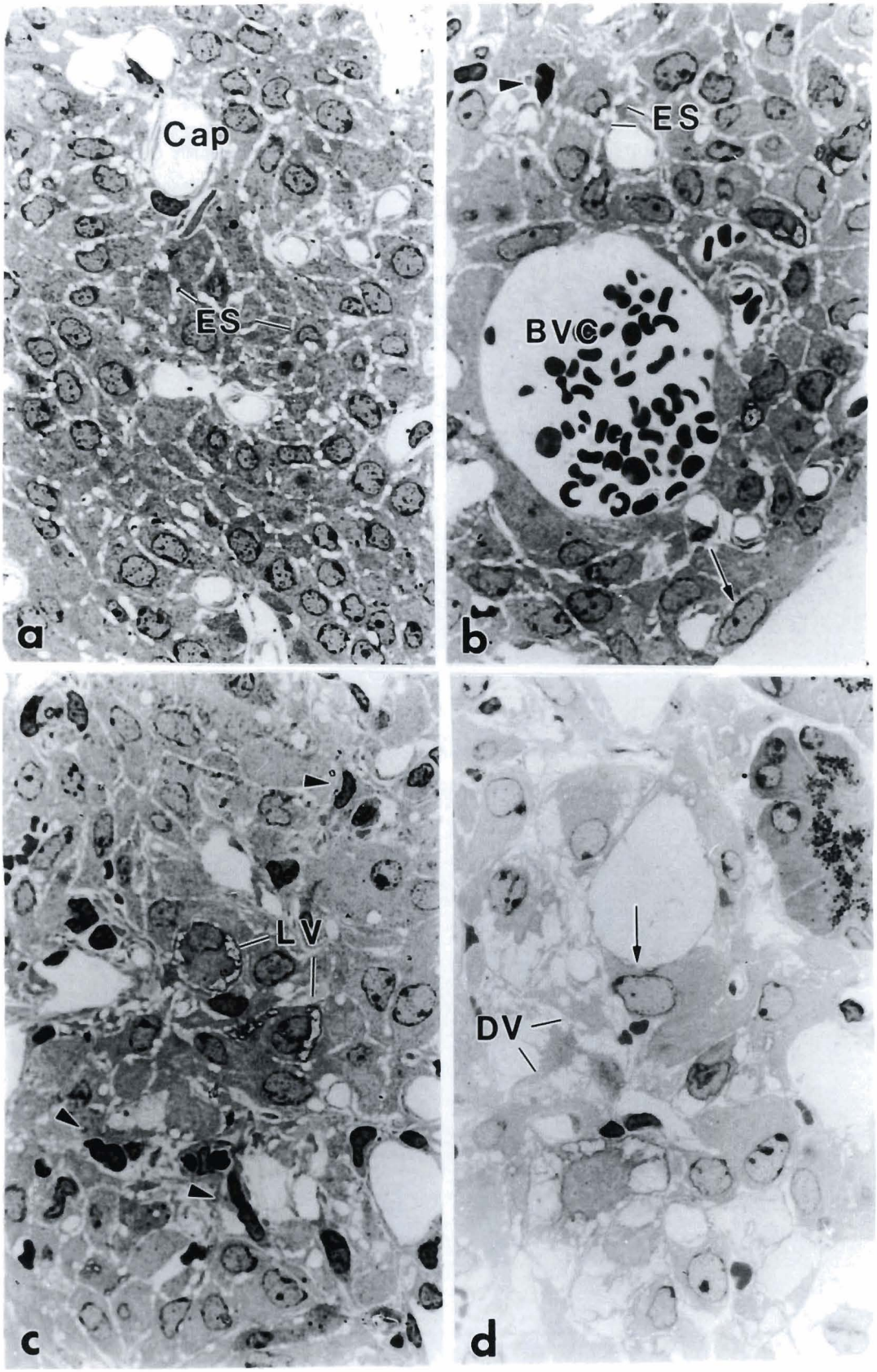

Fig. 2. Vacuolization in pancreatic islets from 139H-infected hamsters. **a.** Pancreatic islet of a control hamster. **b, c** and **d.** Pancreatic islets of 139H-infected hamsters. BVC: blood vessel core; Cap: capillary; DV: diffuse vacuolization; LV: localized vacuolization; we can see cellular hypertrophy, cytoplasmic vesicles, and enlarged extracellular spaces (ES). Nuclear pathological changes such as swelling (small arrow) and changes in shape are seen. Many nuclei (arrow head) that occur between islet cells appear to be similar to the nuclei of macrophages. x 820

