Histol Histopathol (1995) 10: 995-1021

Histology and Histopathology

Invited Review

The pathological changes in peripheral organs of scrapie-infected animals

X. Ye and R.I. Carp

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

Summary. Scrapie is an unconventional neurodegenerative disease in sheep and goats that has been known in Europe for over 260 Years. The scrapie agents affect the brain and are transmissible from animal to animal. Key features of scrapie infections are abnormal behavior and deficits in motor function. These clinical findings can be related to the damage found in the central nervous system. In some scrapie strain-host model systems there are other manifestations of disease that appear to be related to pathological changes found in the peripheral organs, especially in the endocrine organs such as pituitary, adrenal glands, the islet of Langerhans and ovary. In those model systems in which extensive histopathological changes have been seen in peripheral organs, the titers of scrapie infectivity and the levels of the scrapie specific protein, PrP^{Sc}, are relatively low in the affected organs. These data suggest but do not prove that changes in peripheral organs are secondary to the scrapie-induced neurodegeneration that is occurring in the brain. In some scrapie strain-host combinations, obesity and aberrant glucose metabolism are seen in the preclinical and clinical phases of the incubation period. There appear to be two pathways that lead to these particular clinical manifestations. In SJL mice infected by the ME7 or 22L strains of mouse-adapted scrapie and in some scrapie-infected sheep, the mechanism is related to changes induced in the hypothalamic-pituitary-adrenal axis. The other pathway is exemplified by hamsters infected with two hamster-adapted scrapie strains, 139H and 22CH; it appears that lesions found in the hypothalamic-islets of Langerhans axis are critical. A number of reviews on the pathological changes in the central nervous system have been published and therefore, in this review article, we focus on the gross and histopathological changes in peripheral organs in several scrapie strain-host combinations. The changes induced in peripheral organs in a number of scrapie strain-host combinations expand the number of diseases in which the unconventional slow infections could serve as a model. Further work in this area could help us to understand the mechanisms and pathways of the pathological changes found in the peripheral organs of the scrapie-infected animals

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

Key words: Scrapie, PrP, Histopathology, Pituitary, The islet of Langerhans

A. Introduction

Scrapie is a disease that affects sheep and goats (Sigurdsson, 1954; Dickinson, 1977). The disease is caused by an uncharacterized infectious agent which has been passaged and studied in a variety of laboratory animals, with particular emphasis on investigations in mice and hamsters (Kimberlin and Marsch, 1975; Dickinson and Fraser, 1977). Studies in both the natural and experimental disease have included the key elements of the interaction between infectious agents and hosts: epidemiology, pathology, pathogenesis, genetics. Scrapie is the archetype of a group of diseases referred to by several names: unconventional slow infections, transmissible spongiform encephalopathies, subacute spongiform encephalopathies, prion diseases. The group includes three human diseases, Creutzfeldt-Jakob Disease (CJD), Gerstmann-Straussler-Shenker syndrome and perhaps fatal familial insomnia, and a number of animal diseases, including transmissible mink encephalopathy, chronic wasting disease of mule deer and elk and bovine spongiform encephalopathy (Gibbs et al., 1968; Gajdusek, 1977; Marsh and Hanson, 1979; Williams and Young, 1980, 1982; Masters et al., 1981; Wells, 1987). Note that all subsequent discussions will use scrapie as the model system for this group of diseases so that, unless otherwise stated, our discussion will refer to results obtained with scrapie.

A key feature of scrapie and the other unconventional slow infections is the histopathological changes with these conditions. The histopathological changes which are seen in the brains of scrapie positive animals include gliosis, astrocytosis, vacuolation and, in some instances, amyloid plaques (Fraser, 1979; Carp et al, 1987). Neuronal loss has also been noted, although other than observations of the stratum pyramidale of the hippocampus and a loss in Purkinje cells in mice injected stereotaxically in the cerebellum, the reduction of neuronal cell number has not been assessed in detail (Scott and Fraser, 1984; Kim et al., 1990). All of the histopathological changes appear to be influenced by the genetics of both scrapie strain and host (Dickinson and Fraser, 1977; Carp and Rubinstein, 1991). The two changes that have been most thoroughly analyzed in the context of genetic influence are vacuolation and amyloid plaque formation. For the latter, there are scrapie strain-host combinations that yield extensive plaque formation, others that have comparatively few plaques and still others in which plaque formation can not be demonstrated (Bruce and Dickinson, 1985; Carp et al., 1987). For vacuolation, genetic control affects the concentration of vacuoles in various brain areas. For example, if the brain is divided into nine regions form posterior (medulla) to anterior (anterior cerebral cortex) and each region is given a score of vacuolar concentration, a "lesion profile" can be developed for each scrapie strain-inbred mouse strain combination (Fraser and Dickinson, 1973; Fraser, 1979). These profiles can be so distinctive that they can serve as a "signature" for a specific scrapie strain-host combination. It is often stated that scrapie pathological changes are restricted to the central nervous system (CNS). For some scrapie strain-host combinations this is correct, however, there are model systems in which profound changes occur in visceral organs, and it is these non-CNS or peripheral pathological changes (including the changes in pituitary) which will be a major focus of this presentation.

The variation in lesion location and concentration in the brain which, as stated, is based on genetic characteristics of both agent and host, indicates that differential targeting of cells within the CNS occurs. In assessing the effects of various toxins and infectious agents on brain, a concept of specific targeting of brain cells for damage by these disease including agents was established and the term "pathoclisis" was coined to refer to the concept (Vogt and Vogt, 1937). In the case of scrapie, this differential targeting is, at least in part, a function of the genetic characteristics of the infecting agent. Thus, within a single disease entity there are differences with regard to the cells affected with pathological change and, quite probably, there is differential targeting of cells that show functional changes in the absence of detectable histopathological effects. It is probable that the differences noted in pathological and physiological changes in peripheral organs, which occur as a function of the scrapie strain and host genotypes, are related to the nerve cells targeted in the central nervous system (Carp et al., 1994b). In ways still not clear, the genetic characteristics that define host cell susceptibility and scrapie strain-specific characteristics can lead to either a successful or an unsuccessful infectious process.

In recent years there has been extensive work on a cellular protein that has altered characteristics after scrapie infection. The protein is termed protease resistant protein, PrP^{Sc}, and the cellular isoform is termed PrP^{C} (Prusiner, 1982; McKinley et al., 1983). There has been extensive speculation concerning the relationship of this protein to the infectious agent. A number of investigators believe that the infectious agent in scrapie is a standard virus and that PrP is a protein that is altered during the infectious process (Rohwer, 1984; Braig and Diringer, 1985; Manuelidis et al., 1988). A second postulated, termed the virino theory, states that the scrapie infectious agent contains a non-coding nucleic acid, probably RNA, which causes pathology by

Table 1. The pathological changes in peripheral organs of scrapie-infected animals.

ORGANS	SHEEP	MICE(SJL)			HAMSTER (LVG/LAK)		
	Scrapie	139A	ME7	22L	139H	22CH	263K
Pituitary							
Weight					-		
PN and PI*	+	-	-	-	-		-
PD vacuolation	-	-	-	-	+++		±
PD cytopathology	±	-	-	-	+++		±
Adrenal							
Adrenal enlarged	++	-	++	++	++	++	-
ZF enlarged*	++	-	++	++	++		
Cytopathology	++	-	-	-	±		
Pancreas							
Weight		-	-	-	↑	↑	-
Acini changes		-	-	-	-	-	-
Islet #, size ↑		-	-	-	+++	+++	-
B cell #, size ↑		-	-	-	+++		-
Cytopathology		-	-	-	+++		-
(insulin) ↑		-	-		+++	+++	-
Uterus							
Weight		-	-		-		
Cytopathology		-	-	-	-	-	
Ovary							
Weight	-	↓	↓		-	↑	
Luteinize	-	-	-	-	-		
Cytopathology	-	-	-	-	-		
Spleen							
Weight		↓	↓		-	-	
Cytopathology		-	-	-	-	-	
Liver							
Weight	↑	↓	↓		↑	↑	
Cytopathology	-	-	-	-	±		
Kidney							
Weight		-	-		↑	↑	
Cytopathology		-	-	-	±		
*Others***							
Cytopathology		-	-	-	-		

*: PN, pars nervosa; PI, pars intermedia; PD, pars distalis; ZF, zona fasciculata. **: esophagus, trachea, and intestine.

disrupting normal RNA processing and/or function (Dickinson and Outram, 1979; Kimberlin, 1982). This agent-specific nucleic acid is the informational molecule which provides the basis for genetic differences among scrapie strains. It is postulated that this nucleic acid is surrounded and protected by a host-coded protein; PrP is a candidate for this role. In the prion theory concerning the nature of the agent, the altered form of PrP, i.e., PrP^{Sc}, is the infectious agent (Prusiner, 1982; Prusiner et al., 1990). In this theory, no additional macromolecule is required to form the infectious agent. Replication is said to occur by having the incoming PrP^{Sc} molecule act as a template, based upon its tertiary structure, for the conversion of PrP^{C} to the abnormal, "infectious" form of the protein (Prusiner et al., 1990; Weissmann, 1991). Genetic specificity would be impaired by variations in the tertiary structure of the "infecting" PrP^{Sc} molecules. There have been numerous reviews on the "nature of the agent" issue in recent years (Manuelidis et al., 1988; Carp et al., 1989a, 1994b; Prusiner, 1989; Kascsak et al., 1991). Regardless of its role in the infectious agent (from none in the virus theory to primary in the prion theory), it is clear that PrP is important in determining the pathogenesis of the disease; there have been a number of findings that support this concept but probably the most convincing is found in works with transgenic mice and knock-out mice. In the transgenic mice: a hamster-adapted scrapie strain, 263K, does not readily replicate in our cause disease in mice. However, if the hamster PrP gene is used as a transgene, the transgenic mice produced are susceptible to infection by the 263K scrapie strain and to the induction of disease (Scott et al., 1989). In knock-out mice: Prn-$p^{o/o}$ mice devoid of PrP shows normal development and behavior. When inoculated with scrapie agent, they are free of scrapie symptoms and with no detectable PrP^{Sc} accumulation (Büeler et al., 1993). Whether this is a function of PrP acting as a receptor for the infectious agent or inducing pathological changes within specific cells or a combination of these is not clear at the present

time. it could well be that the PrP molecule plays a role in targeting within the CNS.

As noted above there have been extensive studies of histopathological changes within the CNS (Fraser and Dickinson, 1973; Fraser, 1979; Scott and Fraser, 1984; Bruce and Dickinson, 1985; Carp et al., 1987) but comparatively few analyses of changes in peripheral organs. For the commonly studied scrapie strain-host combinations it has been reported that peripheral organs are free of histological changes (Bendheim et al., 1992). There are, however, a number of combinations in which the peripheral changes are evident (Carp et al., 1984, 1990; Kim et al., 1987; Ye et al., 1994a,b). Because these changes in organs other than the brain and spinal cord are not universal findings, they cannot be central to the pathogenesis of the disease; however, their occurrence in some model systems expands the spectrum of potential clinical presentations and may provide clues to the mechanisms involved in scrapie targeting and host neuroendocrine processes. Since disease can occur with or without involvement of peripheral organs depending on scrapie strain and host there must be a genetic component(s) that controls the occurrence of peripheral changes. These model systems might provide an efficient way to assess the pertinent genetic influences. In this review we will emphasize the findings of changes in peripheral organs including pituitary (see Table 1).

B. Scrapie-infected sheep

Scrapie, as an untreatable neurodegenerative disease of sheep, has been known in Europe for over 260 years. In their book entitled «Scrapie disease in Sheep», Parry and Oppenheimer (1983) summarized an extensive study of this disease in sheep. According to their observations, the syndromes of scrapie-infected sheep can be related to five main physiological disturbances in:

(1) metabolism, animals showed inanition and loss of muscle mass, but occasionally became obese, with abnormal intake of water and sodium chloride, but without loss of appetite;

(2) motor function: dysmetric ataxia of the limbs, but without disturbances of body righting reflexes;

(3) sensation: compulsive rubbing or nibbling of certain parts of the body, without evidence of skin disease, and elicitable by pressure on deep tissues;

(4) behavior: signs of emotional instability, anxiety, and confusion;

(5) autonomic nervous control: tendency to tachycardia and cardiac arrhythmia, distortion of normal alimentary mobility, and inability to maintain bodily homeostasis under conditions of moderate environmental perturbations.

Most of these syndromes can be related to the extensive pathological changes found in the central nervous system, but some of the disturbances may be related to pathological changes found in the peripheral organs, especially in those organs concerned with metabolic and homeostatic regulation.

1. Pituitary gland

When stained with the Gomori chrome-alum-hematoxylin (CAH) method, Bignami et al. (1970) found that in normal sheep, there was abundant CAH-positive neurosecretory material in the posterior lobe (neurohypophysis, or pars nervosa). However, there were only scanty remnants of CAH-positive material in the posterior lobes of scrapie-infected sheep. On the other hand, there was a significant increase of CAH-positive material in Herring bodies and in beaded fibers in the median eminence, pituitary stalk as well as in the pars tuberalis in scrapie-infected sheep. In contrast, CAH-positive material was never seen in these regions in normal sheep (Beck et al., 1964).

By using immunofluorescent staining, Parry and Livett (1977) found that in scrapie-infected sheep there was a marked reduction in the amount of neurophysin throughout the pathway leading to the distal neurohypophysis including the internal infundibular zone; in contrast, there was a two- to three-fold increase in neurophysin in the proximal neurohypophysis, seen throughout the external infundibular zone. No immunofluorescence was seen in the pars intermedia and pars distalis. In scrapie, the density of the cells is considerably increased in the anterior lobe (pars distalis), however, individual cells appear to be normal. Vasopressin activity was reduced in scrapie-infected animals, especially in the distal neurohypophysis. It is interesting to note that we have also observed that vasopressin immunostaining is reduced in the lateral hypothalamus of 139H-infected hamsters (Ye et al., 1994c). This finding will be discussed in detail latter in this presentation. The changes in the pituitaries of scrapie-infected sheep may be responsible for the metabolic disturbances such as abnormal water and sodium chloride intake, obesity and adrenal abnormality.

2. Adrenal gland

There were pathological changes in animals which had shown clinical disease for a long time: adrenals were enlarged, owing to increased mass and width of the cortex, which was a pale grey-white instead of a light pink-brown. The zona fasciculata was enlarged, with cells in the region showing swelling, with foamy cytoplasm; the cells were tightly packed together (Parry and Oppenheimer, 1983). Scrapie strain-mouse strain combinations which were obese also showed abnormal adrenal enlargement. This suggests that adrenal glands play an important role in scrapie induced obesity which will be discussed in detail later.

3. Muscular system

Depending upon the location, muscles showed various degrees of atrophy. Parry and Oppenheimer (1983) reported that some muscles such as the iliopsoas, facial, auricular, external eye muscles, and those of the

larynx, pharynx, and tongue, were often extremely atrophic and showed dissolution. These changes correlated well with the clinical disturbances observed, e.g. sheep which had great difficulty in raising their heads revealed severe lesions of some of the dorsal neck muscles. Under light microscope, the lesions had features of both polymyositis and dystrophy.

4. Other peripheral organs

Beck et al. (1964) found no gross macroscopic or microscopic abnormalities in other organs, although in some cases of scrapie-infected sheep, the liver and adrenal glands were enlarged, while the thyroid glands were small. Parry and Oppenheimer (1983) reported that the thyroid glands in scrapie animals were translucent and showed a normal flat basal epithelium without any obvious changes in the colloid accumulation or in follicles. The ovaries and testes appeared to be normal in scrapie-positive sheep (Parry and Oppenheimer, 1983).

C. Scrapie-Infected Mice

Scrapie agents have been passaged in a number of laboratory animal species, including mice and hamsters (Chandler, 1963; Kimberlin and Walker, 1986). In mice, characteristic clinical features may include a waddling gait, reluctance to move, dullness, loss of condition, impaired grooming, abnormal posture, ataxia and stiffness of the tail. For many combinations of scrapie strain and mouse strain, total body weight during the preclinical phase of disease was similar to the average weight for controls. For some combinations there was a significant increase in weight compared to controls during the latter part of the preclinical phase and through most of the clinical phase of the disease. The clinical differences in these scrapie strain-host interactions may be directly related to the pathological changes in the peripheral organs seen in those combinations that yield obesity; this will be discussed in the following sections.

