Histol Histopathol (1999) 14: 673-678

http://www.ehu.es/histol-histopathol

Histology and Histopathology

Abnormal periodic acid-Schiff (PAS)-positive substance in the islets of Langerhans, pituitaries and adrenal glands of 139H scrapie-infected hamsters

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Summary. Previous studies showed that the 139H strain of scrapie injected intra-cerebrally in hamsters caused obesity, and extensive histopathological changes in islets of Langerhans and pituitaries. In the current study, we report that an abnormal granular substance, which stained positively with periodic acid-Schiff (PAS-positive substance; PPS), was found in the islets of Langerhans, pituitaries, adrenal glands, in the lumens of blood vessel cores (BVCs) and in blood vessels in 139H-infected hamsters, but not in either 263K-infected or control hamsters. This substance was found in the endocrine organs, forming grape-like or plaque-like structures, which were small, round to ovoid, and homogenous measuring up to 7μm in diameter and usually grouped in clusters. PPS was not found in the brains of control or scrapie-infected hamsters. Using immunostaining for amyloid protein (PrP, ßA4), as well as Congo red and thioflavin-S stains, no evidence was found of amyloid plaque formation in the islets of Langerhans, the adrenal glands, or the pituitaries of 139H- or 263K-infected hamsters. PPS might relate to the pathological changes in the endocrine organs in 139H-infected hamsters.

Key words: Scrapie, Hamster, PAS-positive substance, Pituitary, Islet of Langerhans

Introduction

Scrapie is a natural disease in sheep and goats and is the archetype of a group of diseases referred to as transmissible spongiform encephalopathy or prion diseases. The scrapie agents have been transmitted to a variety of laboratory animal species, including mice and hamsters (Chandler, 1963; Kimberlin et al., 1989). In some scrapie isolate-mouse strain or hamster strain combinations, there is an increase in body weight that starts prior to the onset of the typical motor dysfunction that signals the start of clinical disease (Carp et al., 1990). Hamsters injected intra-cerebrally (IC) with scrapie strains 139H or 22CH become obese prior to the appearance of the motor changes. During the latter part of the pre-clinical and throughout the clinical phase of the disease, animals were hypoglycemic and showed marked hyperinsulinemia with values as much as x 49 higher than those seen in controls (Carp et al., 1990). At autopsy, there was marked hyperplasia and hypertrophy of the cells of the islets of Langerhans. The thyroid, adrenal glands, liver and kidneys were also enlarged. In contrast, hamsters injected with the commonly used 263K strain of hamster-adapted scrapie did not show any of the above changes. The total body weight of these animals was the same as that of the ones injected with normal hamster brain throughout the preclinical and clinical periods (Carp et al., 1990; Kascsak et al., 1991).

The differences observed between 139H- or 22CH- and 263K-infected hamsters are most probably a function of differences in targeting of brain regions and/or of neuronal cell types by the different scrapie strains. It is known that targeting can vary for different scrapie strains (Carp et al., 1990; Hecker et al., 1992; Ye and Carp, 1995a).

While the data for 139H and 22CH strains suggested that they induced a severe generalized endocrinopathy, the most prominent pathological changes were found in the islets of Langerhans (Carp et al., 1990). It has been reported that pancreatic changes play an important role in the obesity of 139H-infected hamsters (Ye and Carp, 1995a).

In previous publications, it was reported that 139H-infected hamsters had extensive pathological changes in the islets of Langerhans and the pituitaries (Ye et al., 1994a,b; Ye and Carp, 1996b). The changes included blood vessel cores formation, cellular atrophy; fibrosis; cytoplasmic vesicles and pyknosis, plus karyorrhexis and karyolysis of the nuclei. In the current study, by using immunostaining, Congo red and thioflavin-S staining, no evidence of amyloid plaque formation in the islets of Langerhans, adrenal glands and pituitaries of 139H-infected hamsters was found. However, by using periodic acid Schiff (PAS) stain and PAS with orange G

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stain, abnormal PAS-positive substance (PPS) was found in these endocrine glands in 139H-infected hamsters but not in control or 263K-infected hamsters. This substance might relate to the pathological changes in the endocrine organs of 139H-infected hamsters.