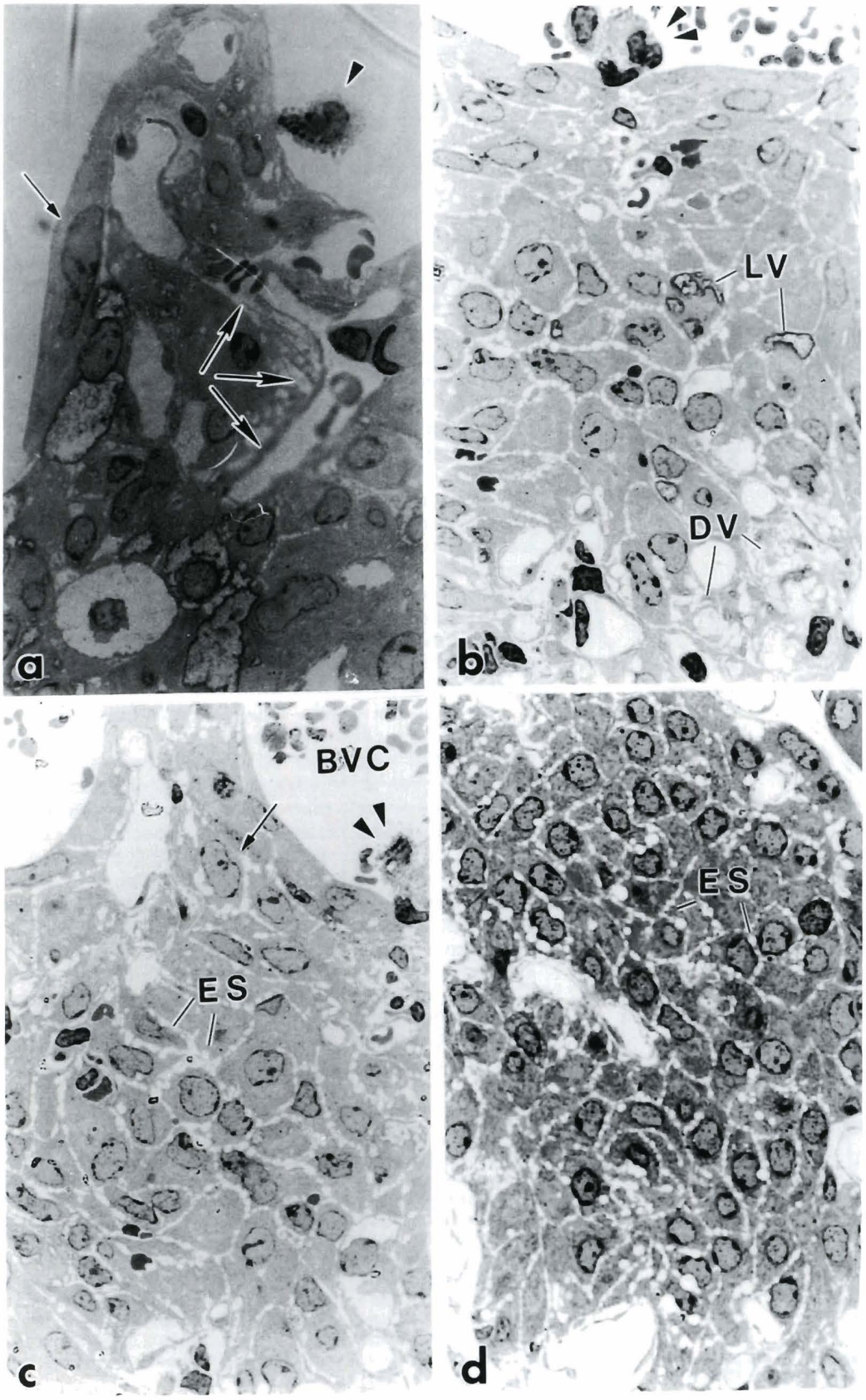

Fig. 3. Margination and diapedesis of macrophages through islet B-cells (the wall of the BVCs) in islets of 139H-infected hamsters. **a, b** and **c.** Pancreatic islets of 139H-infected hamsters (semi-thin section). **d.** Pancreatic islet of a control hamster (semi-thin section). BVC: blood vessel core; DV: diffuse vacuolization; ES: extracellular space; LV: localized vacuolization; Nuclear swelling and changes in nuclear shape (small arrow). A single macrophage (arrowhead) and a group of macrophages (double arrowheads) are shown attached to B-cells at the wall of BVC, with the nuclear headed down to the islet parenchyma through the junction of the islet cells. A channel connection between the BVC and the central part of the islet (three medium arrows) is evident. x 820

outside of cells (Figs. 2d and 3b). These DV may span intracellular and extracellular regions of the tissues.