1. Adrenal gland

In early studies employing stereotactic inoculation, Carp et al. (1989a) observed that in SJL mice injected with the 22L scrapie strain (22L-SJL), both the cortex and hypothalamus injection groups developed preclinical weight increases to a similar extent with the increase starting slightly earlier in the hypothalamus group. In the ME7-SJL combination, both groups became obese, but a higher level of obesity developed in the hypothalamus injection group than in the cortex group and the increase started earlier in the former group. For the ME7-C57BL combination, the increase in weight was seen only in the groups injected in the hypothalamus. These results suggest that scrapie induced obesity is dependent, at least in part, on an effect on the hypothalamus. Furthermore, the potential association between obesity and altered glucose tolerance was assessed. Virtually all obese mice showed reduced glucose tolerance as shown by significantly higher blood glucose levels 2 hr after a glucose overload. Mice injected with a scrapie strain that did not cause obesity showed normal tolerance. Following increasing dilution of the inoculum, the increase in body weight and the development of aberrant glucose tolerance reached an end-point that was similar to that of scrapie infectivity (Carp et al., 1989a). In those scrapie-mouse strain combinations that showed an increase in body weight, the adrenal gland was the only organ that showed a significant increase in weight. Adrenalectomy prevented both the increase in total body weight and aberrant glucose tolerance but had no other effect on the course of the disease in two strains of mice injected with the ME7 scrapie strain (Kim et al., 1987, 1988; Carp et al., 1989a). These findings suggested that abnormal adrenal gland function played an important role in reduced glucose tolerance and obesity in scrapie-infected mice. The only changes seen in the adrenals were a marked enlargement of the zona fasciculata and a slight enlargement of the zona glomerulosa of the cortex. Individual cells did not reveal any cytopathic changes. Adrenal weight decreased in the 139A-SJL group, which did not show any obesity and aberrant glucose tolerance (Kim et al., 1988). The importance of adrenal function in other obesity syndromes, such as in OB/OB mice, has been documented (Solomon and Mayer, 1973; Bailey et al., 1986).

2. Ovary and uterus

The weights of the ovaries in 139A-SJL and ME7-SJL mice were decreased compared to the organ weight in control mice (Kim et al., 1988). Sturman (1972) reported that at necropsy, the ovaries of Chandler agent (139A) infected BALB/c mice were bright orange. Under light microscopy, there was a striking change in the ovarian parenchyma. Interfollicular cells of the cortex and medulla were enlarged and epithelioid. These cells resembled well-developed, luteinized cells with distinct nuclei and abundant foamy cytoplasm. Some groups of cells formed a lobulated or alveolar arrangement. In areas enclosed by luteinized stroma, cells of the theca interna of graafian follicles were also luteinized. By periodic acid Schiff and acid-fast stains, accumulations of lipofuscin pigment could be demonstrated with some luteal-like interstitial cells. Sturman (1972) also observed that the ovarian luteinization was accompanied by enlargement of the uterus. Oedema, a decrease in the number of cells in the endometrial stroma, and an increased acidophilic staining of the endometrial cells were seen microscopically. Mitoses of uterine epithelial cells were rare and leucocytes few in number. The vaginal epithelium was generally in a metoestrus secondary stage. With routine histostaining, we did not find any cytopathological changes in the ovary and uterus of the 139A-, ME7-, and 22L-infected SJL mice (Ye and Carp, in preparation). In 139A-SJL and ME7-SJL

combinations the weight of the uterus remained the same compared to the weight of controls (Kim et al., 1988). The reason for this differences in results obtained by Sturman (1972) and Kim et al. (1988) is almost certainly a function of the different mouse strains that were studied.

3. Spleen and lymph nodes

The weight of the spleen in 139A-SJL and ME7-SJL mice was decreased significantly compared to controls (Kim et al., 1988). Reduction in the size of spleens is a routine finding in the terminal stage of scrapie infection. Under the light microscope, pathological changes were not apparent in these spleens.

Chandler (1969) reported observations on the ultrastructure of the spleen and lymph nodes of BSVS mice infected with 139A scrapie. There were no qualitative differences between scrapie and normal spleens or lymph nodes. A variety of particles and vesicles were observed in both scrapie and control lymphoid organs. Crystalline structures resembling rods were seen within the cytoplasm of some macrophage cells of spleen and lymph nodes in both groups of mice; the largest accumulations of crystals were observed in clinically affected scrapie mice. The crystals stained with varying density and in some cases appeared to be surrounded by a membrane. They were usually associated with collections of dense particulate material resembling ferritin, which may represent a product of hemoglobin metabolism.

4. Other peripheral organs

Kim et al. (1988) monitored the weights of several peripheral organs in mice from a scrapie strain-mouse strain combination that showed an increase in total body weight during scrapie (ME7-SJL) as well as in a combination (139A-SJL) in which the body weight was similar to controls during preclinical and early clinical phase of disease. They found that the weight of a number of organs such as spleen, ovary, brain, liver, and kidney were decreased compared to organ weights of mice injected with normal mouse brain. This was seen in scrapie strain-mouse combinations that became obese as well as in those that did not. The weights of pancreas and uterus were the same as those of controls.

Recently, we also investigated the histostaining of pituitary, thyroid, esophagus, trachea, intestine, pancreas, spleen, ovary, liver, adrenal and kidney in scrapie-infected mice, this includes SJL mice infected with 139A, ME7, or 22 L strain, C57BL mice infected with the sheep isolates, C602 or C605, as well as SJL and C57BL mice injected with normal mouse brain homogenate as controls. Thus far, our preliminary results do not reveal any abnormal cytopathological changes in these organs except the liver cells from some C602 and C605-infected C57BL mice showed swelling and vacuolization. The pancreas of the scrapie-infected mice did not show any histopathological changes compared with the controls. The exocrine part of the pancreas appeared to be normal. In scrapie-infected mice, the structure of the islets of Langerhans as well as the islet cells themselves did not show any abnormalities when compared to controls. With antibodies to insulin, glucagon, somatostatin and pancreatic polypeptide, the immunostaining patterns of the islets of Langerhans in the scrapie-infected mice were similar to those seen in the islets of control animals (Ye and Carp, in preparation). These results are markedly different from those we will describe subsequently for 139H-infected hamsters.

D. Scrapie-Infected Hamsters

Recent studies have demonstrated that in some scrapie strain-hamster strain combinations, there was an increase in body weight that started prior to the onset of typical motor dysfunction that signals the start of clinical disease (Carp et al., 1990). For these studies, hamsters were injected intracerebrally with scrapie strains 139H or 22CH or with normal hamster brain (NHB). Animals were then assessed for body weight, insulin level and glucose tolerance periodically throughout the incubation period. Animals injected with the scrapie strains became obese prior to the appearance of the motor changes that are indicative of the start of clinical disease. During the later part of the preclinical and throughout the clinical phase of disease, animals were hypoglycemic and showed marked hyperinsulinemia, (immunoreactive insulin values as much as x49 higher than those seen in controls). At necropsy, there was marked hyperplasia and hypertrophy of the cells of the islets of Langerhans. Thyroid, adrenal glands, liver and kidney 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 (Carp and Rubenstein, 1991; Kascsak et al., 1991). For example, the total body weight of these animals was the same as those injected with normal hamster brain throughout the preclinical and clinical periods.

While the data for the 139H and 22CH strains suggest that they induce a severe generalized endocrinopathy, the pathological changes in the islets of Langerhans were most prominent (Carp et al., 1990). It is probable that the pancreatic changes could play an important role in the obesity of scrapie infected hamsters. We studied the pathological changes in the pituitaries, adrenal glands and islets of Langerhans in 139H scrapie infected hamsters.

1. Pituitary gland

a. Histochemical staining

The pituitary is generally considered to be structurally and functionally the most complex organ of the endocrine system. It controls many of the endocrine

glands in the body. In the present studies, coronal sections of the pituitary were stained with hematoxylin and eosin, and were examined with light microscopy. Hamsters infected with the 139H scrapie strain showed extensive vacuolization in the pituitary. Most vacuoles were located in the ventral and/or ventrolateral parts of the pars distalis. The pituitaries of 139H infected hamsters also showed cellular hypertrophy, cellular atrophy, and cytoplasmic vesicles. There were nuclear pathological changes such as swelling, vesicular changes, pyknosis, karyorrhexis and karyolysis. The cellular and nuclear pathological changes were most pronounced in the regions with vacuolation (Fig. 1). These pathological changes were not found in 263K-infected hamsters, which did not became obese or show generalized endocrinopathy, indicating that the pathology of the pituitary in 139H-infected hamsters might play an important role in the severe generalized endocrinopathy in these animals (Ye et al., 1991).

Using PAS and orange G stain, we noted an

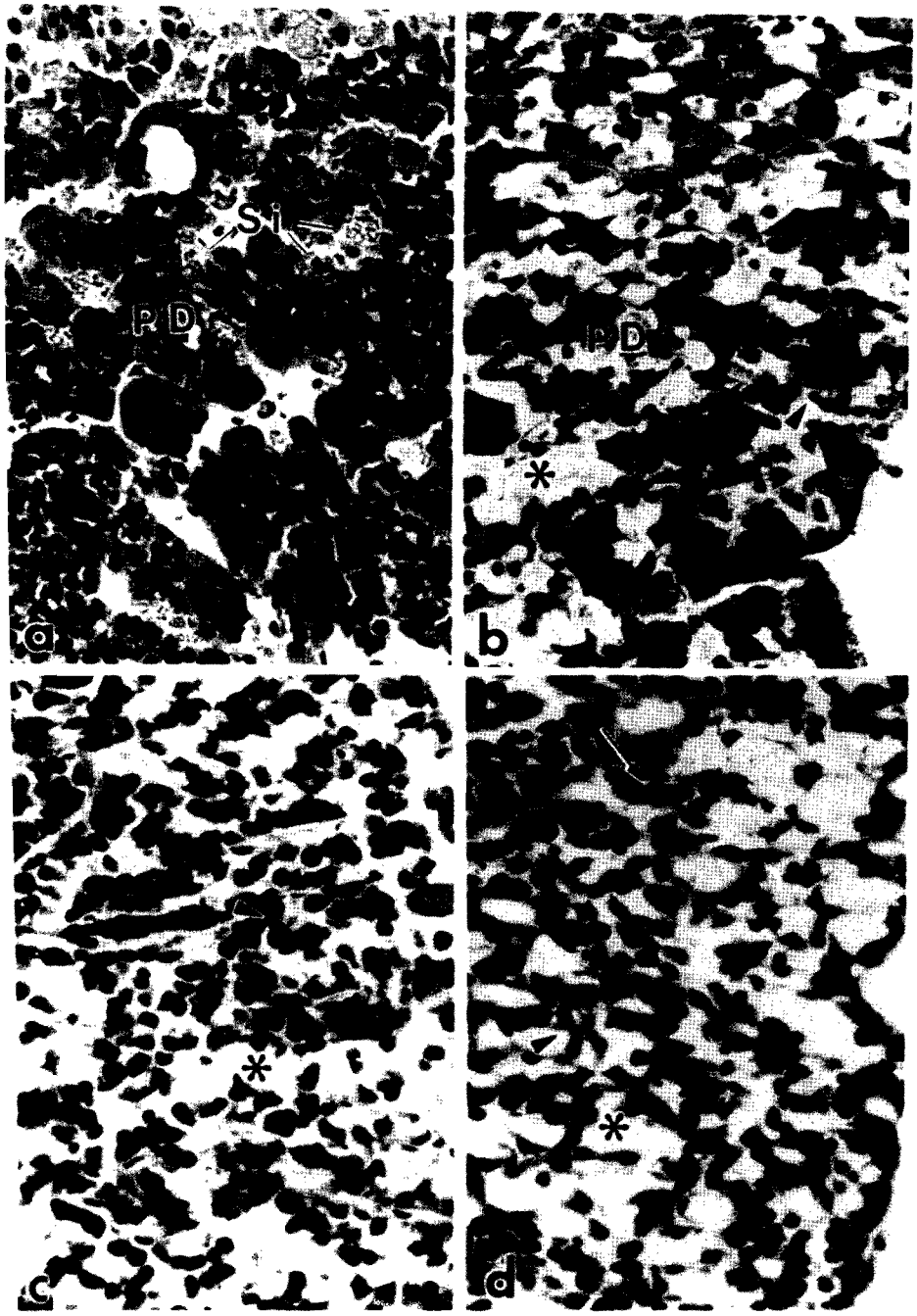

Fig. 1. Hematoxylin and Eosin stain of pituitaries. **a.** Pars distalis of the pituitary of a control hamster shows the normal structure. **b, c, d.** Pars distalis of the pituitaries of 139H-infected hamsters show extensive extracellular vacuolization (EV) (*). Note: most vacuoles (*) are located on the ventral and/or ventro-lateral parts of the pars distalis (PD) in the 139H-infected hamsters. Pituitaries of 139H-infected hamsters show cellular hypertrophy (small arrow). There are nuclear pathological changes such as swelling, ring-form changes (arrowhead); vesicular changes, pyknosis, karyorrhexis and karyolysis (arrowhead) in the 139H-infected hamsters. The cellular and nuclear pathological changes are most pronounced in the regions with vacuolation. Si: sinusoid. x 244

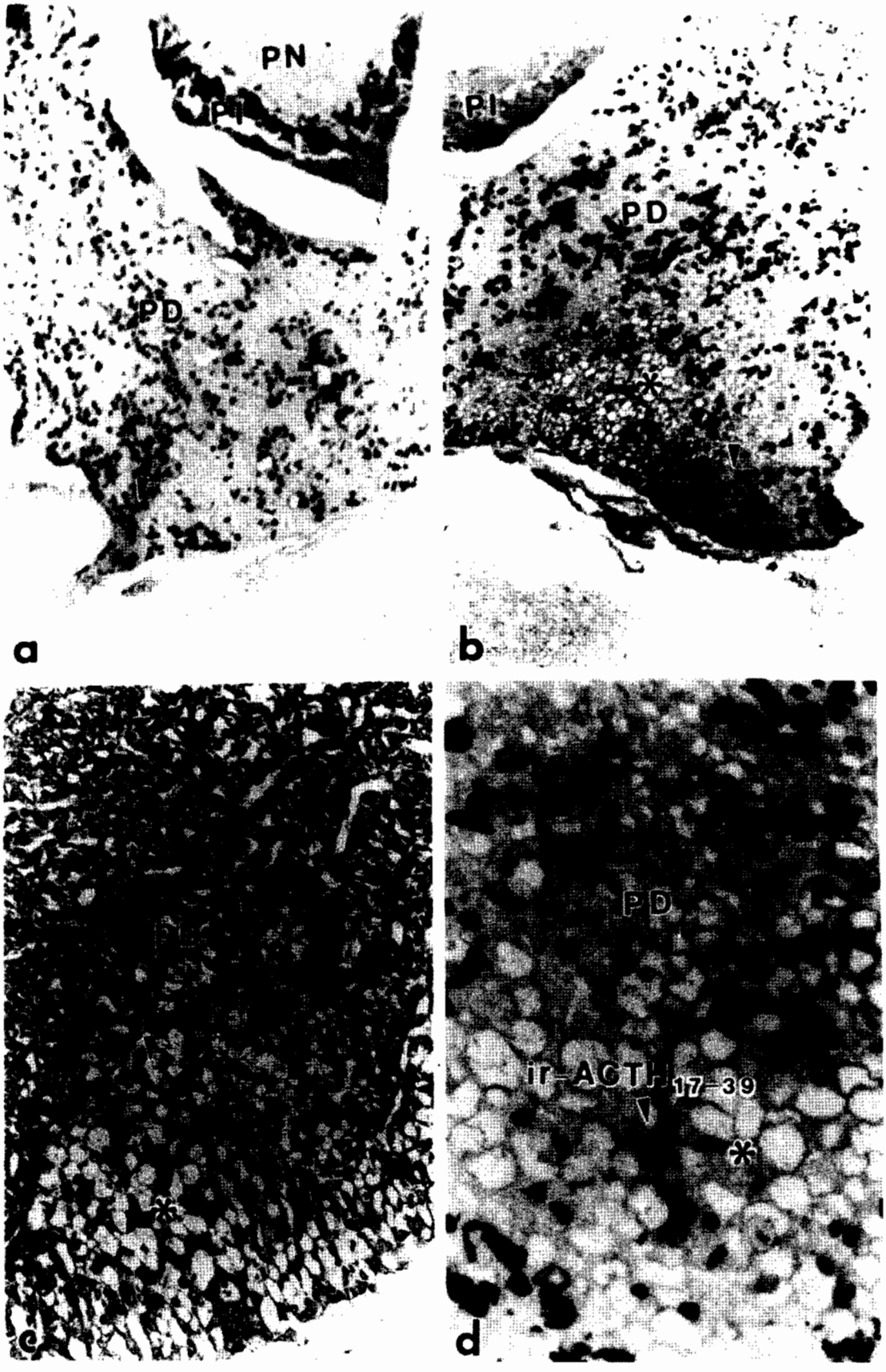

Fig. 2. Immunostaining patterns of $ACTH_{17-39}$ in the PD and PI of pituitaries. **a.** Control hamster. **b, c, d.** 139H-infected hamster. (PI: pars intermedia; PD: pars distalis). Note: The immunostaining of $ACTH_{17-39}$ is distributed throughout the PD and PI in both the control hamster and the 139H-infected hamster. Arrowhead shows $ACTH_{17-39}$ immunostaining in PD and PI. There was a reduction in the number of cells stained for $ACTH_{17-39}$ in those focal areas (*) that had extensive vacuolation in the 139H pituitary. a, b, x 82; c, x 164; d, x 328.