Materials and methods

Animals

Female, weanling LVG/LAK hamsters were obtained from Charles River (Wilmington, MA) and maintained in a temperature- and humidity-controlled room. Hamsters were given food and water *ad libitum*. Animals were injected with 40 μl intra cerebrally 1-2 weeks after arrival.

Inocula

Three inocula were used: normal hamster brain (NHB), and scrapie strains 139H or 263K. Scrapie strains were provided by Dr. Richard H. Kimberlin (SARDAS, Edinburgh, UK), and are maintained in the laboratory by hamster-to-hamster passage. The characteristics of passaged materials were exactly the same as those of the strains obtained from Dr. Kimberlin. Passages were done by routine intracerebral injection of 40μl of brain homogenate prepared in cold (4 °C) phosphate-buffered saline. Homogenization was done using 20 strokes of a hand-operated Ten-Broeck homogenizer (see Carp et al., 1990).

Tissue preparation

These experiments used paraffin sections for histological staining, and paraffin sections and vibratome sections for immunostaining. At the end of the incubation period (about 115-125 days post-injection for 139H), the animals were anesthetized with sodium pentobarbital (intra peritoneally, 70 mg/kg body wt).

The anesthetized animals were perfused through the heart with saline (0.9%) at room temperature for a few minutes (15 ml/min) then with 4% paraformaldehyde and 0.05% glutaraldehyde in 0.1M sodium phosphate buffer (pH 7.4, 15 ml/min) for 10-15 min, and then decapitated. The pituitary was removed with the brain. The other organs, such as a splenic portion of the pancreas and adrenal glands, were removed immediately, then immersed in the fixative solution for 24 hours at 4 °C. They were then placed in the buffer solution for one day. The tissues were drained thoroughly. Tissues were then cut into small blocks, which were put into labeled carriers. Brain and pancreas blocks were dehydrated in graded alcohols and xylene and embedded in paraffin wax. Six micrometer's thick serial sections were mounted on gelatin-coated slides and allowed to dry overnight at 37 °C.