In normal physiological condition, islet cells were separated each other by extracellular spaces, under light microscopy, these extracellular spaces look like necklace chains inside islets (Fig. 2). In spite of many necklace-like chains, which correspond to the extracellular spaces, between cells, islet cells maintained intimate contacts with each other at multiple points in both control and 139H-infected hamsters (Figs. 1-3). The contact points indicate a close relationship of islet cell plasma membranes. Such points might correspond to tight junctions (Orci, 1976). In our study, these extracellular space and contact points were changed significantly in 139H-infected hamsters compared with control hamsters. In the case of cellular hypertrophy, the increase of the cellular volume caused cell-to-cell contact closely, therefore the extracellular space was narrow or did not exist (Figs. 2c, 3a), while cellular atrophy or necrosis caused the extracellular space enlarged and the tight junctions disappear (Fig. 2d).

In the central portion of the enlarged islets of 139H-infected hamsters there were large cavities with a large mass of blood cells, termed blood vessel cores (BVCs) (Ye et al., 1994a). In present study, endothelial cells and basement membrane were not seen around BVCs. We used immunocytochemical staining for insulin antibody on the semi-thin sections, it is found that BVCs were surrounded by the B-cells of the islets (not shown). We also observed channels containing red blood cells which followed a course from the BVC into other portion of the islets; in these channels, red blood cells, which closely contact with islet cells, may come from a damaged capillary (Fig. 3a, medium arrows).

By semi-thin section, margination and diapedesis of inflammatory cells (macrophages, neutrophils and lymphocytes) were observed relatively more clear than paraffin section at the edge of BVCs. Most of the time, we observed a single macrophage-like inflammatory cell (Fig. 3a, arrowhead) attached to the pancreatic B-cell at the edge of BVC. A clump of condense lysosomes was observed at the side of the inflammatory cell which contact closely with B-cell, while the nucleus was found at the other side. A most remarkable observation was that a group of two or more inflammatory cells attached each other and formed a ball-shape structure. This group of inflammatory cells attached closely to the pancreatic B-cells. One of the nucleus headed into the junction between B-cells and leading the group of inflammatory cells transverse through the cellular junction into the