abnormal PAS positive substance (PPS) in grape-like or plaque-like form located in the pituitaries from 139H scrapie infected hamsters, but not in those from control hamsters. This substance was located both inside and outside the cells of the pituitary. The significance of the PPS in the pathological changes in the pituitary of 139H-infected hamsters is unknown. We also found PPS in the adrenal glands and the islets of Langerhans of 139H-infected hamsters. The relationship between PPS found in the pituitaries and that found in the adrenals and the islets is not clear (Ye and Carp, in preparation).

b. Immunostaining

The synthesis and secretion of many pituitary hormones such as prolactin (PLT), follicle stimulating hormone (FSH), luteinizing hormone (LH), adreno-corticotropic hormone (ACTH), and growth hormone (GH) are influenced by a variety of stimuli (e.g. sleep, stress) and, are episodic and pulsatile. In female hamsters during estrus, the levels of several pituitary hormones (LH, FSH, PLT, and GH) in the plasma can change many fold within a few minutes. In our own immunostaining for the pituitary hormones mention above, considerable animal to animal variation was seen, which was probably a function of the episodic effects of various stimuli. In these studies, there were decreases in the number of cells immunostained for several hormones in the vacuolar areas in 139H-infected hamsters (Fig. 2). This indicated that these pituitaries could no longer function normally. Abnormal function of the pituitaries would almost certainly affect other endocrine organs including the adrenals and the islets of Langerhans (Ye and Carp, in preparation).

c. Semithin sections

Analysis of pituitary semithin sections stained with toluidine blue, revealed profound pathological changes in the ventral or ventrolateral parts of the pars distalis in the pituitaries of 139H-infected hamsters. There was extensive cellular lysis. The cytoplasm of these cells had disappeared, but the cellular membranes still existed and formed a membrane network. Some of the nucleus still existed and was attached to the cellular membranes. These vacuolation areas looked like a honey-comb under the light microscope. In adjacent areas, there was cellular vacuolization, with numerous necrotic cell bodies (Ye and Carp, in preparation).

d. Electron microscopy

Using routine electron microscopy, we observed more details of the histopathological changes in the pars distalis of the pituitaries in the 139H-infected hamsters. Individual cell lysis was found. Dilation and vacuolation were first observed in the mitochondria and rough endoplasmic reticulum (RER). The width of a portion of some mitochondria was increased from 250-400 nm to 800-1500 nm, with the cristae showing gradual dispersal in the dilated portion. In some cases, the dilated mitochondria lost all the inner cristae, leaving only the double membranes. Some of the mitochondrial membranes appeared to have dissolved completely. Some of the RER were also dilated. The width of the RER lumens were about 50-150 nm in normal animals, whereas in 139H-infected hamsters, the width of some RER lumens were increased up to 4500 nm in diameter. The dilation and vacuolation of the mitochondria and RER can be observed even when the remaining cellular structures look normal (Fig. 3a). The subsequent abnormal events seen include vacuolation and break-down of the secretory vesicles, lysosomal breakdown and finally cell digestion and lysis. There were numerous pieces of membrane debris in the empty cell space which had a diameter of 100-2500 nm. Most of the time in the damaged cells, we could still find the nucleus attached to the cellular membranes, suggesting that the nuclei were protected to some degree from the effects of cytoplasmic toxins by nuclear membranes. Some of the cells adjacent to dead cells showed cellular lesions, but others still looked normal, with normal cell-cell contacts, including gap junctions. Based upon these observations, we believe that the cellular death seen in the pituitaries of 139H-infected hamsters is due to necrosis rather than apoptosis (Ye and Carp, in preparation).

Immuno-electron microscopy studies are still in progress, however, preliminary results indicate that at least ACTH- and TSH-releasing cells are affected by 139H-infection (Fig. 3b).

2. Islets of Langerhans

a. Histochemical staining

By gross examination, the size of the pancreas in 139H-affected hamsters was greater than in controls or in 263K-affected hamsters. The pancreas from 139H-affected animals showed small, brown and red-brown nodules scattered all over the surface. There were no nodules on the pancreata of hamsters inoculated with the 263K strain or with the homogenate of normal hamster brain. The exocrine part of the pancreas appeared to be normal in both 139H- and 263K-affected animals, however, the islets of Langerhans in 139H-affected hamsters were markedly enlarged, showing extensive histopathological changes (Fig. 4) (Carp et al., 1990; Ye et al., 1994a,b).

In our studies of the histopathological changes in the pancreatic islets (Ye et al., 1994a,b), the islets were classified into three sizes with an image analyzer. The number and total area covered by"small" (area $<0.01 mm^2$) islet profiles were reduced in 139H-affected hamsters compared to profiles in control hamsters. In contrast, the number and the area of "medium" (area ≥ 0.01 mm^2 and <0.1 mm^2) and "large" (area ≥ 0.1 mm^2) islet profiles were significantly increased in 139H

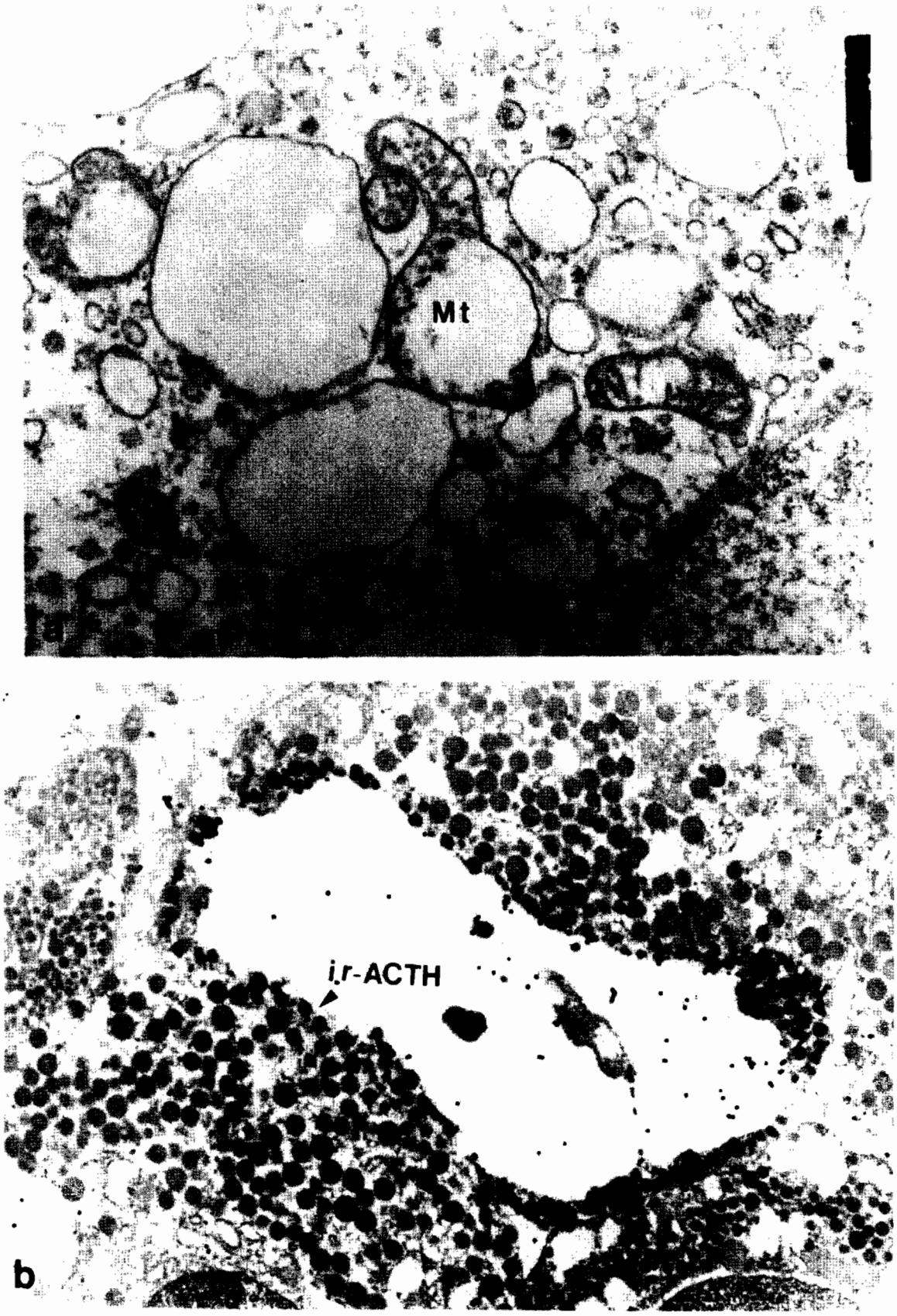

Fig. 3. EM and Immuno-EM studies of the pituitary of 139H-infected hamsters. **a.** EM studies: cellular degeneration in the pars distalis, mitochondria (Mt) and endoplasmic reticulum (ER) are found swelling, dilated and damaged while the nucleus still look normal. x 25,070. **b.** Immuno-EM studies: ACTH-releasing cell is affected by 139H-infection. The ACTH-releasing cell is vacuolated, leaving the secretion granules attached to the membrane of the death cell, while other non-ACTH releasing cells still look normal. x 15,230

hamsters (Figs. 5, 6).

Additional histopathological changes in the islets of Langerhans in 139H-infected hamsters included fibrosis, vacuolization, cellular atrophy, cellular elongation, changes in cell shape and in cell orientation. There were also nuclear pathological changes such as swelling, changes in shape, pyknosis, karyorrhexis and karyolysis. Although diabetes mellitus was found in 263K-infected

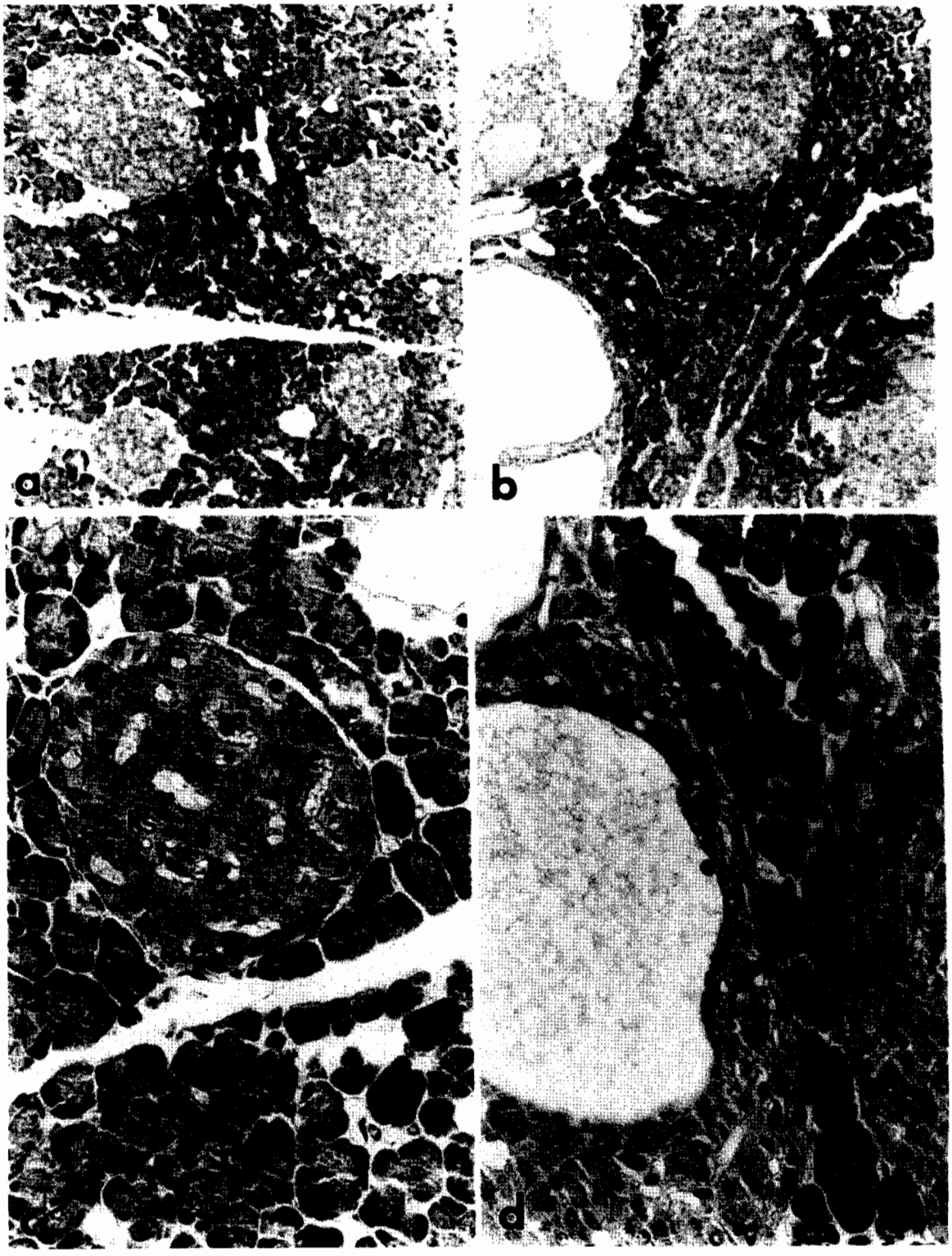

Fig. 4. Hematoxylin and eosin stain of pancreatic islets. **a.** Pancreatic islets of a control hamster at low power. **b.** Pancreatic islets of a 139H-affected hamster at low power. **c.** Pancreatic islets of the control hamster at high power. **d.** Pancreatic islets of 139H-affected hamster at high power. The sizes of the islets in the 139H-affected hamster were considerably larger than those in the control hamster. Islet cells in 139H-affected hamsters are hypertrophied (Fig. 1d) compared to those in controls (Fig. 1c). The cystic areas called «blood vessel core» (BVC) are in the islets of 139H-affected hamsters. Aci: acini, Is: islet. a, b, x 82; c, d, x 328

hamsters, there was no morphological changes in the islets of Langerhans in these animals (Srinivasappa et al., 1989).

The vascular pattern was disturbed significantly in the islets of 139H-infected hamsters. Blood filled spaces, vary in size from 30μm to 300μm in diameter, were present in most of the islets of 139H-infected hamsters. Histologically, these space did not appear to be lined by endothelium. Therefore, these structures were referred to as "blood vessel cores" (BVCs). BVCs were usually centrally located within the islets and were surrounded by B cells, some of which were elongated abnormally. The number of BVCs was 13.0 per 50 mm^2 pancreatic area. The average diameter of each BVC was 0.13 mm (Ye et al., 1994a,b). Carp et al. (1990) showed that some of the islets in 139H-infected hamsters contained hemorrhages. The pathogenesis of hemorrhage and/or BVC formation in the islets is still not clear.

There are two possibilities for the fate of the blood within the BVC (Fig. 7). One possibility is hemorrhage; which means that the blood within the BVC escapes from capillaries inside the islet. The blood oozing from the capillary will leave the circulation and be trapped in the BVC. The blood cells will eventually die and will be cleared by macrophages. In this case the BVC is not a part of the circulatory system. The other possibility we refer to as "pseudo-hemorrhage"; which means that the blood within the BVC is from one or more capillaries, and sooner or later the blood cells will re-enter the circulation through other capillaries inside the islet. In this case the BVC is a part of the circulatory system. In assessing these two possibilities, the following points are relevant: (1) the endocrine pancreas is richly vascularized by an extensive labyrinth of capillaries. (2) It has been estimated that islets receive 13%-20% of the entire pancreatic blood flow, in spite of the fact that islets comprise only 1.5% of pancreatic volume (Lifson et al., 1980). (3) In our studies, we observed that rather than clear up the blood cells in the BVC, many macrophages migrate from the BVC into the islets. In our opinion, the possibility of pseudo-hemorrhage is much higher than that of hemorrhage (Ye et al., 1994b).

b. Immunostaining

There was no changes in the immunostaining of insulin, glucagon and somatostatin in 263K-infected hamsters compared to the controls (Srinivasappa et al., 1989). However, the immunostained areas of insulin (B cells) and of glucagon (A cells) were increased significantly in 139H-infected animals compared to the areas in control animals (Ye et al., 1994b). The immunostained areas of somatostatin (D cells) and pancreatic polypeptide (F cells) did not differ significantly between the two groups. The proportion of B, A, D and F cells was determined. With somatostatin positive cells arbitrarily given a value of 1, the ratio of B:A:D:F cells in the islets of normal hamsters was 27:5:1:0.04, respectively, whereas, in the islet of 139H-infected hamsters the ratio was 122:7:1:0.04. Our results indicate that the increase in B cells could serve as a major component in the enlargement of pancreatic islets and play an important role in the hypoglycemia-hyperinsulinemia seen in hamsters infected with the 139H scrapie strain (Fig. 8, Table 2).