Immunocytochemistry and histology staining methods

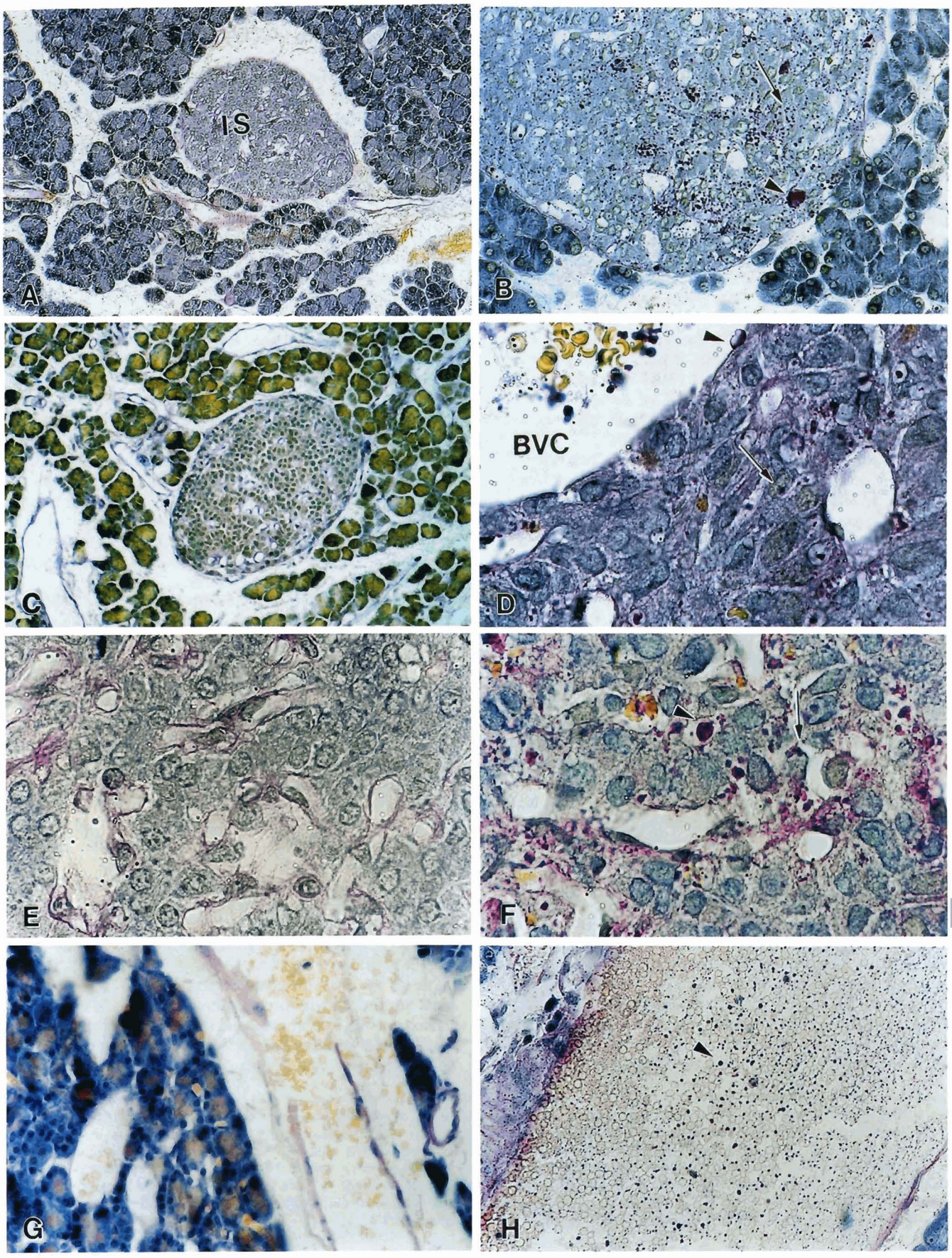
Sections of the adrenal gland, pancreas, pituitary, and brain were stained with immunocytochemistry methods using antibodies for amyloid protein (antibody 3F4 for PrPSc, antibody 4G8 for ßA4) (Ye et al., 1998), as well as routine histological stains including hematoxylin and eosin, PAS, PAS and orange G, Gomori's one step trichrome, Congo red, and thioflavin-S (see Sheehan and Hrapchak, 1980). Gomori's one step trichrome stains collagen fibers yielding a blue-green color. Congo red and thioflavin-S have been used clinically for diagnosis of amyloidosis. Congo red stains amyloid with orange color and has an intense green-red birefringence when viewed with polarized light. Thioflavin-S stains amyloid with fluorescence when observed with U.V. light. The slides were also observed with standard light microscopy for other changes. All the photos and slides of the histological and immunoreactive stained sections in this study were taken with a Carl Zeiss Axiophot.

Results

As shown before (Ye et al., 1994a,b), there were histopathological changes in the islets of Langerhans in hamsters infected with the 139H scrapie strain. Fibrosis, vacuolization, cellular atrophy, cellular elongation, changes in cell shape and orientation could be seen. There were also nuclear pathological changes such as swelling, changes in shape, pyknosis, karyorrhexis and karyolysis. There were no such changes in 263K-infected hamsters.

As shown in Figure 1, using PAS and orange G stain, an abnormal PAS-positive substance was noted in plaque-like forms located in the islets of Langerhans and in blood vessel cores (BVCs) (Fig. 1B,D) in 139H-infected hamsters, but not in 263K-infected hamsters (Fig. 1C) or controls (Fig. 1A). This substance stained red with either PAS stain alone or PAS and orange G stain. It was located both inside and outside the cells in the endocrine organs. Some of the deposits were small (< 0.1μm), others were much larger (>7 μm). This PAS-positive substance was referred to as "PPS"(Fig. 1B,D,

Fig. 1. Periodic Acid-Schiff and Orange G stain of the Islets of Langerhans. **A.** A pancreatic islet of a control hamster (61X) shows the normal structure and no PPS. IS: islet. x 61. **B.** A pancreatic islet of a 139H-infected hamster shows small and large PPS inside (arrow) and outside (arrowhead) of cells. x 244. **C.** A pancreatic islet of a 263K-infected hamster shows no pathological changes or PPS. x 61. **D.** A pancreatic islet of a 139H-infected hamster shows PPS located inside (arrow) and outside the islet cells and in the lumen of a blood vessel core (BVC) (arrowhead). x 610. **E.** A pancreatic islet of a control hamster shows normal structure and capillaries. x 610. **F.** A pancreatic islet of a 139H-infected hamster shows small and large (> 7μm) PPS inside (arrow) and outside islet cells (arrowhead) and near the capillaries.x 610. **G.** A pancreatic acini and blood vessel (BV) of a control hamster shows no PPS in blood vessel. x 610. **H.** A pancreatic blood vessel in a 139H-infected hamster shows PPS in the lumen of a blood vessel (arrowhead) and the wall of a blood vessel. x 610


IS
A
B
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F,H, arrowhead).