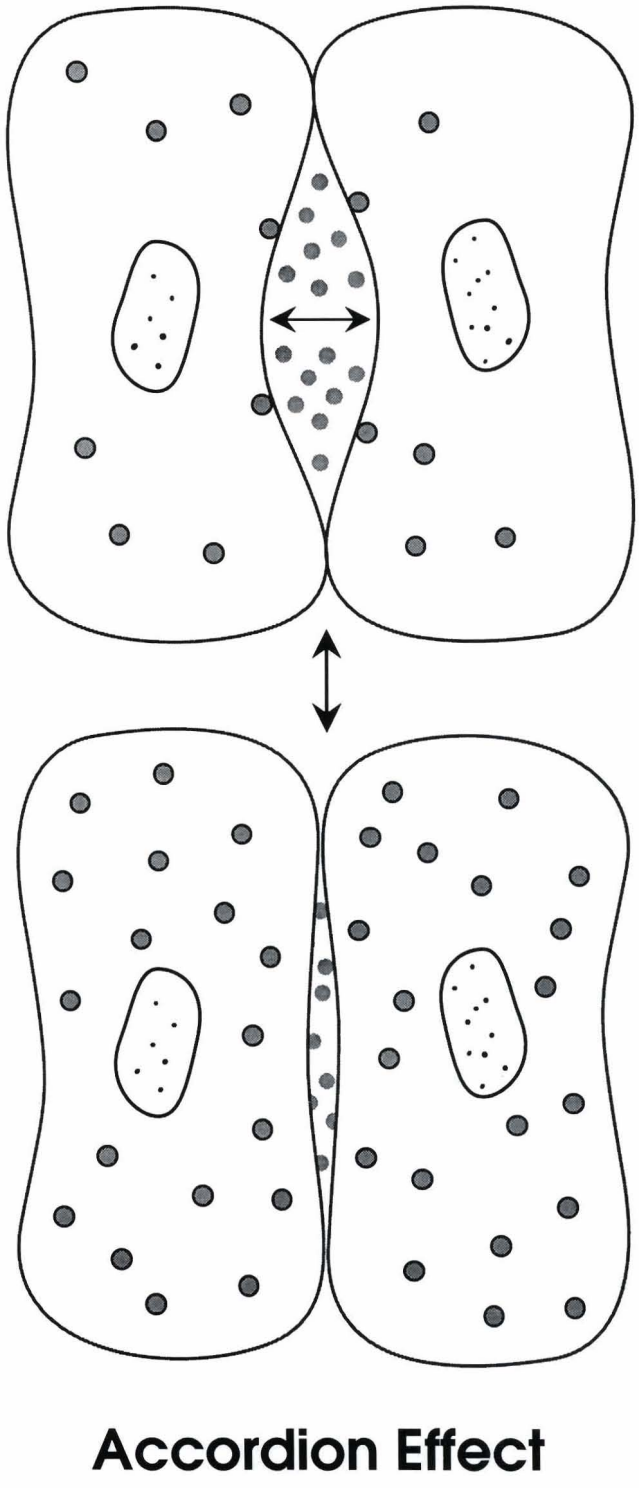

Fig. 4. The concept of "the accordion effect". Under normal physiological conditions, as the synthesis of secretory hormone increases in cells, the volume of cells would increase and the volume of extracellular space would decrease. After a synchronized secretory response from the stimulated endocrine cells, the secretory product would move from the intracellular space into the extracellular space, the volume of the cells would be decreased and the volume of the extracellular space would be increased. Some of the secretory product might be released into the blood stream, whereas the rest would remain in the enlarged extracellular space. The cycle would then repeat. Thus, the expandable extracellular space would serve as a buffer system and a reservoir to collect and store some secretory products for future use. We refer to this concept as "the accordion effect".

islet (Fig. 3b,c, double arrowheads). There were also nuclei which phenotypically consistent with those of macrophages between the islet cells in 139H-infected hamsters (Fig. 2c, arrowhead). There were no BVCs and no inflammatory reaction in the islets of control animals.

Discussion

As reported previously (Ye et al., 1994a), there are a number of cytopathological changes in the islets of hamsters infected with the 139H strain of scrapie including vacuolization, cellular atrophy, necrosis, abnormal cell shape and orientation. The semi-thin sections provide more detail morphological changes in the islets of Langerhans infected with 139H strain of scrapie.

While islets of Langerhans contain cords and irregular clumps of cells and capillaries, previous studies indicate that there is spatial restriction and compartmentalization of islet secretory products. For example, as reported by Samols and Harrison (1976), very low concentrations of somatostatin or glucagon introduced into the arterial supply of islets can profoundly alter the release of other islet hormones. It is thought that the concentration of somatostatin in that portion of interstitial space into which D cells release somatostatin must be extremely high, almost certainly more than 1,000-fold higher than those quantities which can influence A and B cell functions (Samols et al., 1983). Therefore, if there were not some form of physiologic and anatomic compartmentalization, the concentration of somatostatin released by D cells would be high enough to inhibit all of the A and B cell functions within the islets (Samols et al., 1983).

One kind of compartmentalization could be created by tight junctions, which are narrow areas of fusion between adjacent homogeneous and heterogeneous islet cell types (Orci, 1976). These tight junctions could create barriers within the interstitial spaces such that secretory products could be shunted through specific channels between the cells. By freeze-fracture replica study, Orci (1976) has shown that the islet cell membrane face contains a network of short ridges or fibrils, which are characteristic of tight junctions. These ridges are amenable to profound modification under different functional conditions.

The cytopathological changes that we have described in the islets of 139H-infected hamsters, such as cellular hypertrophy, changes in cellular shape and orientation, cellular atrophy, necrosis and vacuolization could affect the tight junctions and cellular compartmentalization between islet cells. The effects of cellular hypertrophy and cellular atrophy on tight junctions and cellular compartmentalization would be totally different. Cellular hypertrophy will narrow extracellular space, and block the normal channels connection between cells. Cellular atrophy and necrosis will enlarge the extracellular space and disturb the tight junctions between cells.