The optical density (OD) of insulin immunostaining cells was decreased in the 139H-infected hamsters compared with control animals (Fig. 9). This suggested

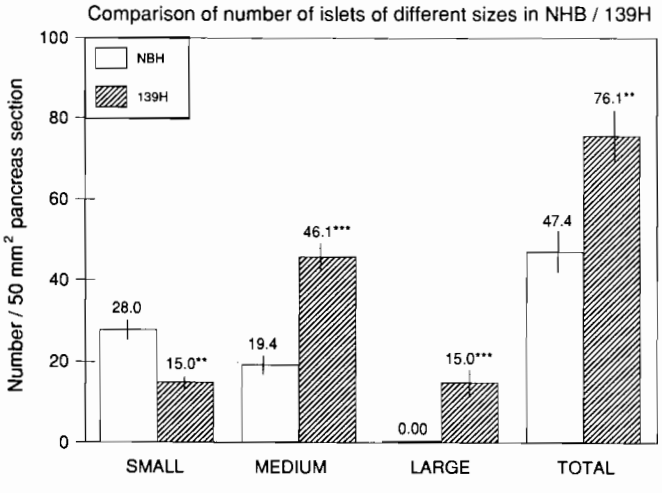

Fig. 5. Number of pancreatic islets. All islets were placed into 3 categories (small, medium and large) based on size. The number per 50 mm^2 of pancreatic section in each category is shown for control hamster (NHB, n=14) and for 139H-infected hamsters (n=20). Small islets: <0.01 mm^2; medium islets: ≥0.01 mm^2 and <0.1mm^2; large islets: ≥0.1mm^2. **: p value <0.01; ***: p value <0.001.

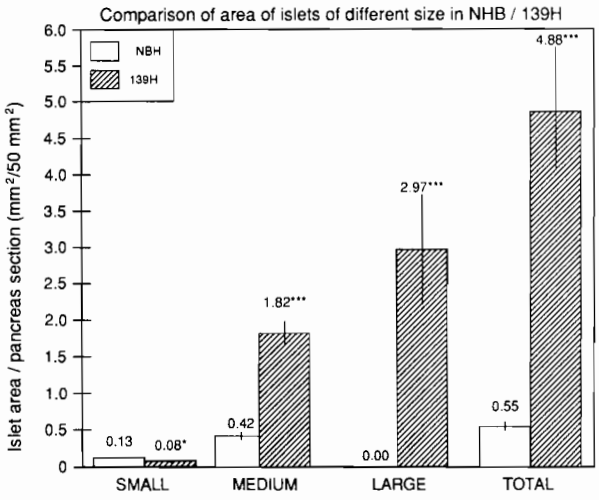

Fig. 6. Area of pancreatic islets. All islets were placed into 3 categories (small, medium and large) based on size. The total area per 50 mm^2 of pancreatic section in each category is shown for control hamsters (NHB, n= 14) and for 139H-infected hamsters (n=20). Small islets: <0.01 mm^2; medium islets ≥0.01 mm^2 and <0.1 mm^2; large islets ≥0.01 mm^2. *: p value <0.05; ***: p value <0.001.

that the concentration of insulin in B granules was decreased or the number of B granules was decreased, or both. The OD of immunostained glucagon, somatostatin and pancreatic polypeptide did not differ between control animals and 139H-infected hamsters.

c. Semithin sections

Using semithin sections stained with toluidine blue, the pathological changes in the islets of 139H-infected hamsters can be seen in more detail. There were cytoplasmic vesicles, and nuclear vacuolization in the islet cells of scrapie infected hamsters. Two types of vacuolization were seen. One type is referred to as "localized vacuolization" (LV). LV has a clear edge and is restricted or confined within the cell. The other type of vacuolization is termed "diffuse vacuolization" (DV). DV has no clear edge, and is scattered within tissues either inside or outside of cells. DVs may span intracellular and extracellular regions of the tissues.

Table 2. Number of B, A, D and F cells in the largest islet profile of each pancreatic section in control (n=14) and 139H-affected (n=18) hamsters.

CELL	NHB	139H	P VALUE
B	439.7±37.2	1123.7±102.9	<0.0001
A	65.5±5.3	77.1±7.2	0.22
D	22.6±2.4	16.4±2.2	0.07
F	0.3±0.1	0.4±0.1	0.63

Margination and diapedesis of inflammatory cells (macrophages or lymphocytes) occurred at the membranes of the large BVCs in 139H-infected hamsters. In these studies, we have observed five different types of cellular combinations of inflammatory cells undergoing margination and diapedesis: (1) only a single lymphocyte; (2) only a single macrophage; (3) a group of two or more lymphocytes; (4) a group of two or more macrophages; (5) a group of mixed lymphocytes and macrophages. These studies showed that lymphocytes can come into close contact with one another and also with macrophages or with pancreatic B-cells (the wall of BVCs). It has been shown that groups of lymphocytes can surround a macrophage, attaching to it intimately (Shelton, 1962). This phenomenon has been described as "peripolesis" in lymph nodes and spleen (Sharp and Burwell, 1960) and can be interpreted as a sign of exchange of information between the cell types. In our studies, we have observed another phenomenon; the interaction between a group of white blood cells (WBCs) and the wall of BVCs. This phenomenon is referred to as "linkage reaction", this group of WBCs as "linkage-WBCs" (Ye and Carp, in preparation). Figure 10 shows the several events of linkage-WBCs migration into areas with lesions in the islets of Langerhans.

d. Electron microscopy

In routine electron microscopy studies, we observed

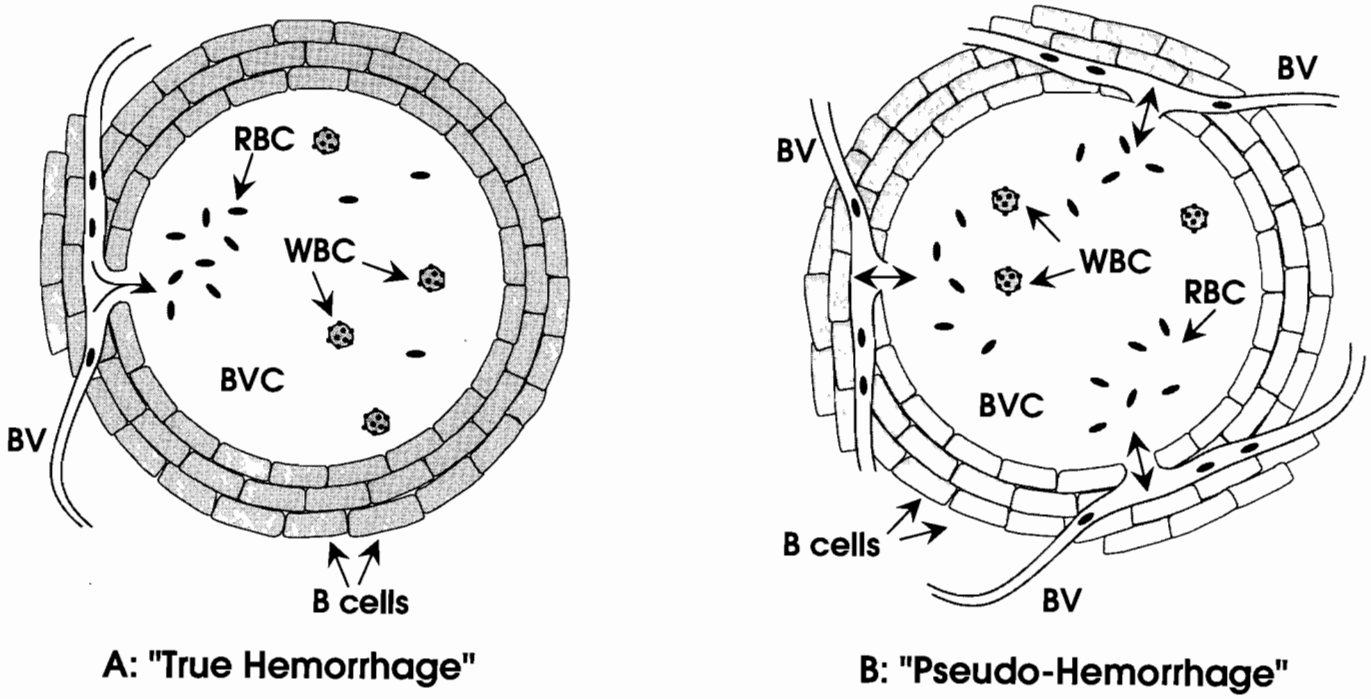

Fig. 7. Schematic representation of two possibilities of the BVC formation **A.** True hemorrhage: the blood oozing from the islet capillaries are being trapped in the BVC. The blood cells would eventually die and be cleared by macrophages, and the BVC would not represent part of the circulatory system. **B.** Pseudo-hemorrhage: the blood within the BVC arrives via one or more capillaries, and that the blood cells eventually re-enter the circulation through other capillaries inside the islets. In this case, the BVC would represent a part of the circulatory system. Since there are extensive labyrinth of capillaries inside the islet, the possibility of pseudo-hemorrhage is much higher than that of true hemorrhage.

more details of the histopathological changes in the cells of islets of Langerhans in the 139H-infected hamsters. Individual cell lysis was found. Mitochondria, smooth endoplasmic reticulum (SER) and RER were found damaged or dilated. The dilation of the mitochondria, SER and RER in the cells if the islets was not as extensive as had been found in the cells of the pituitary. Some of the mitochondrial and RER membranes were undergoing dissolution. The width of the RER and SER lumens were about 50-150 nm in normal samples, whereas in 139H-infected hamsters, the width of the RER and SER lumens were increased up to 4000 nm in diameter. Dilation and vacuolation of the mitochondria, SER and RER were observed, as well as vacuolation and breakdown of secretory vesicles, lysosomal breakdown, and cell digestion and lysis. Most B cells showed degranulation. There were large amounts of membrane debris with approximate diameters of 100-2500 nm in the empty cell spaces. Again some cells adjacent to affected cells showed damage, whereas other adjacent cells appeared to be normal with intact cell-cell contacts, including gap junctions. At the wall of BVCs, we often found some unknown substances attached to the B-cells. Occasionally, we observed mitotic figures in the nuclei of islet cells. These EM observations, confirmed the suggestion that the cellular death seen in the pituitaries as well as the islets of Langerhans in 139H-infected hamsters was due to necrosis, not apoptosis (Ye and Carp, in preparation).

3. Other peripheral organs

Recently, we also investigated the histostaining of thyroid, esophagus, trachea, intestine, spleen, ovary, liver, adrenal and kidney in hamsters infected with 139H, or 263K strain, as well as control hamsters injected with normal hamster brain homogenate. Our preliminary results did not reveal any abnormal cytopathological changes in most of these organs. The pathological changes underlying the enlargement of thyroid, adrenal glands, liver, and kidney in 139H and 22CH-affected hamsters are currently being analyzed. The enlargement of liver and kidney may be due to accumulation of glycogen (Carp et al., 1990). A few of the 139H-infected hamsters showed hemorrhage and/or foamy cytoplasm in the adrenal, kidney and the liver. The PPS found in the pituitaries of 139H-affected hamsters was also found in the adrenal medulla, the islets of Langerhans and the blood stream. This substance is located both inside and outside cells in the endocrine organs of 139H-infected hamsters, but not in control or 263K-affected hamsters (Ye et al., in preparation).

E. Correlations among PrP^{Sc}, infectivity and the pathological changes in the peripheral organs

The normal form of PrP (PrP^C) has a wide distribution in hamsters: it is present in circulating leukocytes, heart, skeletal muscle, lung, liver, pancreas, intestinal tract, spleen, testis, ovary, adrenal gland and kidney (Bendheim et al., 1992). It has been suggested that PrP^C plays a role in lymphocyte activation (Cashman et al., 1990), while in brain it may play a role in glial growth (Oleszak et al., 1988; DeArmond et al., 1992) and a role during embryogenesis (Manson et al., 1992). Knock-out mice devoid of PrP^C appears normal (Büeler et al., 1993). These studies suggest that this protein has a function that is: 1) not unique to brain, and 2) can be dispensable or duplicated by other macromolecules (Büeler et al., 1992). During scrapie infection, PrP^C can

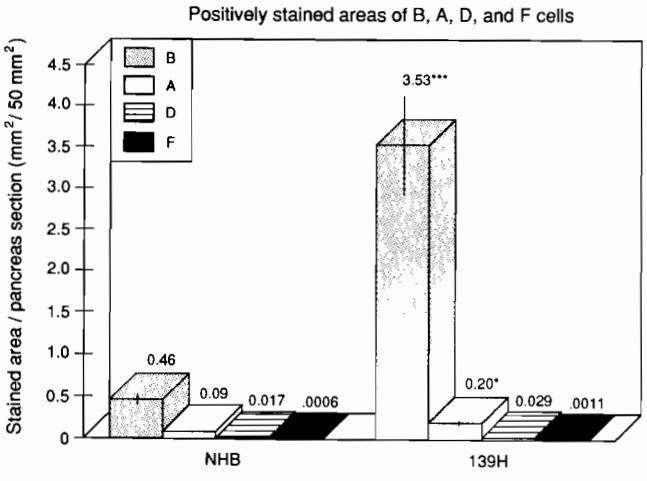

Fig. 8. Comparison of positively stained areas of B, A, D and F cells in control hamsters (NHB, n=14) and 139H-affected hamsters (n=20). *: p value <0.05; ***: p value <0.001.

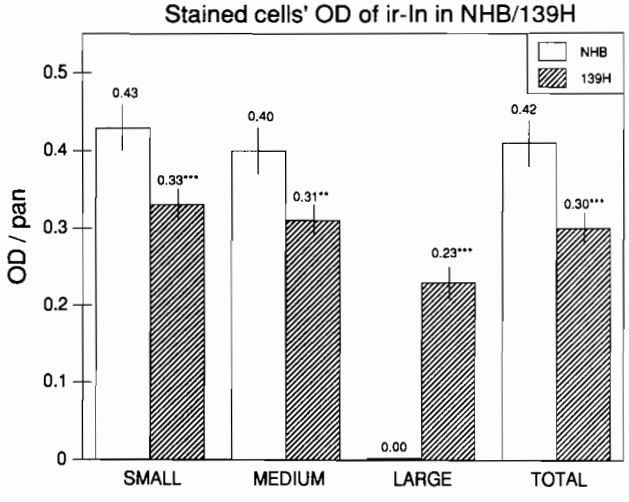

Fig. 9. The average optical density per pancreatic area (OD/pan) of ir-In from 139H-affected hamsters (n=20) and control hamsters (NHB, n=14). S: small islets <0.01 mm²; M: medium islets ≥0.01 mm² and <0.1 mm²; Large islets ≥0.1 mm²; T.OD: average of optical density of stained cells per pancreatic area. **: p value <0.01; ***: p value <0.001.

be transformed to PrP^{Sc} in some tissues. In inherited prion diseases, different mutation in the PrP gene would cause different conformations of PrP^{Sc} and different types of prion diseases (Goldfarb et al., 1992; Gajdusek, 1994). There is general consensus that PrP^{Sc} is essential for the scrapie infectivity (Prusiner, 1982, 1989) and the neuronal pathological changes in scrapie (Taraboulos et al., 1992). It could be speculated that PrP^{Sc} plays a similar pathogenic role in the peripheral organs, which we will discuss below (see Table 3).