In 139H-infected hamsters, there was numerous PPS in the walls of capillaries inside the pancreatic islets and in the lumen of blood vessels outside the islets (Figs 1F and 1H, respectively), but not in those of 263K-infected hamsters and controls. PPS was not found in acinar tissue of either control or scrapie-infected hamsters (Fig. 1A,C,E,G).

As shown in Figure 2, using PAS and orange G stain, the abnormal PAS positive substance was observed in grape-like or plaque-like forms in pituitaries of the 139H-infected hamsters (Fig. 2A,F) but not in control pituitaries (Fig. 2A, 2C) or in pituitaries from 263K-infected hamsters (Fig. 2B). Most of the PAS-positive substance was found in the pars distalis of the pituitary, especially in the areas of vacuolation. Similar PAS-positive substance in grape-like or plaque-like form was found in the adrenal medullas of 139H-infected hamsters (Fig. 2H) but not in those of 263K or control hamsters (Fig. 2G). This substance was located both inside as well as outside the cells of the endocrine organs.

Immunostaining for amyloid protein (antibody 3F4 for PrPSc and antibody 4G8 for ßA4), as well as Congo red and thioflavin-S stains, did not reveal any amyloid staining in pancreatic islets, pituitaries or adrenal glands of 139H-infected hamsters (not shown). PPS was not found in any of the brain sections of scrapie-infected hamsters (not shown).

Discussion

Abnormal depositions of various materials in the islets are regarded as important features of pathological phenomena. Abnormal molecular products may be a reflection of pathological changes that occur at the molecular level. There are several different kinds of pathological changes associated with depositions in pancreatic islets.