Earlier studies showed that pronase treatment of isolated islets led to an augmentation of gluclose-induced insulin release associated with a striking proliferation of tight junctional elements, thus trapping secretory material in pockets of extracellular space (Orci, 1976). These pockets might exist not only between homogeneous cell types but also between heterogeneous cells, and they could serve to store secretion products of the islet cells. Similar to those studies, we found many necklace-like chains of cystic spaces between islet cells; they were part of the extracellular space and might serve a role in hormone storage. It has been suggested that: (1) The activity of B cells changes after islets are exposed to insulin secretagogues (Dean and Matthews, 1970). (2) There are B-cell pacemaker cells which respond to particular glucose concentrations and, in turn, recruit other B cells which otherwise might not have responded to glucose (Meda et al., 1980a,b,c). (3) cAMP and Ca^{++} can pass through gap junctions of B cells which will ensure a synchronized secretory response (Darnell et al., 1986). (4) There are numerous exocytotic stomata in B cell membranes, most of which are situated within the tight junctional domain, and the contents of these stomata are released into the extracellular spaces (Orci, 1976). (5) After stimulation, the intercellular space is expanded at several places so that large amounts of released secretory products may be accommodated. Following stimulation, the B cells appear to be degranulated (Orci, 1976).

According to the above information and our study, in order to explain a possible role of the extracellular space in pancreatic islets, we have developed the following hypothesis for "the accordion effect" (Fig. 4): under normal physiological conditions, as the synthesis of insulin increases in B cells, the volume of B cells will increase and the volume of extracellular space will decrease. After a synchronized secretory response from the stimulated B cells, the secretory product would move from the intracellular space into the extracellular space; the volume of the B cells would be decreased and the volume of the extracellular space would be increased. Some of the secretory product might be released into the blood stream, whereas the rest would remain in the enlarged extracellular space. The cycle would then repeat. As a result, the volume of B-cells will increase slowly and squeeze the remaining secretory products out of the extracellular space gradually. Thus, the expandable extracellular space would serve as a buffer system and a reservoir to collect and store some secretory products for future use. We anticipate that the concept of "the accordion effect" may also be true in other endocrine organs such as pituitary gland and adrenal gland.

Our studies showed that the extracellular space was enlarged in the islets of 139H-infected hamsters compared to control hamsters. The islet cell-to-cell contacts were reduced in DV regions and the extracellular spaces in these regions were enlarged. These findings suggest that the synchronized secretory

Table 1. The pathways for inflammatory cells passing through the blood-tissue barriers.

PATHWAY	CELL TYPE	EXAMPLE	REFERENCE
Between cell-junction	PMLs	Lymphatic tissue	Marchesi and Gowans, 1964; Schoefl, 1972
	Lymphocytes	Lymphatic tissue	Marchesi and Gowans, 1964; Schoefl, 1972; Cho and De Bruyn, 1986
	Mononuclear cells	EAE	Lampert, 1967
	ICs	Islet BVC	Ye and Carp, 1995
Penetrating through endothelial cell	Lymphocytes	Lymphatic tissue	Marchesi and Gowans, 1964; Schoefl, 1972; Cho and De Bruyn, 1986
Parajunctional migration pathway	ICs	Lymph nodes (guinea pig)	Cho and De Bruyn, 1986
	Leukemic cell	Sinusoid (liver)	Azzarelli et al., 1985
	ICs	CREAE	Lossinsky et al., 1989
	PMLs	Brain inflammation produced by α-bungarotoxin	Faustmann and Dermietzel, 1985; Faustman et al., 1987

PMLs: polymorphonuclear leukocytes; EAE: experimental allergic encephalomyelitis; BVC: blood vessel core; ICs: inflammatory cells; CREAE: chronic relapsing EAE.

response of B-cells in DV regions could be damaged and which could alter the accordion effect and impair normal function.

We observed a variety of pathological patterns in islets of 139H-infected hamsters, such as B-cell proliferation, BVC formation, LV, DV, cellular hypertrophy, cellular atrophy, changes in cell orientation and polarity, nuclear swelling, ring-form nuclei, pyknosis, karyorrhexis, and karyolysis. LV is similar to or the same as the intracellular vacuolization seen in stained paraffin sections. DV is related to the extracellular vacuolization seen in stained paraffin sections. In part, DV and extracellular vacuolization might be the result of cell death. All these pathological changes did not occur in the islets of Langerhans of 263K-infected hamsters (Srinivasappa et al., 1989; Ye et al., 1994a,b).