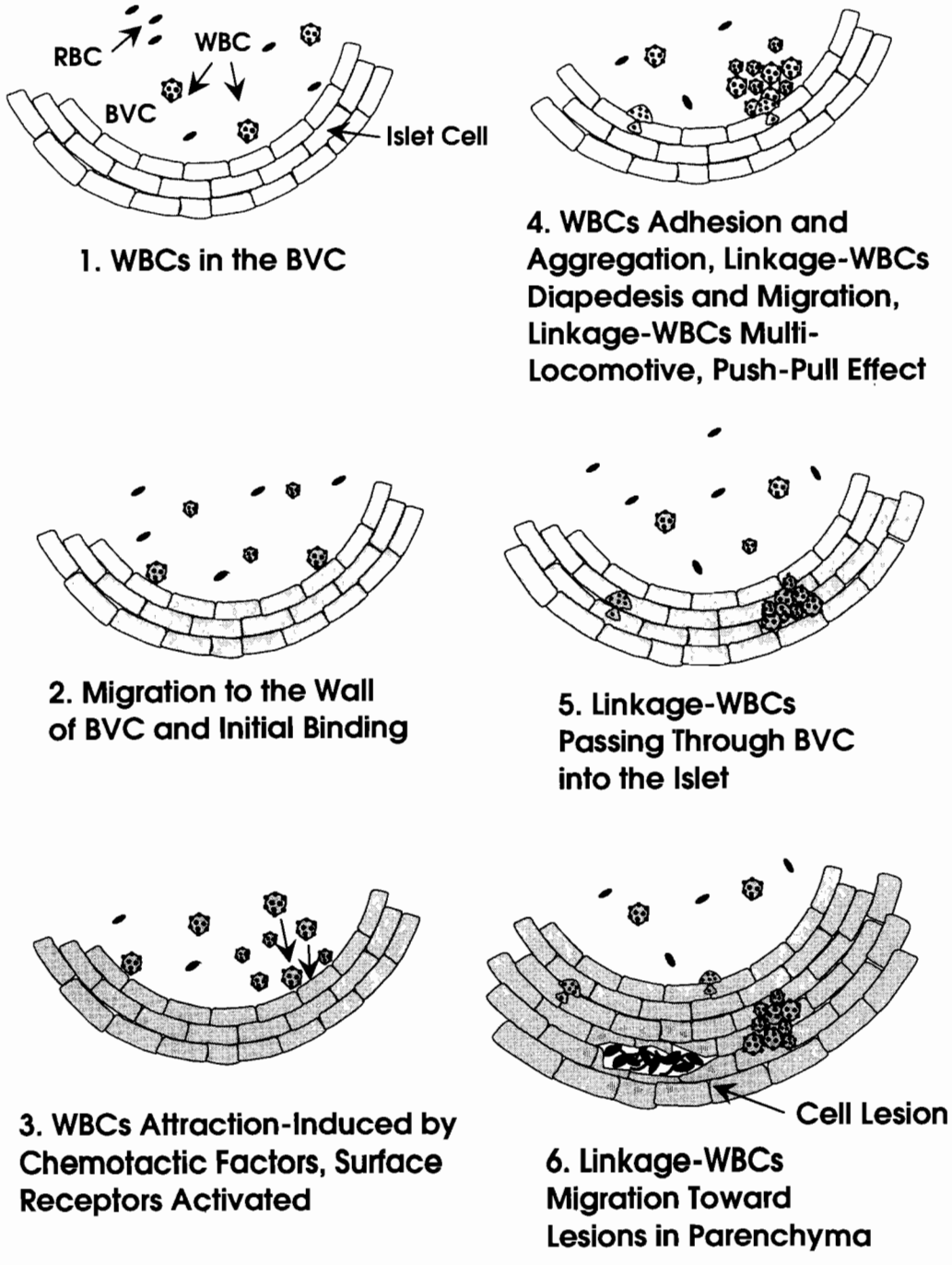

Fig. 10. Schematic representation of several events of linkage-WBCs passing the wall of BVC into the islet. **1.** The red blood cells (RBC) and the white blood cell (WBC) arrive via one or more capillaries into the BVC. **2.** The WBC migrates to the wall of BVC and initiates binding to the islet cell. **3.** A group of WBCs are attracted by chemotactic factors toward the wall of BVC, the intimate contact between WBCs and islet cells at the wall of BVC would further cause the WBC surface receptors activated. **4.** A group of WBCs interacted with each other, causing adhesion and aggregation (at this time, they are termed linkage-WBCs). The migration of WBCs into islet tissues can be considered an energy consuming process. The linkage-WBCs may act like a multi-locomotive and have a push-pull effect for migration, therefore, they may save a great deal of energy. **5.** the linkage-WBCs passing through BVC into the islet. **6.** The ultimate function of WBC is to help set up inflammatory response to the necrotic debris and phagocytosis. If a group of WBCs work together, they may increase the survival rate by decreasing the possibilities of over-phagocytosis.

Table 3. Comparison of pathology, infectivity titers and PrP concentration in peripheral organs of scrapie-infected animals.

ORGANS	SHEEP	MICE (SJL)			HAMSTER (LVG/LAK)		
	Scrapie	139A	ME7	22L	139H	22CH	263K
Pituitary							
Cytopathology	+	-	-	-	+++		±
Infectivity	Low				High		Low
PrP immunostain		±	±	±			
Adrenal							
Cytopathology	++	-	++	++	±		-
Infectivity	Low	Low	Low				
PrP immunostain							
Pancreas							
Pathology-acini		-	-	-	-		-
Infectivity	Low				Low		Low
(PrP^{Sc})					Low		Low
PrP immunostain		-	-	-	-		-
Pathology-islet		-	-	-	+++	+++	-
PrP immunostain		+	+	+	+		
Uterus							
Cytopathology		-	-	-	-	-	
Infectivity	Low						
PrP immunostain		+	+	+	+		
Ovary							
Cytopathology	-	-	-	-	-	-	
Infectivity	Low						
PrP immunostain		-	-	-	-		
Spleen							
Cytopathology		-	-	-	-	-	
Infectivity	High	High	High		High	High	
(PrP^{Sc})		++	++	++	++	++	
PrP immunostain		++	++	++			
Liver							
Cytopathology	-	-	-	-	±		
Infectivity	Low	-	-	-	-		
PrP immunostain		-	-	-	-		
Kidney							
Cytopathology		-	-	-	±		
Infectivity	Low	-	-	-			Low
PrP immunostain		-	-	-	-		

1. Pituitary gland

Recently, using congo red, and thioflavin-S staining and PrP^{Sc} immunostaining, we found little or no amyloid formation or PrP^{Sc} accumulation in the pituitaries of 139A-, ME7-, and 22L-infected mice. Infectivity titers were lower in pituitaries than in brain in scrapie-infected sheep and scrapie-infected hamsters. Comparisons of the pathological changes in the pituitaries of scrapie-infected animals found that only 139H-infected hamsters showed extensive histopathology, while the pituitaries of 263K-infected hamsters and scrapie-infected sheep showed little or mild pathological changes, primarily in the pars distalis in 263K-infected hamsters and in the pars nervosa and pars intermedia in scrapie-infected sheep. The pituitaries obtained from other scrapie strain-host combinations (139A, ME7, and 22L in SJL mice) appeared to be normal. These results suggest either (1) different scrapie strains have differential effects on neuronal cell types in the brain which affect pituitaries cells; or (2) some scrapie strains can directly affect cells in the pituitary, whereas others cannot. There was positive correlation in the pituitaries between histopathological changes and infectivity titers: in the 139H-hamsters combination there were extensive pathological changes and comparatively high infectivity titers, whereas for 263K-infected hamsters there were comparatively low levels for both parameters (Carp et al., 1994a,b). Further experiments should be done to assess the level of PrP^{Sc} in pituitaries of 139H- and 263K-infected hamsters as well as scrapie (139A, ME7 and 22L) infected SJL mice and to compare the level of PrP^{Sc} with pituitary pathology.

2. Adrenal gland

The level of PrP^{C} in adrenal glands is extremely low (Bendheim et al., 1992). Infectivity titers in adrenal glands in scrapie-infected sheep were about 0.1% of the titer in brain (Kimberlin and Wilesmith, 1994). Adrenal glands in mice infected with the ME7 and 139A scrapie

strains had much lower infectivity titers, 0.06% and 1%, respectively, than the titer in brain (Kim et al., 1988).The level of PrP^{Sc} in the adrenal glands of the scrapie-infected animals is still not known. The enlargement of the adrenal cortex in scrapie-infected sheep, in 139H-infected hamsters and in ME7-, and 22L-infected mice may be due to the function of the hypothalamic pituitary-adrenal axis as was suggested in the above section. In those scrapie strain-mouse combinations that show obesity and altered glucose tolerance plus adrenal enlargement, experiments were used stereotaxic injection into the hypothalamus. Further experiments aimed at measuring the level of PrP^{Sc} in adrenal glands in scrapie-infected animals.

3. Pancreas

The infectivity level in pancreas is 6000- to 10000-fold lower than in brain (Carp et al., 1994a,b; Kimberlin and Wilesmith, 1994). Comparatively low levels (vs. brain) of PrP^{Sc} were found in the pancreas of 139H-infected hamsters, which display pathological changes in the islets, whereas PrP^{Sc} was not detectable in the pancreas of 263K-infected hamsters, which showed normal pancreatic islets. The low level of PrP^{Sc} (about 0.6% of the level in brain) in the pancreas of 139H-infected hamsters suggests that the effect is not local. In fact, even if all of the infectivity and all of the PrP^{Sc} was localized to the endocrine portion of the pancreas, immunocytochemical analysis (McBride et al., 1992; Ye and Carp, unpublished results) indicated that PrP^{Sc} is locally within the islets (B cells) in the pancreas of scrapie infected hamsters and mice, the concentrations would still be lower than concentrations found in brain. The pancreatic islets in scrapie-infected mice which stained for PrP^{Sc} immunoreactivity did not show any cytopathological changes (Ye and Carp, unpublished results).

4. Ovary and uterus

As reported by Sturman (1972), the temporal development of ovarian stromal luteinization was determined in 139A-infected BALB/c mice. Six animals were killed at 3 or 4 weeks intervals for histopathologic examination. At 21 weeks after inoculation, all animals had clinical signs of scrapie but not ovarian lesions. by 25 weeks, however, all ten surviving mice showed ovarian stromal luteinization. The infectivity titer of the ovaries was about 0.01% to 0.1% of that in the brain. The late appearance of ovarian lesions and the low infectivity titer suggests that abnormalities in the reproductive system of scrapie-infected BALB/c mice are the consequence of neuroendocrine dysfunction (Sturman, 1972).

5. Spleen and lymph nodes

The data obtained thus far indicate a comparatively low concentration of PrP^{Sc} and infectivity in most of the peripheral organs. Only the peripheral organs of the lymphoreticular system (LRS) system (spleen, tonsil, lymph nodes and intestine) have infectivity titers that approach (1-10%) those of brain and spinal cord. This is true at the clinical stage of experimental scrapie in mice as well as in natural scrapie in sheep and goats. The remaining tissues have comparatively little or no detectable infectivity (Hope et al., 1988; Wilesmith et al., 1991). There are different cell types in the organs of the LRS, including T cells, B cells, macrophages, and follicular dendritic cells (FDCs). It appears that the FDCs plays an important role in the PrP^{Sc} accumulation and scrapie infectivity in the organs of LRS (Kitamoto et al., 1991; McBride et al., 1992; Carp et al., 1994a). Using immunostaining, we also found PrP^{Sc} accumulated in FDCs in the spleens of 139A-, ME7-, and 22L-infected mice. However, we did not observe any abnormal cytopathological changes in the spleens of these animals (Ye and Carp, in preparation).

6. Other peripheral organs

In transgenic mice overexpressing wild-type PrP^{C}, degeneration was found in skeletal muscle as well as in peripheral nerves, and the CNS (Westaway et al., 1994). Overexpression of PrP^{C} was not caused PrP^{Sc} accumulation in these organs. This study suggest that the spontaneous neuromyopathy is a consequence of PrP^{C} overexpression and not a consequence of PrP^{Sc} accumulation (Westaway et al., 1994).

The levels of PrP^{C} in the liver and kidney were found to be extremely low or no detectable in control animals (Oesch et al., 1985; Bendheim et al., 1992). Scrapie infectivity levels in liver and kidney were low (Eklund et al., 1967). The infectivity titers of kidneys in 263K-infected hamsters was approximately one-million fold less than brain (Carp and Robakis, unpublished). We found hemorrhage and cell lesions in the liver and kidney of some 139H-infected hamsters. Peripheral organs studies suggest that the pathological changes found in these organs are the result of specific targetng effects in brain by scrapie agents (Carp et al., 1990). The effects are most probably a function of brain-pituitary-endocrine end organ interactions. For example, in scrapie strain-mouse strain combinations that show obesity and altered glucose tolerance it appears that the hypothalamic-pituitary-adrenal axis is involved.

F. Correlations between obesity and pathological changes in peripheral organs

1. Adrenal gland

Recently, it has been found that corticosterone binds to two types of glucocorticoid receptors in the central nervous system; the MR (type I) receptor is similar to the peripheral mineralocorticoid receptor while the GR (type II) receptor is similar to the peripheral gluco-

corticoid receptor (Reul and DeKloet, 1985). It has been found that GR activity is an essential requirement for the obesity of Zucker (fafa) rats and that the maximum binding of corticosterone to the GR receptor is increased in both the hippocampus and hypothalamus of obese fafa rats, although the affinity of the receptors was consistently reduced (Langley and York, 1990). furthermore, glucocorticoid can regulate the propiomelanocortin (POMC) process in the pituitary and can increase the level of mRNA for malic enzyme and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) in liver and adipose tissue in the obese rats (York, 1991). These studies indicate that adrenal glucocorticoids are essential for the development of many forms of obesity. The cells in the zona fasciculata produce glucocorticoids. Excessive amounts of these hormones will cause abnormal fat distribution. As mentioned above, adrenalectomy prevented both the obesity and aberrant glucose tolerance in ME7-infected SJL mice. It is possible that obesity in scrapie-infected sheep and scrapie-infected mice is due to a marked enlargement of the adrenal glands.

2. The islets of Langerhans

Insulin also plays an important regulatory role in the development of obesity in many instances. Hyperinsulinemia is a common feature of most obesities (Bray and York, 1979). In scrapie, lesions of the islets of Langerhans and hypoglycemia-hyperinsulinemia were found only in 139H- and 22CH-infected hamsters, which also showed marked obesity. Using immunostaining methods, we found 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 indicates that increased B cell proliferation precedes hyperinsulinemia and obesity in yellow A^{Vy}/-mice (Warbritton et al., 1994). When analysis of the relationship among obesity, plasma insulin, and hepatic lipogenic enzymes in "viable yellow obese" mice (A^{Vy}/a) (Yen et al., 1976), it is found that hyperinsulinemia correlated positively with increase body weight and liver weight, and increase specific activities of malic enzyme and citrate cleavage enzyme. The hyperinsulinemia would change cellular enzymes activity. It would increase lipogenesis and inhibit lipolysis in liver and adipose tissue. It would also increase lipoprotein lipase activity and the elevated lipoprotein lipase would accelerate deposition of lipids into adipose tissue.

Another question concerns the function of the molecule called amylin. Amylin is synthesized in and co-released with insulin from the B cells of the islets of Langerhans (Johnson et al., 1988; Banks, 1990). Amylin is a novel hormone which may control carbohydrate metabolism in partnership with insulin and other glucoregulatory factors (Cooper et al., 1990). Amylin inhibits insulin-stimulated glycogen synthesis in skeletal muscle, while leaving insulin-stimulated carbohydrate metabolism in adipose tissue unchanged (Cooper et al., 1990). In well-nourished mammals, the body requires nutrients sufficient to provide energy and substance for immediate requirements and storage. Usually, the excess dietary carbohydrate is converted to glucose and then either initially stored as glycogen for later use or converted to fatty acids for longer-term storage as triacylglycerol in adipose tissue. The major sites of glycogen storage in the body are liver and skeletal muscle. Skeletal muscle accounts for 40% of the total body mass, therefore, it has the greater capacity for glycogen storage. After feeding, 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 (NIDDM) (Cooper et al., 1990). Under certain condition, amylin can form amyloid fibril and it has also been found that amylin fibrils could be toxic to islet B cells (Lorenzo et al., 1994). In our studies, we did not find amyloid formation in the islets of 139H-infected hamsters by Congo red or thioflavin-S staining. Based on the fact that their amino acid sequences are different, only some mammals such as cats, raccoons and humans form amyloids in islets. Rat amylin does not form amyloids in vivo (Cooper et al., 1990). It is possible that the synthesis and release of amylin from B-cells is also increased in 139H-infected hamsters. This is based on these findings: (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 would be interesting to determine comparative amylin concentrations in 139H-infected and control hamsters and to assess its role in induction of obesity in 139H-infected animals in future studies.

G. The possible relationship between pathological changes in brain and peripheral organs

1. Sheep

In scrapie-infected sheep, disturbances in five main clinical symptom-complexes have been described by Parry and Oppenheimer (1983): (1) metabolism: loss of muscle mass, or obesity; (2) motor dysfunction: ataxia; (3) sensation: rubbing or nibbling; (4) behavioral disorders: emotional instability, anxiety, and confusion; and (5) autonomic disorder: cardiac arrhythmia. Most of these syndromes can be related to the extensive pathological changes found in the central nervous system. At least three anatomic-physiological systems have been found to be affected: (1) the cerebellar; (2) the hypothalamo-neurohypophysial and (3) the central autonomic system (Fig. 11).