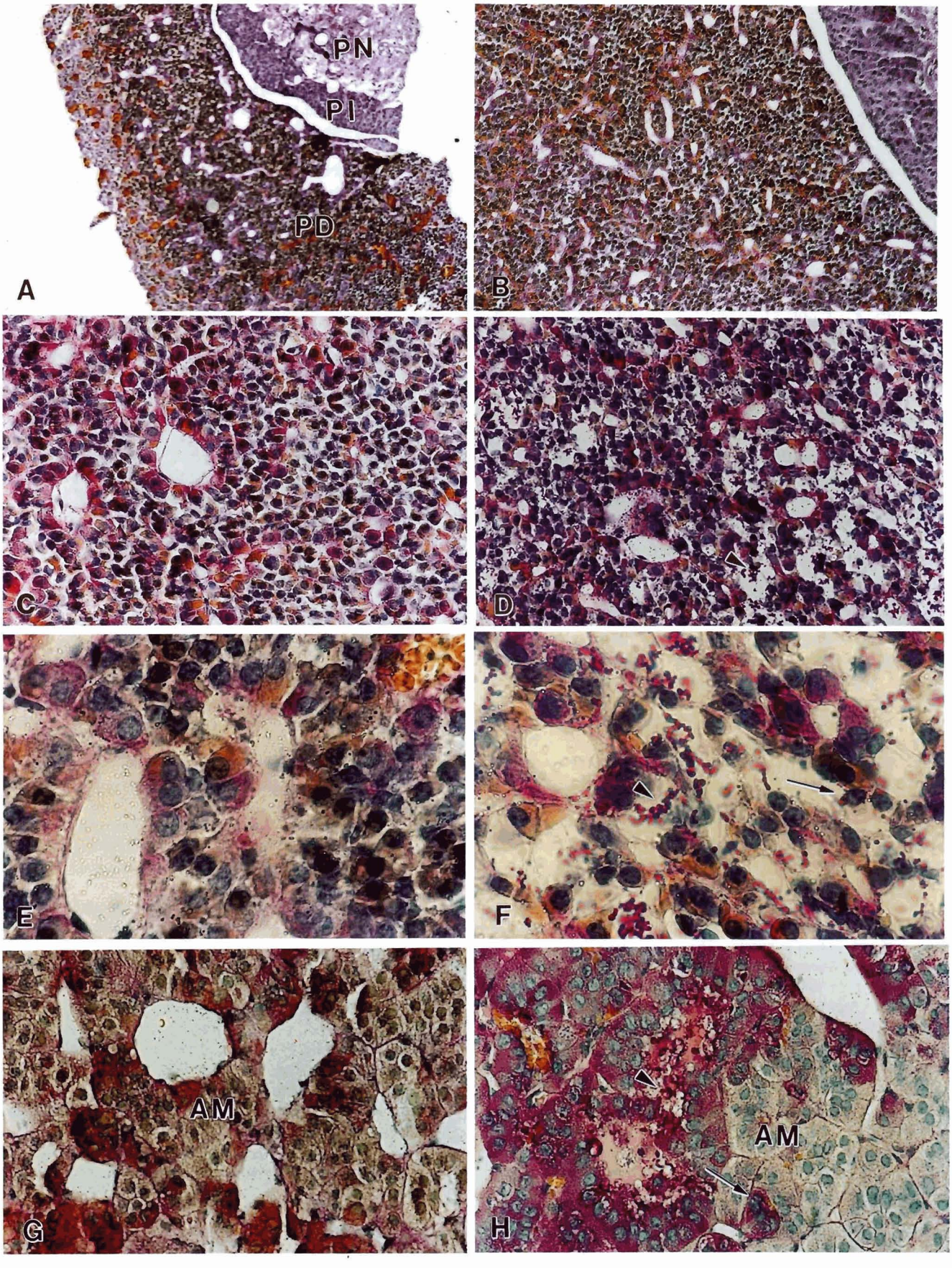
Hyalinization of the pancreatic islets has been described for many years and is now generally considered the most typical pancreatic lesion in diabetes mellitus. Gellerstedt (1938), chiefly on the basis of studies with the methyl violet reaction, concluded that hyaline is actually amyloid and the condition should be called "insular para-amyloidosis". Schwartz (1965) using fluorescent dyes such as thioflavin-T confirmed that hyaline deposits in islets represent a manifestation of senile amyloidosis which was also found in the brain and heart. Under electron microscopy, the amyloid appeared as tightly packed fibrillar bundles which extend into deep recesses in the cells, formed by infolding of the cell plasma membrane. Lacy (1964) found the same type of fine fibrillar structure in hyaline material in islets.

Although the presence of hyaline (amyloid-like material) was first noted in specimens of a human pancreas as early as 1901 by the pathologist Opie, it was not until 1986 that efforts to solubilize this material were successful (see Nishi et al., 1990). This amyloid is not the same as the amyloid found in Alzheimer Disease, nor is it the same as the amyloid found in scrapie-like diseases. It is related to the 37-amino acid neuroendocrine peptides, calcitonin gene-related peptides 1 and 2 (Leighton and Cooper, 1988), and is called islet amyloid polypeptide or amylin. Amylin is a novel hormone which may control carbohydrate metabolism in partnership with insulin and other glucoregulatory factors (see Cooper et al., 1989). Amylin is synthesized in and co-released with insulin from the B-cells of the islets of Langerhans (see Banks, 1990). Amylin inhibits insulin-stimulated glycogen synthesis in skeletal muscle, while leaving insulin-stimulated carbohydrate metabolism in adipose tissue unchanged (see Cooper et al., 1989). After eating, if the rate of glycogen synthesis in skeletal muscle is decreased by amylin, glucose will either be diverted into triacylglycerol storage in adipose tissue, which in pathological situations may lead to obesity, or will remain in the blood and result in hyperglycemia, as in non-insulin-dependent diabetes mellitus (see Cooper et al., 1989).

A second type of deposit is fibrosis, which is perhaps the simplest and most frequently occurring change in islets of Langerhans. It is present primarily in the atrophic type of islet. When pancreatic sections were stained with Gomori's one step trichrome, a few of the islets of Langerhans in 139H-infected hamsters showed fibrosis. The areas of fibrosis were located around the elongated islet cells. Fibrosis might help to change islet cell shape and orientation. Fibrosis might also impede cell-to-cell communication within the islets. The cause of fibrosis in the islets is difficult to explain. It could be partly due to islet inflammation or to a collapse of the framework of the islets after the disappearance of B cells (Ye and Carp, 1996a).