Different types of pathological changes can occur in the same region of a tissue. It is not clear whether these different pathological patterns are a function of the sequence of events related to injury of a specific cell type or are caused by different pathologic agents acting in the same region. Different structures and functions of the cells in the particular tissue regions could lead to several pathological patterns being caused by the same agent. This agent might be (1) endogenous, i.e. produced by the cell itself, such as a toxic by-product (such as prion protein PrP^{Sc}) or "free radical" (such as nitric oxide); or (2) exogenous, i.e. received from outside the cell. Although the direct agents causing cell injury in scrapie are unknown, it is possible that the pathologic agents can transmit from one cell to another, thereby causing a domino or a cascade effect in a particular area. The transmission pathway can be gap junctions, endocytosis or diffusion.

The pathogenesis of the lesions in the islet of Langerhans and in the brain might be totally different. It is found that PrP^{Sc} is essential for infectivity and neuronal lesions in scrapie (Prusiner, 1987; Taraboulos et al., 1992). However, the level of PrP^{Sc} and infectivity in pancreas were at least 1000 times less than in the brain (Carp et al., 1990; Ye et al., 1994a,b). This suggests that scrapie agent might not directly cause lesions in the pancreas islets, instead, it might target on specific neurons in hippocampal, hypothalamic and/or cells in pituitary that are regulating normal functions of the islet of Langerhans.

Another phenomenon observed was the interaction between groups of white blood cells (WBCs) with B-cells at the wall of BVCs and inside the islets of Langerhans. We have referred to this phenomenon as "linkage-reaction"; this group of WBCs as "linkage-WBCs". There were also nuclei which resemble those of macrophages between the islet cells in 139H-infected hamsters. Our study indicates that cellular injury and inflammatory reactions occur in the islets of Langerhans of 139H-infected hamsters.

Different types of inflammatory cells might select different routes to penetrate the various blood-tissue barriers (Table 1). Such as: (1) by passing between endothelial cells junctions, the examples of this include: (a) polymorphonuclear leukocytes (PMLs) cross the endothelium cell barrier in lymphatic tissue (Marchesi and Gowans, 1964; Schoefl, 1972); (b) mononuclear cells traverse blood-tissue barriers in experimental allergic encephalomyelitis (EAE) (Lampert, 1967). (2) by penetrating through the endothelial cell, such as: lymphocytes were also shown to either cross through the endothelium cell or passing between endothelium cells (Marchesi and Gowans, 1964; Schoefl, 1972; Cho and De Bruyn, 1986). (3) by a parajunctional migration pathway, this type of emigration has been observed in many studies, including: (a) emigration of inflammatory cells in post-capillary high-endothelial venules from guinea pig lymph nodes (Cho and De Bruyn, 1986); (b) hepatic sinusoidal emigration of leukemic cells

(Azzarelli et al., 1985); (c) inflammatory cells transport across the blood-brain barrier in a murine model of chronic relapsing experimental allergic encephalomyelitis (CREAE) (Lossinsky et al., 1989); (d) PMLs emigrate across the cerebral microblood vessels in a model of brain inflammation produced by α-bungarotoxin (Faustmann and Dermietzel, 1985; Faustmann et al., 1987). Tumor cells can also migrated in leptomeningial veins specially at regions adjacent to the junctions (Azzarelli et al., 1984). Based on these studies, it appears plausible that sensitized inflammatory cells select a trans-endothelial cell rather than a trans-junctional route for passage from the blood circulation to the brain, therefore, supports the concept that inflammatory cells probably avoid attempts to break open tight endothelial cell junctions (Lossinsky et al., 1989).

The common characteristic of the above studies are the interaction between inflammatory cells and blood vessel endothelial cells. Our study revealed other scenario, that is: (1) inflammatory cells can also interact with non-blood vessel cells (islet B-cells); (2) the linkage-inflammatory cells migrate from BVC into islet tissue through the junction of islet cells. However, this does not exclude other migration pathways (such as: penetrating through islet cells or parajunctional migration pathway). Furthermore, we observed one of the nucleus in the linkage-inflammatory cells leads downward into the islet tissue while the others follow. It is interesting to known, in vitro, lymphocytes also have a distinct polarity and, frequently, the nucleus leads while most of the cytoplasm is trailed behind (De Bruyn, 1946; Trowell, 1965). In lymphoid tissue, lymphocytes were often seen in groups and migrate across through the vascular endothelium together (Schoefl, 1972). The process might also involve chemokinetic reaction, receptors activation and recognition between the inflammatory cells and between the inflammatory cells and the islet cells in this study and the endothelium cell in lymphoid tissue. Whether the cellular and molecular mechanism of the interaction between inflammatory cells with B-cells is similar to that with endothelial cells is still not known.