In cerebellum, the loss of nerve cells was found in the granular layer, and in the Purkinje cells of the flocculo-nodular lobe, especially in the paleocerebellum.

Mossy fibers were degenerated; fibrous gliosis was found in grey and white matter. The inferior olives and other brainstem nuclei showed vacuolation. Upon electron microscopy examination, membrane-bound vacuoles were found within neuronal cell bodies, dendrites, and axonal terminals in the medulla, pons, granule layer of the cerebellum, supraoptic nucleus (SON), lateral mammillary nucleus, and the pyramidal layer of the hippocampus. The large numbers of degenerating boutons terminaux around apparently

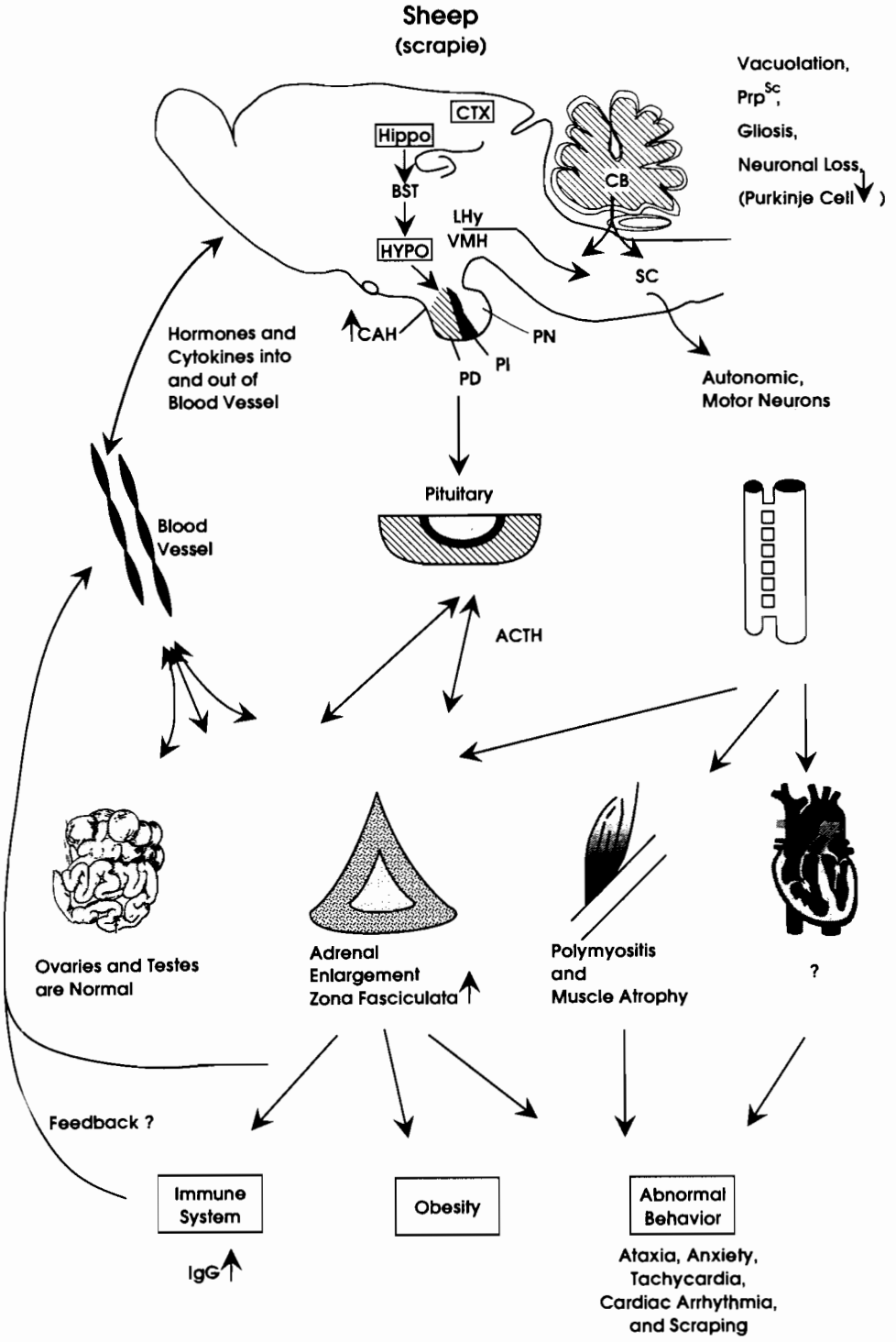

Fig. 11. Schematic representation of possible relationship between the pathological changes in the brain and the peripheral organs in scrapie-infected sheep. CTX: cortex; Hippo: hippocampus; BST: bed nucleus of the stria terminalis; HYPO: hypothalamus; LHy: lateral hypothalamus; VMH: ventral-medium hypothalamus; CB: cerebellum; Sc: spinal cord; CAH: Gomori chrome-alum-hematoxylin positive neurosecretory material; PN: pars nervosa; PI: pars intermedia; PD: pars distalis; ACTH: adrenocorticotropic hormone.

normal neurons of the pontine and papilliform nuclei were associated with a marked degree of ataxia, polymyositis and muscular atrophy (Parry and Oppenheimer, 1983).

In the hypothalamo-neurohypophysial (HNH) system, both in sheep infected with scrapie and in sheep with hypophysectomy or pituitary stalk section, there was a similar loss of neurons in the SON and paraventricular nuclei (PVN). Nerve fibers in the HNH tract were degenerated, with excessive CAH and neurophysin depositions. Degeneration in other hypothalamic nuclei such as the suprachiasmatic nucleus (SCN) was also found. Vacuolated neurons in the ventromedial hypothalamic nucleus (VMH, the "satiety center") and in the lateral hypothalamic area (LHy) may be due to retrograde degeneration. Lesions in VMH can cause obesity in animals either by the adrenal gland pathway (Debons et al., 1982) or by the pancreatic islets pathway (York and Bray, 1972). However, since the pathways, interrelationships and functions in the hypothalamic nuclei are still not fully understood, it is hard to explain how the hypothalamic lesions correlated with the metabolic and behavioral disorders such as abnormal water and sodium chloride uptake, obesity, tachycardia, and cardiac arrhythmia (Beck et al., 1964; Parry and Oppenheimer, 1983).

The central autonomic system also contained lesions in scrapie-infected sheep. The dorsal nucleus of the vagus nerve frequently showed neuronal vacuolation and fibrous gliosis. Some loss of neurons was found in the intermediolateral columns of the thoracic and lumbosacral cord (Wight, 1960). Direct hypothalamo-autonomic connections have been documented from the PVN to the central parasympathetic and sympathetic nuclei of the brainstem and cord of the rat, cat and monkey (Saper et al., 1976). Neurophysin was found in the hypothalamo-autonomic pathways (Swanson, 1977). These studies suggest that scrapie-induced lesions in different anatomic-physiological systems has some type of "retrograde" connection. However, it is not known whether the lesions in the adrenal glands of scrapie-infected sheep mentioned above are due to the hypothalamo-pituitary (CRF-ACTH) pathway or the hypothalamo-autonomic pathway.

It has been found that the concentration of IgG in serum was increased significantly and that there was a shift in the major IgG isotype from IgG1 to IgG2 in a high proportion of scrapie-infected Herdwick sheep (Collis and Kimberlin, 1983). Similar increases in serum immunoglobulin concentrations have also been observed in certain scrapie agent-mouse strain combinations (see later discussion). The significance of the increases in serum IgG in scrapie-infected animals is still not known. It may be due to (1) a specific immune response either to antigens related to scrapie agent (if there are any, i.e. PrP^{Sc}) or to host antigens associated with cell injury (i.e. brain or some peripheral organs) (Collis and Kimberlin, 1983); or (2) non-specific activation of cells within LRS. The ELISA assay was used to monitor antibody response to PrP^{Sc} in mice. Plasma from mice infected with several different scrapie agents (ME7- or 139A-infected C57BL/6J mice, and 87V-infected IM/Dk mice) was examined before and during clinical disease; antibody response to PrP^{Sc} was not detected (Kascsak et al., 1987). It is not known whether peripheral hormones, such as cortisol or cytokines, may feedback influence the neuroendocrine system, which in turn, leads to the increase of serum IgG in scrapie-infected animals.

2. Mice

Recently, it has been found that PrP 106-126 residues caused apoptosis in cultured rat hippocampal neurons (Forloni et al., 1993). Infection with a mouse scrapie strain can cause apoptosis and vacuolation in GT1-1-trk9 neurons (a hypothalamic cell line which has NGF receptors and secretes GnRH hormone). In contrast, other currently available scrapie-infected neuronal cell lines do not show distinct morphological changes (Schätzl et al., 1994). This suggests that (1) PrP^{Sc} only causes cellular pathologic changes in certain types of cells in the brain; or (2) a certain concentration of PrP^{Sc} is necessary for damaging a specific cell type. A similar conclusion comes from the observation that neuronal loss in vivo appears to occur primarily in very specific areas.

The lesions in the CNS of scrapie-infected mice have been studied for many years. Figure 12 summarizes the possible relationships between the pathological changes in the central and the peripheral organs in scrapie-infected mice. The most characteristic histopathological changes in scrapie infection are vacuolation, plaque formation (Carp et al., 1987; Jeffrey et al., 1994), gliosis, and neuronal and neurite degeneration in the brain, including cerebral cortex, and hippocampus (Scott and Fraser, 1984). There are also changes in brain neurochemical system (Cross, 1986), interleukin-1ß (IL-Iß) and NOS systems (Ye and Carp, unpublished results). Abnormal behaviors such as ataxia, waddling gait, and dullness in scrapie-infected mice may be due to muscle atrophy or neuronal damage. The neuronal or hormonal pathways might play an important role in the pathological changes in peripheral organs in scrapie-infected mice. The titer of scrapie in the adrenals was comparatively low. Adrenalectomy prevented both obesity and aberrant glucose tolerance. These results suggest that scrapie-induced obesity is based on an effect on the hypothalamic-pituitary-adrenal axis (Kim et al., 1987; Carp et al., 1989a,b), or on the axis termed hypothalamic-autonomic system-adrenal. Sturman (1972) reported endocrinopathy, especially ovarian luteinization in 139A-infected BALB/c mice. The scrapie titer in the ovaries was very low. The author suggested that ovarian pathology was due to neuroendocrine dysfunction as a result of scrapie effects on the CNS.

It has been found that some scrapie infected sheep and mice (87V-infected MB, VM, or IM mice) may

increase IgG production, while there was no changes in IgG concentration in 139A-infected CW mice, 22C-infected CW mice, 22A-infected IM mice and 87V-infected CW mice (Collis and Kimberlin, 1989). The level of IgA in the serum of 139A-infected mice (C57BL/6J and SJL/J) was markedly reduced compared to the levels in controls and the levels in ME7-infected mice (Carp et al., 1994a). Since ME7, but not 139A, caused morphological changes in the adrenal cortex, it was our expectation that if there was a change from

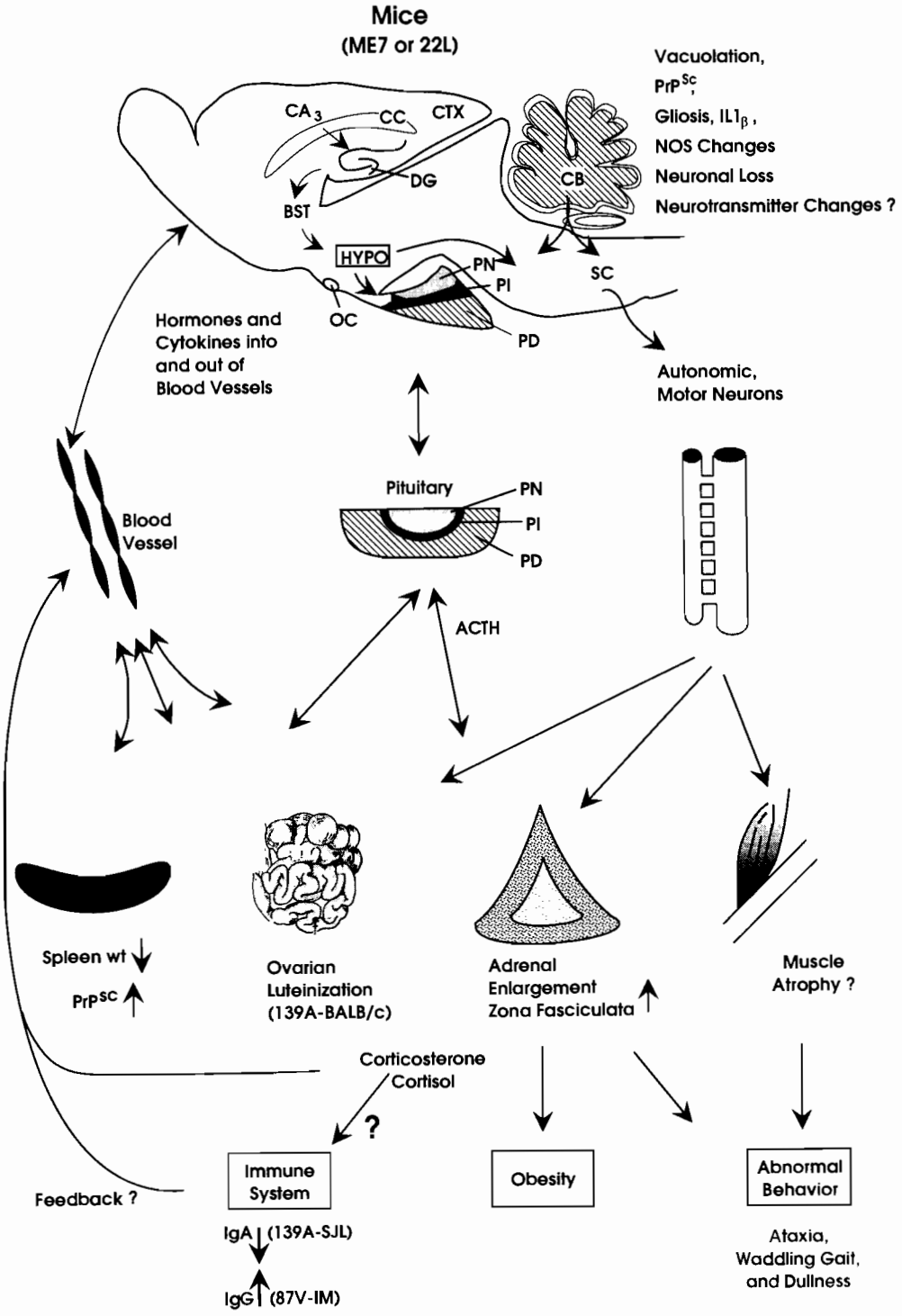

Fig. 12. Schematic representation of possible relationships between the pathological changes in the brain and the peripheral organs in scrapie-infected mice. CTX: cortex; cc: corpus callosum CA3, hippocampus; DG: Dentate gyrus; BST: Bed nucleus of the stria terminalis; HYPO: Hypothalamus; OC: optic chiasma; CB: cerebellum; Sc: Spinal cord; CAH: Gomori chrome-alum-hematoxylin positive neurosecretory material; PN: Pars nervosa; PI: Pars intermedia; PD: Pars distalis; ACTH: Adrenocorticotropic hormone.

normal in immunoglobulin concentration it would be induced by ME7 rather than 139A. It is certainly possible that the 139A scrapie strain changed adrenal hormone production without inducing histopathological changes. Another possibility is that the changes in IgG or IgA may not be due to changes of adrenal functions during scrapie infection. It has been found that FDCs, but not the T and B cells, play an important role in the PrP^{Sc} accumulation and scrapie infectivity in the organs of the LRS such as spleen (Kitamoto et al., 1991;