It is interesting to note that 139H-infected hamsters showed hyperinsulinemia and obesity (Carp et al., 1990) (in these studies, the body weight was increased from 205.8±9.9 g in control animals to 262.4±4.9 g in 139H-infected hamsters), but there were no body weight changes in 263K-infected hamsters. This study did not find amyloid formation in the islets of 139H-infected hamsters by PrP immunostaining and Congo red or thioflavin-S staining. Based on the fact that their amino acid sequences are different, only some mammals, such

Fig. 2. Periodic Acid-Schiff and Orange G stain of the pituitaries and adrenal glands. **A.** Pituitary of a control hamster shows normal structure and no PPS. PN: pars nervosa; PI: pars intermedia; PD: pars distalis. x 61. **B.** Pituitary of a 263K-infected hamster shows no pathological changes and no PPS. x 82. **C.** Pituitary (pars distalis) of a control hamster, shows normal structure and no PPS. x 224. **D.** Pituitary of a 139H-infected hamster shows PPS (arrowhead) located inside and outside the pituitary cells and in the vacuolation area. x 224. **E.** Pituitary of a control hamstershows normal structure and capillaries. **x 610. F.** Pituitary of a 139H-infected hamster shows small and large (> 7μm) PPS in grape-like form located both inside (arrow) and outside the cells (arrowhead). x 610. **G.** An adrenal medulla (AM) of a control hamster shows no PPS. x 610. **H.** An adrenal medulla (AM) of a 139H-infected hamster shows PPS inside (arrow) and outside of cells (arrowhead).x 610


PN
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AM
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as cats, raccoons and humans, form amyloid in islets. Neither rat amylin, nor any of the calcitonin gene-related peptides are known to form amyloid in vivo (see Cooper et al., 1989). It is not surprising then that amyloid formation was not found in the islets of 139H-infected hamsters. However, since (1) 139H-infected hamsters show hyperinsulinemia, and a significant increase in B-cells, and (2) amylin is co-localized and co-released with insulin in B-cells, it is possible that the synthesis and release of amylin from B-cells are also increased in 139H-infected hamsters. It would be interesting to determine comparative amylin concentrations in 139H-infected and control hamsters and to assess the role of amylin in induction of obesity in 139H-infected animals.

Using PAS and Orange G stain, deposits of PPS were found in the islets of Langerhans, the adrenal medulla, the pituitary and the blood stream of 139H-infected hamsters. Many substances will show a PAS-positive reaction, such as: glycogen, neutral muco-substances, certain epithelial sulfomucins, certain epithelial sialomucins and certain hyalin substances (Sheehan and Hrapchak, 1980). PPS deposits were not seen in control or 263K-infected hamsters. This substance is located both inside and outside cells in endocrine organs. At this time it cannot be said definitely whether or not the PPS is hyalin substances (amylin). At present, amylin has only been found in B-cells in islets, however, in addition to islets PPS is found in adrenal medullas and pituitaries of 139H-infected hamsters. Unless amylin can also be produced and/or taken up from blood by cells of the adrenal medulla and the pituitary, there is no reason to presume that PPS is amylin. The significance of PPS in the pathological changes found in these organs is still not clear. PPS may be related to the activation of inflammatory cells in the islets of Langerhans (Ye and Carp, 1996a). PPS is not found in the brains of scrapie-infected hamsters nor is it immunostained with PrP and ßA4 antibodies. PPS may serve as a biomarker for the diagnosis of 139H-infected hamsters. Further experiments should be done to identify the nature of PPS and the occurrence of PPS in different organs in various stages of the scrapie incubation period.

Acknowledgements. The authors wish to thank Drs. T.J. Bucci, C. Okerberg, Z.K.Binienda and L.Schmued for critically reading the manuscript and for their comments. This work was partially supported by the New York State Department of Mental Hygiene and NIH grant AG09017. Dr. Ye was also supported by an appointment to the Postgraduate 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.

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Accepted October 27, 1998