In summary, 139H scrapie strain induced hyperinsulinemia and obesity in hamster. Hyperinsulinemia is a common feature of most obesities. Our studies suggested that the increase in B cells in the islets would account for hyperinsulinemia and obesity in 139H-infected hamsters (Ye et al., 1994a,b). Recent study also indicated that increased B cell proliferation precedes hyperinsulinemia and obesity in yellow $A^{vy/-}$-mice (Warbritton et al., 1994). Mice infected with canine distemper virus (CDV) became obese, with greatly enlarged pancreatic tissue and hyperinsulinemia (Nagashima et al., 1992). All these studies suggested insulin play an important regulatory role in the development of obesity in many instances. Because scrapie infectivity and PrP^{Sc} level in the pancreas of 139H-infected hamsters is very low (Carp et al., 1990; Ye et al., 1994a,b), it is unlikely that the scrapie agent directly induces an inflammatory response in the islets of Langerhans. It would seem, therefore, that the inflammatory reaction in the islets of 139H-infected hamsters is due to the secondary effect of B cell damage seen in this study and reported previously (Ye et al., 1994a,b).

Acknowledgements. We thank Drs. A. Scallet, J. Fikes and B. Bolon for editing and excellent assistance in preparation of this manuscript. Dr. J. Shek and Mr. R. Weed for help and Ms M.E. Cafaro for assistance with the layout of figures. This work was supported by the New York State Department of Mental Hygiene and NIH grant AGO 9017. X. Ye was supported in part by a fellowship from the CSI/IBR Center for Developmental Neuroscience and by an appointment to the Post-graduate Research Program at the National Center for Toxicological Research administered by the Oak Ridge Institute for Science and Education through an interagency agreement between the U.S. Department of Energy and the U.S. Food and Drug Administration.

References

Azzarelli B., Muller J., Mirkin L.D. and Goheen M. (1985). Transvascular migration of L2C leukemic cells studied in the liver of the guinea pig. Virchows Arch. (A) 406, 425-440.

Carp R.I., Kim Y.S. and Callahan S.M. (1990). Pancreatic lesions and hypoglycemia-hyperinsulinemia in scrapie-injected hamsters. J. Infect. Dis. 161, 462-466.

Chandler R.L. (1963). Experimental scrapie in the mosue. Res. Vet. Sci. 4, 276-285.

Cho Y. and De Bruyn P.P.H. (1986). Internal structure of the post-capillary high-endothelial venules of rodent lymph nodes and Peyer's patches and the transendothelial lymphocyte passage. Am. J. Anat. 177, 481-490.

Darnell J., Lodish H. and Baltimore D. (1986). Molecular cell biology. Scientific American books, Inc.

Dean P.M. and Matthews E.K. (1970). Glucagon-induced electrical activity in pancreatic islet cells. J. Physiol. 210, 255-264.

DeBruyn P.P.H. (1946). The amoeboid movement of mammalian leukocyte in tissue culture. Anat. Rec. 96, 177-184.

Faustmann P.M. and Dermietzel R. (1985). Extravasation of polymorphonuclear leukocytes from the cerebral micro-vasculature. Inflammatory repsonse induced by alpha-bungarotoxin. Cell Tissue Res. 242, 399-407.

Faustmann P.M., Teutrine S. and Dermietzel R. (1987). Functional ultrastructure of the blood-brain barrier under acute inflammatory reactions. In: Stroke and microcirculation. Cervós-Navarro J., Ferszt R. (eds). Raven Press. New York. pp 285-288.

Gajdusek D.C. (1977). Unconventional viruses and the origin and disappearance of Kuru. Science 197, 943-960.

Kimberlin R.H. (1989). Introduction to scrapie and perspectives on current scrapie research. In: Alzheimer's disease and related disorders. Igbal K., Wisniewski H.M. and Winblad B. (eds). Alan R. Liss Inc. pp 559-566.

Kimberlin R.H. and Walker C.A. (1986). Pathogenesis of scrapie (strain 263K) in hamsters infected intracerebrally, intraperitoneally or intraocularly. J. Gen. Virol. 67, 255-263.