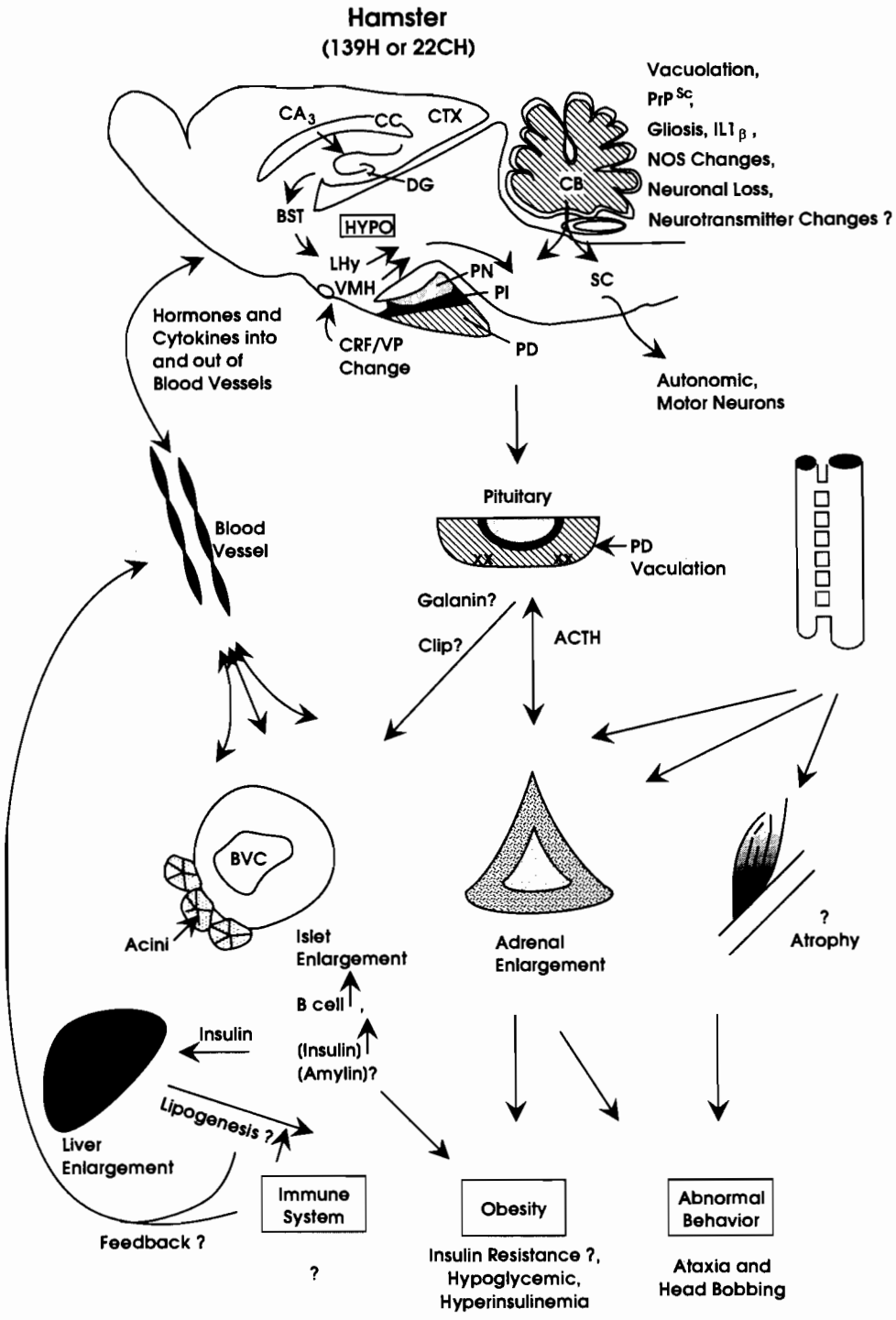

Fig. 13. Schematic representation of possible relationship between the pathological changes in the brain and the peripheral organs in scrapie-infected hamster. CTX: cortex; cc: corpus callosum CA3, hippocampus; DG: Dentate gyrus; BST: Bed nucleus of the stria terminalis; HYPO: Hypothalamus; LHy: lateral hypothalamus; VMH: ventral-medium hypothalamus; CB: cerebellum; SC: Spinal cord; CAH: Gomori chrome-alum-hematoxylin positive neurosecretory material; PN: Pars nervosa; PI: Pars intermedia; PD: Pars distalis; ACTH: Adrenocorticotropic hormone; CRF: corticotropin releasing factor; Clip: corticotropin-like intermediate peptide; VP: vasopressin; BVC: blood vessel core.

McBride et al., 1992; Carp et al., 1994a). A major function of FDCs is thought to be the stimulation of memory B cell production by immobilized immune complexes on FDC processes (Klaus et al., 1980). The role that scrapie-infected FDCs play in the changes in serum IgG and IgA levels is not clear.

3. Hamsters

Figure 13 summarizes the possible relationships between the pathological changes in the central and the peripheral organs in scrapie-infected hamsters. In the brain, vacuolation, PrP^{Sc} formation, gliosis, NOS changes (Ye and Carp, 1994), and neuronal loss were found in scrapie-infected hamsters. There are different patterns of PrP^{Sc} accumulation which correlate with the intensity of pathological changes in the brains and with the pattern of astrocytosis in scrapie-infected animals (Hecker et al., 1992; Ye et al., 1993). We also observed that increased IL-1ß immunostaining and PrP^{Sc} immunostaining and co-localized with GFAP immunostaining in reactive astrocytes in scrapie-infected hamsters (Ye and Carp, in preparation). The hypothalamo-pituitary system is important in regulating peripheral endocrine functions. It has been found that there are changes in the pattern and/or number of cells positive for corticotropin-releasing factor (CRF) and vasopressin (VP) in the hypothalamus of 139H-infected hamsters (Ye et al., 1994c). Using immunocytochemical techniques in 139H-infected hamsters, we observed a significantly increased number of CRF immunostained neurons in the preoptic nucleus and a significantly decreased number of VP immunostained neurons in the lateral hypothalamus compared to controls (Ye et al., 1994c). Lesions in the ventral hippocampal structure would increase CRF activity (Herman et al., 1992; Uno et al., 1992). Increase IL-1 may also stimulate the hypothalamic CRF activity (Berkenbosh et al., 1987; Sapolsky et al., 1987) and endothelium-independent production of nitric oxide (Beasley, 1990). Therefore, IL-1 increases in astrocytes may up-regulate hypothalamo-pituitary-adrenal (CRF-ACTH-corticosterone) activity and brain NOS activity. It is interesting to note that a novel cysteine protease, termed IL-1ß-converting enzyme, is responsible for cleaving the 31 kDa IL-1ß precursor to the mature form (Cerretti et al., 1992). This protease is also thought to have a role in regulating apoptosis in both neuronal and non-neuronal cells (Miura et al., 1993; Wilson et al., 1994). Considering that prion protein can also cause apoptosis in cultured rat hippocampal neurons (Forloni et al., 1993) and in GT1-trk9 cultured hypothalamic neurons (Schätzl et al., 1994), it would be interesting to determine whether IL-1ß-converting enzyme is increased in neurons, and whether apoptosis accounts for neuronal loss is scrapie-infected animals.

The fact that lesions in the VMH in rats can cause obesity has been known for many years (York and Bray, 1972; Debons et al., 1982). However, it is still not certain how the lesions of the CNS cause the pathological changes in the peripheral organs such as pituitary, islets of Langerhans, and adrenal glands. Both neuronal and hormonal pathways must be considered. In the neuronal pathway, lesions in VMH would stimulate the motor neurons of the vagus in the brain stem, increasing parasympathetic activity. Acetylcholine released from the vagus nerve can stimulate B cell activity and increase insulin secretion (Jeanrenaud, 1985). In the hormonal pathway, an insulin-secretion-promoting factor released from the ventral hypothalamus can stimulate insulin release (Bobbioni-Harsch and Jeanrenaud, 1989). Galanin is a 29-amino acid hormone, known to inhibit insulin secretion and cause hyper-glycemia. Galanin has been found in anterior pituitary cells (Kaplan et al., 1988). Another hormone, termed corticotropin-like intermediate peptide (CLIP), is located in the pars intermedia and stimulates insulin release. These hormones may be affected by the cellular damage in the hypothalamus and the pituitary in 139H-infected hamsters.

Mice infected with canine distemper virus (CDV) became obese (Nagashima et al., 1992). The pathological changes in the peripheral organs in CDV-affected mice are very similar to those of 139H-affected hamsters including increased liver, kidney and pancreas weights, with greatly enlarged pancreatic islets. These CDV-induced obese animals were also hyper-insulinemic. Examination of the brains of these mice revealed a significant reduction in tyrosine hydroxylase immunoreactivity and in pro-opiomelanocortin mRNA-positive perikarya in the arcuate area. Nagashima et al. (1992) suggested that the loss of critical populations of hypothalamic neurons as a result of CDV infection led ultimately to the development of morbid obesity.

Based on all these observations, it is probable that the obesity and pathological changes in the peripheral organs in scrapie-infected animals are a function of brain neuronal death and dysfunction. Further experiments should be done on this important issue.

H. Conclusion

In the current presentation, we detailed the gross and histopathological changes seen in a number of scrapie strain-host combinations and in sheep naturally infected with scrapie. These changes were seen in a number of organs, with particular predilection for endocrine organs. It is clear that changes in endocrine organs do not play a fundamental role in agent replication or in the induction of diseases, since in some model systems scrapie-induced changes are not observed in these organs.

The peripheral changes seen in some model systems are intriguing from a number of standpoints. First, their occurrence is determined by genetic influences from both agent and host. In this context, they provide additional approaches for the dissection of mechanisms involved in genetic control of events in scrapie

pathogenesis. This leads to a second general point: a suggested means of genetic control would be through effects upon targeting of neurons within the CNS. The mechanisms whereby specific neurons are targeted would then become critical to understanding the basis of differences in histological and clinical manifestations. A final point is that this broad spectrum of possible clinical and histopathological effects broadens the spectrum of diseases and syndromes that could be based upon slow infection processes.

Further work in this area should help to provide additional important information about this fascinating group of infectious diseases.

Acknowledgemetns. We wish to thank Drs. R.J. Kascsak, A. Scallet, J. Fikes and B. Bolon for critical reading this review. This study was supported in part by the New York State Department of Mental Hygiene and NIH grant AGO 9017. Dr. Xuemin Ye was supported in part 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.

References

Bailey C.J., Day C., Bray G.A., Lipson L.G. and Flatt P.R. (1986). Role of adrenal glands in the development of abnormal glucose and insulin homeostasis in genetically obese (ob/ob) mice. Horm. Metabol. Res. 18, 357-360.

Banks P. (1990). Amyloid deposits of diabetes spark new interest. J. NIH Res. 2, 34-35.

Beasley D. (1990). Interleukin 1 and endotoxin activate soluble guanylate cyclase in vascular smooth muscle. The American Physiological Society. R38-R44.

Beck E., Daniel P.M. and Parry H.B. (1964). Degeneration of the cerebellar and hypothalamic-neurohypophysial systems in sheep with scrapie and its relationship to human system degenerations. Brain 87, 153-176.

Bendheim P.E., Brown H.R., Rudelli R.D., Scala L.J., Goller N.L., Wen G.Y., Kascsak R.J., Cashman N.R. and Bolton D.C. (1992). Nearly ubiquitous tissue distribution of the scrapie agent precursor protein. Neurology 42, 149-156.

Berkenbosh F., Oers J.V., Rey A.D., Tilders F. and Besedovsky H. (1987). Corticotropin-releasing factor-producing neurons in the rat activated by interleukin-1. Science 238, 524-526.

Bignami A., Beck E. and Parry H.B. (1970). Neurosecretion-like material in the hindbrain of aging sheep and sheep affected with natural scrapie. Nature 225, 194-196.

Bobbioni-Harsch E. and Jeanrenaud B. (1989). The hypothalamic origin of an insulin secretion promoting factor present in the plasma of normal rats. J. Neuroendocrinol. 1, 103-108.

Braig H.R. and Diringer H. (1985). Scrapie: concept of a virus-induced amyloidosis in brain. EMBO J. 4, 2309-2312.

Bruce M.E., Dickinson A.G. (1985). Genetic control of amyloid plaque production and incubation period in scrapie-infected mice. J. Neuropathol. Exp. Neurol. 44, 285-294.

Büeler H., Fischer M., Lang Y., Blüthmann H., Lipp H.-L., DeArmond S.J., Prusiner S.B., Aguet M. and Weissmann C. (1992). The neuronal cell surface protein PrP is not essential for normal development and behavior of the mouse. Nature 356, 577-582.

Büeler H., Aguzzi A., Saller A., Greiner R.A., Autenried P., Aguet M. and Weissmann C. (1993). Mice devoid of PrP are resistant to scrapie. Cell 73, 1339-1347.

Carp R.I. and Rubestein R. (1991). Diversity and significance of scrapie strains. In: Seminars in Virology. Vol. 1. Haase A.T. (ed). Academic Press. New York. pp 203-213.

Carp R.I., Callahan S.M., Sersen E.A. and Moretz R.C. (1984). Preclinical changes in weight of scrapie-infected mice as a function of scrapie agent-mouse strain combination. Intervirology 21, 61-69.

Carp R.I., Moretz R.C., Natelli M. and Dickinson A.G. (1987). Genetic control of scrapie: incubation period and plaque formation in I mice. J. Gen. Virol. 68, 401-407.

Carp R.I., Kascsak R.J., Wisniewski H.M., Merz P.A., Rubenstein R., Bendheim P. and Bolton D. (1989a). The nature of the unconventional slow infection agents remains a puzzle. Alzheimer disease and associated disorders. 3 (1/2), 79-99.

Carp R.I., Kim Y.S. and Callahan S.M. (1989b). Scrapie-induced alterations in glucose tolerance in mice. J. Gen. Virol. 70, 827-835.

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

Carp R.I., Callahan S.M., Patrick B.A. and Mehta P.D. (1994a). Interaction of scrapie agent and cells of the lymphoreticular system. Arch. Virol. 136, 255-268.

Carp R.I., Ye X., Kascsak R.J. and Rubenstein R. (1994b). The nature of the scrapie agent: Biological characteristics of scrapie in different scrapie strain-host combinations. In: Slow infections of the central nervous system. Bjornsson J., Carp R.I., Love A. and Wisniewski H.M. (eds). New York Academy of Sciences. New York. pp 221-234.

Cashman N.R., Loertschrer R., Nalbantoglu J., Shaw I., Kascsak R.J., Bolton O.C. and Bendheim P.E. (1990). Cellular isoform of the scrapie agent protein participates in lymphocyte activation. Cell 61, 185-192.

Cerretti D.P., Kozlosky C.J., Mosley B., Nelson N., Ness K.V., Greenstreet T.A., March C.J., Kronheim S.R., Druck T., Cannizzaro L.A., Huebner K. and Black R.A. (1992). Molecular cloning of the interleukin-1ß converting enzyme. Science 256, 97-100.

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

Chandler R.L. (1969). Observations on the ultrastructure of the spinal cord, spleen and lymph nodes of mice inoculated with scrapie. Res. Vet. Sci. 10, 292-293.

Collis S.C. and Kimberlin R.H. (1983). Further studies on changes in immunoglobulin G in the sera and CSF of herdwick sheep with natural and experimental scrapie. J. Comp. Pathol. 93, 331-338.

Collis S.C. and Kimberlin R.H. (1989). Polyclonal increase in certain IgG subclasses in mice persistently infected with the 87V strain of scrapie. J. Comp. Pathol. 101, 131-141.

Cooper G.J.S., Day A.J., Willis A.C., Roberts A.N., Reid K.B.M. and Leighton B. (1990). Amylin and the amylin gene: structure, function and relationship to islet amyloid and to diabetes mellitus. Biochim. Biophys. Acta 1014, 247-258.

Cross A.J. (1986). Neurochemical changes in scrapie: comparison with conventional virus infections. In: unconventional virus diseases of the central nervous system. Court L.A., Dormont D., Brown P. and Kingsbury D.T. (eds). Commissariat à i Énergie Atomique. Paris. pp

617-626.

Debons A.F., Siclari E., Das K.C. and Fuhr B. (1982). Gold thioglucose-induced hypothalamic damage, hyperphagia and obesity: dependence on the adrenal gland. Endocrinology 110, 2024-2029.

DeArmond S.J., Mobely W.C., DeMott D.L., Barry R.A., Beckstead J.H. and Prusiner S.B. (1987). Changes in the localization of brain prion proteins during scrapie infection. Neurology 37, 1271-1280.

DeArmond S.J., Kristensson K. and Bowler R.P. (1992). PrP^{Sc} causes nerve cell death and stimulates astrocyte proliferation: a paradox. In: Progress in Brain Research. Vol. 94. Yu A.C.H., Hertz L., Norenberg M.D., Sykova E. and Waxman S.G. (eds). Elsevier Science Publishers. Amsterdam. pp 437-446.

Dickinson A.G. (1977). Scrapie in sheep and goats. In: Slow virus diseases of animals and man. Kimberlin. R.H. (ed). North-Holland. Amsterdam. pp 209-241.

Dickinson A.G. and Fraser H. (1977). Scrapie pathogenesis in inbred mice: An assessment of host control and response involving many stains of agent. In: Slow virus infections of the central nervous system. Ter Meulen V. and Katz M. (eds). Srpinger-Verlag. New York. pp 3-14.