Kimberlin R.H., Walker C.A. and Fraser H. (1989). The genomic identity

of different strains of mouse scrapie is expressed in hamsters and preserved on reisolation in mice. J. Gen. Virol. 70, 2017-2025.

Lampert P. (1967). Electron microscopic studies on ordinary and hyper-acute experimental allergic encephalomylitis. Acta Neuropathol. 9, 99-126.

Lossinsky A.S., Badmajew V., Robson J.A., Moretz R.C. and Wisniewski H.M. (1989). Sites of egress of inflammatory cells and horseradish peroxidase transport across the blood-brain barrier in a murine model of chronic relapsing experimental allergic encephalo-myelitis. Acta Neuropathol. 78, 359-371.

Marchesi V.T. and Gowans J.I. (1964). The migration of lymphocytes through the endothelium of venules in lymph nodes: and electron microscope study. Proc. Roy. Soc. Lond. B 159, 283-290.

Meda P., Halban P., Perrelet A., Renold A.E. and Orci L. (1980a). Gap junction development is correlated with insulin content in the pancreatic B cell. Science 209, 1026-1028.

Meda P., Denef J.F., Perrelet A. and Orci L. (1980b). Non-random distribution of gap junctions between pancreatic B cells. Am. J. Physiol. 238, C114-C119.

Meda P., Perrelet A. and Orci L. (1980c). Gap junctions and B cell function. In: Biochemistry and biophysics of the pancreatic B cells. Malaisse W.J., Taljedal I.B. (eds). Thieme. Stuttgart. pp 157-162.

Nagashima K., Zabriskie J.B. and Lyons M.J. (1992) Virus-induced obesity in mice: association with a hypothalamic lesion. J. Neuropathol. Exp. Neurol. 51, 101-109.

Orci L. (1976). The microanatomy of the islets of Langerhans. Metabolism 25, 1303-1313.

Parry H.R. (1983). Scrapie disease in sheep. Historical, clinical, epidemiological, pathological and practical aspects of the natural disease. Oppenheimer D.R. (ed). Academic Press, Inc. London.

Prusiner S.B. (1987). Prions and neurodegenerative diseases. N. Engl. J. Med. 317, 1571-1581.

Samols E. and Harrison J. (1976). Remarkable potency of somatostatin as a glucagon suppressant. Metabolism (Suppl 1) 25, 1495-1497.

Samols E., Weir G.C. and Bonner-Weir S. (1983). Intraislet insulin-glucagon-somatostatin relationships. In: Glucagon II. Handbook of experimental pharmacology, 66. Lefebvre P.J. (ed). Springer-Verlag. Berlin. pp 133-173.

Schoefl G.I. (1972). The migration of lymphocytes across the vascular endothelium in lymphoid tissue. J. Exp. Med. 136, 568-578.

Srinivasappa J., Asher D.M., Pomeroy K.L., Murphy L.J., Wolff A.V., Yoon J.W., Gajdusek D.C. and Notkins A.L. (1989). Scrapie-induced diabetes mellitus in hamsters. Microb. Pathogenesis 7, 189-194.

Taraboulos A., Jendfroska K., Serban D., Yang S.L., DeArmond S.J. and Prusiner S.B. (1992). Regional mapping of prion proteins in brain. Proc. Nalt. Acad. Sci. USA 89, 7620-7624.

Trowell O.A. (1965). Lymphocytes. In: Cells and tissue culture. Willmer E.N. (ed). Academic Press, Inc. New York. p 95.

Warbritton A., Gill A.M., Yen T.T., Bucci T. and Wolff G.L. (1994). Pancreatic islet cells in preobese yellow $A^{Zy/-}$ mice: relation to adult hyperinsulinemia and obesity. Proc. Oncol. Exp. Biol. Med. 206, 145-151.

Ye X. and Carp R.I. (1995). The pathological changes in peripheral organs of scrapie-infected animals. Histol. Histopathol. 10, 995-1021.

Ye X., Carp R.I. and Kascsak R.J. (1994a). Histopathological changes in the islets of Langerhans in scrapie 139H-affected hamsters. J. Comp. Pathol. 110, 153-167.

Ye X., Carp R.I., Yu Y., Kozielski R. and Kozlowski P. (1994b). Hyperplasia and hypertrophy of B cells in the islets of Langerhans in hamsters infected with the 139H strain of scrapie. J. Comp. Pathol. 110, 169-183.

Accepted September 1, 1995