Dickinson A.G. and Fraser H. (1979). An assessment of genetics of scrapie in sheep and mice. In: Slow Transmissible diseases of the nervous system. Vol. 1. Prusiner S.B. and Hadlow W.J. (eds). Academic Press. New York. pp 367-385.

Dickinson A.G. and Outram G.W. (1979). The scrapie replication site hypothesis and its implications for pathogenesis. In: Slow transmissible diseases of the nervous system. Vol. 2. Prusiner S.B. and Hadlow W.J. (eds). Academic Press. New York. pp 13-32.

Eklund C.M., Kennedy R.C. and Hadlow W.J. (1967). Pathogenesis of scrapie virus infection in the mouse. J. Infect. Dis. 117. 15-22.

Fraser H. (1979). Neuropathology of scrapie: The precision of the lesions and their diversity. In: Slow transmissible diseases of the nervous system. Vol. 1. Prusiner S.B. and Hadlow W.J. (eds). Academic Press. New York. pp 387-406.

Fraser H. and Dickinson A.G. (1973). Scrapie in mice: agent-strain differences in the distribution and intensity of grey matter vacuolation. J. Comp. Pathol. 83, 29-40.

Forloni G.N., Angeretti R., Chiesa E., Monzani M., Salmona O., Bugliani O. and Tagliavini F. (1993). Neurotoxicity of a prion protein fragment. Nature 362, 543-546.

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

Gajdusek D.C. (1994). Nucleation of amyloidogenesis in infectious and noninfectious amyloidoses of brain. In: Slow infections of the central nervous system. Bjornsson J., Carp R.I., Love A. and Wisniewski H.M. (eds). New York Academy of Sciences. New York. pp 173-190.

Gibbs C.J., Jr., Gajdusek D.C., Asher D.M., Alpers M.P., Beck E., Daniel P.M. and Matthews W.B. (1968). Creutzfeldt-Jakob disease (spongiform encephalopathy): Transmission to the chimpanzee. Science 161, 388-389.

Goldfarb L.G., Petersen R.B., Tabaton M., Brown P., LeBlanc A.C., Montagna P., Cortelli P., Julien J., Vital C., Pendelbury W.W., Haltia M., Wills P.R., Hauw J.J., McKeever P.E., Monari L., Schrank B., Swergold G.D., Autilio-Grambetti L., Gajdusek D.C., Lugaresi L. and Gambetti P. (1992). Fatal familial insomnia and familial Creutzfeldt-Jakob disease: disease phenotype determined by a DNA polymorphism. Science 258, 806-808.

Hecker R., Taraboulos A., Scott M., Pan K.M., Yang S.L., Torchia M., Jendroska K., DeArmond S.J. and Prusiner S.B. (1992). Replication of distinct scrapie prion isolates is region specific in brains of transgenic mice and hamsters. Gene. Dev. 6, 1213-1228.

Herman I.P., Cullinan W.E., Morano M.I., Watson S.J. (1992). Involvement of the ventral hippocampus in regulation of the hypothalamo-pituitary-adrenocortical axis. Soc. Neurosci. 18, 1541 (Abstr.).

Hope J., Reekie J.D., Hunter N., Multhaup G., Beyreuther H., White H., Scott A.C., Stack M.J., Dawson M. and Wells G.A.H. (1988). Fibrils from brains of cows with new cattle disease contains scrapie-associated protein. Nature 336, 390-392.

Jeanrenaud B. (1985). An hypothesis on the aetiology of obesity: dysfunction of the central nervous system as a primary cause. Diabetologia 28, 502-513.

Jeffrey M., Goddsir C.M., Bruce M.E., McBride P.A. and Farquhar C. (1994). Morphogenesis of amyloid plaques in 87V murine scrapie. Neuropathol. Appl. Neurobiol. 20, 535-542.

Johnson K., O'Brien T.D., Hayden D.W., Jordan K., Ghobrial H.K.G., Mahoney W.C. and Westermark (1988). Immunolocalization of islet amyloid polypeptide (IAPP) in pancreatic beta cells by means of peroxidase-antiperoxidase (PAP) and protein A-gold techniques. Am. J. Pathol. 130, 1-8.

Kaplan L.M., Gabriel S.M., Koenig J.I., Sunday M.E., Spindel E.R., Martin J.B. and Chin W.W. (1988). Galanin is an estrogen-inducible, secretory product of the rat anterior pituitary. Proc. Natl. Acad. Sci. USA 85, 7408-7412.

Kascsak R.J., Rubenstein R., Merz P.A., Tonna-DeMasi M., Fersko R., Carp R.I., Wisniewski H.M. and Diringer H. (1987). Mouse polyclonal and monoclonal antibody to scrapie-associated fibril proteins. J. Virol. 61, 3688-3693.

Kascsak R.J., Rubenstein R. and Carp R.I. (1991). Evidence for biological and structural diversity among scrapie strains. In: Current topics in microbiology and immunology. Vol. 172. Chesebro B.W. (ed). Springer-Verlag. Berlin-Heidelberg. pp 139-152.

Kim Y.S., Carp R.I., Callahan S.M. and Wisniewski H.M. (1987). Scrapie-induced obesity in mice. J. Infect. Dis. 156, 402-405.

Kim Y.S., Carp R.I., Callahan S. and Wisniewski H.M. (1988). Adrenal involvement in scrapie-induced obesity. Proc. Soc. Exp. Biol. Med. 189, 21-27.

Kim Y.S., Carp R.I., Callahan S. and Wisniewski H.M. (1990). Pathogenesis and pathology of scrapie after stereotactic injection of strain 22L in intact and bisected cerebella. J. Neuropathol. Exp. Neurol. 49, 114-121.

Kimberlin R.H. (1982). Scrapie agent: prions or virions? Nature 297, 107-108.

Kimberlin R.H. and Marsch R.F. (1975). Comparisons of scrapie and transmissible mink encephalopathy in hamsters. I. Biochemical studies of brain during development of disease. J. Infect. Dis. 131, 97-103.

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. and Wilesmith J.W. (1994). Bovine spongiform encephalopathy, epidemiology, low dose exposure and risks. In: Slow infections of the central nervous system. Björnsson J., Carp R.I., Lòve A. and Wisniewski H.M. (eds). Ann. NY. Acad. Sci. pp 211-220.

Kitamoto T., Muramoto T., Mohri S., Doh-Ura K. and Tateishi J. (1991). Abnormal isoform of prion protein accumulates in follicular dendritic

cells in mice with Creutzfeldt-Jakob disease. J. virol. 65, 6292-6295.

Klaus G.G.B., Humphrey J.H., Kunkl A. and Dongworth D.W. (1980). The follicular dendrite cell: its role in antigen presentation in the generation of immunological memory. Immunol. Rev. 53, 3-28.

Langley S. and York D.A. (1990). The effects of the antiglucocorticoid RU-486 on the development and obesity in the obese fafa rat. Am. J. Physiol. 259, R539-544.

Lifson N., Kraming K.G., Mayrand R.R. and Lander E.J. (1980). Blood flow to the rabbit pancreas with special reference to the islets of Langerhans. Gastroenterology 79, 466-473.

Lorenzo A., Razzabonl B., Weir G.C. and Yankner B.A. (1994). Pancreatic islet cell toxicity of amylin associated with type-2 diabetes mellitus. Nature 368, 756-760.

Manson J., West J.D., Thomson V., McBride P., Kaufman M.H. and Hope J. (1992). The prion protein gene: a role in mouse embryogenesis? Development 115, 117-122.

Manuelidis L., Murdoch G. and Manuelidis E.E. (1988). Potential involvement of retroviral elements in human. In: Novel infectious agents and the central nervous system. Bock G. and Marsh J. (eds). Ciba Foundation Symposium No. 135. Academic Press. New York. pp 117-134.

Marsh R.F. and Hanson R.P. (1979). On the origin of transmissible mink encephalopathy. In: Slow transmissible diseases of the nervous system. Vol. 1. Prusiner S.B. and Hadlow W.J. (eds). Academic Press. New York. pp 451-460.

Masters C.L., Gajdusek D.C. and Gibbs C.J., Jr. (1981). Creutzfeldt-Jakob disease virus isolations from the Gerstmann-Straussler syndrome. Brain 104, 559-588.

McBride P.A, Eikelenboom P., Kraal G., Fraser H. and Bruce M.E. (1992). PrP protein is associated with follicular dendritic cells of spleens and lymph nodes in uninfected and scrapie-infected mice. J. Pathol. 168, 413-418.

McKinley M.P., Bolton D.C. and Prusiner S.B. (1983). A protease-resistant protein is a structural component of the scrapie prion. Cell 35, 57-62.

Miura M., Zhu H., Rotello R., Hartwieg E.A. and Yuan J. (1993). Induction of apoptosis in fibroblasts by IL-1ß-converting enzyme, a mammalian homolog of the *C. elegans* cell death gene ced-3. Cell 75, 635-660.

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

Oesch B., Westaway D., Walchli M., McKinley M.P., Kent S.B.H., Aebersold R., Barry R.A., Tempst P., Teplow D.B., Hood L.E., Prusiner S.B. and Weissmann C. (1985). A cellular gene encodes scrapie PrP 27-30 protein. Cell 40, 735-746.

Oleszak E.L., Murdoch G., Manuelidis L. and Manuelidis E.E. (1988). Growth factor production by Creutzfeldt-Jakob disease cell lines. J. Virol. 62, 3103-3108.

Parry H.B. and Livett B.G. (1977). Neurophysin in the brain and pituitary gland of normal and scrapie-affected sheep. II. Its occurrence in the cerebellum in dystrophic axon terminals, with lysosome-lipofuscin accumulation. Neuroscience 2, 53-72.

Parry H.B. and Oppenheimer D.R. (1983). In: Scrapie disease in sheep. Oppenheimer D.R. (ed). Academic Press. London. pp 1-20.

Prusiner S.B. (1982). Novel proteinaceous infectious particles cause scrapie. Science 216, 136-144.

Prusiner S.B. (1989). Scrapie prions. Annu. Rev. Microbiol. 43, 345-374.

Prusiner S.B., Scott M., Foster D., Pan K.-M., Groth D.F., Mirenda C.A., Torchia M., Yang S.-L., Serban D., Carlson G.A., Hoppe P.C., Westaway D. and DeArmond S.J. (1990). Transgenic studies implicate interactions between homologus PrP isoforms in scrapie prion replication. Cell 63, 673-686.

Reul J.M.H.M. and deKloet E.R. (1985). Two receptor systems for corticosterone in rat brain: microdissection and differential occupation. Endocrinology 117, 2505-2511.

Rohwer R.G. (1984). Scrapie infectious agent is virus-like in size and susceptibility to inactivation. Nature 308, 658-662.

Saper C.B., Loewy A.D., Swanson L.W. and Cowan W.M. (1976). Direct hypothalamo-autonomic connections. Brain Res. 117, 305-312.

Sapolsky R., Rivier C., Yamamoto G., Plotsky P. and Vale W. (1987). Interleukin-1 stimulates the secretion of hypothalamic corticotropin-releasing factor. Science 238, 522-524.

Schätzl H.M., Weiner R.J., Laszlo L., Holtzman D.M., Mobely W.C. and Prusiner S.B. (1994). Apoptosis, vacuolation and infectivity in a novel cell line producing scrapie prions. Soc. Neurosci. 18, 1257 (Abstr.).

Scott J.R. and Fraser H. (1984). Degenerative hippocampal pathology in mice infected with scrapie. Acta Neuropathol. 65, 62-68.

Scott M., Foster D., Mirenda C.A., Serban D., Coufal F., Walchli M., Torchia M., Groth D., Carlson G.A., DeArmond S.J., Westaway D. and Prusiner S.B. (1989). Transgenic mice expressing hamster prion protein produce species-specific scrapie infectivity and amyloid plaques. Cell 59, 847-857.

Shelton E. (1962). Prolonged survival of rabbit thoracic duct lymphocytes in a diffusion chamber. J. Cell Biol. 12, 652-655.

Sharp J.A. and Burwell R.G. (1960). Interaction («peripolesis») of macrophages and lymphocytes after skin homografting or challenge with soluble antigens. Nature 188, 474-475.

Sigurdsson S.B. (1954). Rida, a chronic encephalitis of sheep with general remarks on infections which develop slowly and some of their special characteristics. Br. Vet. J. 110, 341-354.

Solomon J. and Mayer J. (1973). The effect of adrenalectomy on the development of the obese-hyperglycemic syndrome in OB/OB mice. Endocrinology 93, 510-513.

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.

Sturman L.S. (1972). Endocrinopathy in mice affected with scrapie. Nature New Biol. 238, 208-209.

Swanson L.W. (1977). Immunohistochemical evidence for a neurophysin-containing autonomic pathway arising in the paraventricular nucleus of the hypothalamus. Brain Res. 128, 346-353.

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

Uno H., Sakai A., Shelton S. and Eisele S. (1992). Hippocampal regulation of the pituitary-adrenal axis in monkeys. Soc. Neurosci. 18, 1541 (Abstr.).

Vogt C. and Vogt O. (1937). Sitz and Wesen der Krankheiten im Lichte der topistischen Hirnforschund und des Variierens der Tiere. J. Psychol. Neurol. 47, 237.

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

Weissmann C. (1991). A «unified theory» of prion propagation. Nature

352, 679-374.

Wells G.A.H., Scott A.C., Johnson C.T., Gunning R.F., Hancock R.D., Jeffrey M., Dawson M. and Bradley R. (1987). A novel progressive spongiform encephalopathy in cattle. Vet. Rec. 121, 419-420.

Westaway D., DeArmond S.J., Cayetano-Canlas J., Groth D., Foster D., Yang S.L., Torchia M., Carlson G.A. and Prusiner S.B. (1994). Degeneration of skeletal muscle, peripheral nerves, and the central nervous system in transgenic mice overexpressing wild-type prion proteins. Cell 76, 117-129.

Wight P.A.L. (1960). The histopathology of the spinal cord in scrapie disease of sheep. J. Comp. Pathol. 70, 70-83.

Wilesmith J.W., Ryan J.M.B. and Atkinson M.I. (1991). Bovine spongiform encephalopathy: Epidemiological studies on the origin. Vet. Res. 128, 199-203.

Williams E.S. and Young S. (1980). Chronic wasting disease of captive mule deer: A spongiform encephalopathy. J. Wildl. Dis. 16, 89-98.

Williams E.S. and Young S. (1982). Spongiform encephalopathy of Rocky Mountain elk. J. Wildl. Dis. 18, 465-471.

Wilson K.P., Black J.F., Thomson J.A., Kim E.A., Griffith J.P., Navia M.A., Murcko M.A., Chambers S.P., Aldape R.A. Raybuck S.A. and Livingston D.J. (1994). Structure and mechanism of interleukin-1ß converting enzyme. Nature 370, 270-275.

Ye X. and Carp I.R. (1994). Scrapie-induced increase in nitric oxide synthase activity in the hamster brain. Brain Pathol. 4, p 525.

Ye X., Carp R.I., Callahan S. and Shak J. (1991). Histopathological changes in the pituitary of female hamsters infected with the 139H strain of scrapie. Soc. Neurol. 17, 1274 (Abstr.).

Ye X., Carp R.I., Kascsak R., Kozielski R., Kozlowski P. (1993). Astrocytosis and amyloid deposition in scrapie-infected hamsters. In: Biology and pathology of astrocyte-neuron interactions. Fedoroff S., Juurlink B.H.J. and Doucette R. (eds). Plenum Press. New york. London. p 443.

Ye X., Carp R.I., Yu Y., Kozielski R. and Kozlowski P. (1994a). 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.

Ye X., Carp R.I., Yu Y., Kozielski R. and Kozlowski P. (1994b). Histopathological changes in the islets of Langerhans in 139H-infected hamsters. J. Comp. Pathol. 110, 153-167.

Ye X., Carp R.I., Yu Y., Kozielski R. and Kozlowski P. (1994c). Effect of infection with the 139H scrapie strain on the number, area and/or location of hypothalamic CRF- and VP-immunostained neurons. Acta Neuropathol. 88, 44-54.

Yen T.T., Greenberg M.M., Yu P., and Pearson D.V. (1976). An analysis of the relationship among obesity, plasma insulin, and hepatic lipogenic enzymes in "viable yellow obese" mice (A^{vy}/a). Horm. Metabol. Res. 8, 159-166.

York D.A. (1991). Neuroendocrine and metabolic derangements in animal models of obesity. In: Obesity and cachexia. Rothwell N.J. and Stock M.J. (eds). John Wiley and Sons Ltd. pp 103-118.

York D.A. and Bray G.A. (1972). Dependence of hypothalamic obesity of insulin, the pituitary and the adrenal gland. Endocrinology, 90, 885-894.