

Aging, Metabolism, and Alzheimer Disease: Review and Hypotheses

CALEB E. FINCH^{*,1} AND DAVID M. COHEN^{†,2}

^{*}Neurogerontology Division, Andrus Gerontology Center and Department of Biological Sciences, University of Southern California, Los Angeles, California 90089-0191; and [†]Metabolic Research Unit, Department of Physiology and Biophysics, School of Medicine, University of Southern California, Los Angeles, California 90033

Relationships are considered among aging, metabolism, and Alzheimer disease (AD). In particular, after 60 years, human populations show progressive age-related trends for increased blood glucose that are concurrent with the accelerating incidence of AD. The accumulation of glycated products in the AD brain, such as is also found in peripheral tissues during diabetes, suggests interactions of AD with age-related changes in metabolism. A review of 13 recent studies on AD and diabetes shows no consensus, although most studies indicate an apparent exclusion of AD and diabetes. We argue that longitudinal studies are needed to evaluate the possibility that an initial age-related hyperglycemic state is reversed by the cachexia and weight loss common to later stages of AD. A review of literature on chronic food restriction in rodents shows the slowing of some aspects of aging in the nervous system and generally supports interactions of peripheral metabolism with brain aging. Finally, we discuss aspects of intermediary metabolism that could ensue from oxidative damage to enzymes by glycation or oxidative stress which include excess production of ammonia from the inhibition of glutamine synthetase and the production of glyceraldehyde-3-phosphate, a glycating agent that could contribute to damage in addition to the hyperglycemic trends during aging.

© 1997 Academic Press

INTRODUCTION

Alzheimer disease (AD) is primarily a disease of aging, with an incidence that doubles every 5 years between 65 and 85 years in all human populations examined (79). AD is generally viewed as originating within the brain, with a slow inexorable progression that has been generally presumed to arise autonomously of systemic influences because it occurs in both sexes. We outline an alternative and complementary view that considers possible relationships of AD to

systemic and local metabolic changes during aging. As discussed below, changes in metabolism and metabolic hormones are also prominent in this age group.

First, we summarize features of AD that are the basis for our argument, including the apparent rarity (exclusion) of diabetes in AD. Nonetheless, it is striking that senile plaques (SP) and neurofibrillary tangles (NFT) display advanced glycation endproducts (AGEPs), oxidation products of proteins which are associated with hyperglycemia and diabetes. Smith *et al.* (190, 191) observe that, because AGEPs are increased by prolonged exposure to elevated glucose, as occurs in maturity onset diabetes (Type II, or non-insulin-dependent diabetes mellitus), the presence of AGEP in AD brains implies a role of glycation in AD pathogenesis.

Next, we discuss age-related changes in the regulation of blood glucose and glucocorticoids, and the manipulation of age changes in the nervous system of rats by diet restriction and hypophysectomy. This literature, which is rarely mentioned in relation to AD, is a source of valuable insights on interrelationships of aging, AGEP, and AD. Finally, we discuss changes of intermediary metabolism that could also promote the formation of AGEP, particularly through the inhibition of glutamine synthetase and glyceraldehyde-3-phosphate dehydrogenase. A possible excess of ammonia from the inhibition of glutamine synthetase could favor the production of amyloidogenic fragments of amyloid precursor protein (β -APP). Increased production of nitric oxide (NO) could inhibit glyceraldehyde-3-phosphate dehydrogenase, leading to increased levels of glyceraldehyde-3-phosphate, a substrate for the formation of AGEP that is more reactive than glucose. This discussion is intended to broaden the concepts of glucose toxicity, which have arisen in the analysis of diabetes. We do not, however, consider gender differences in AD and many other hormonal changes of aging which would overextend the already broad sweep of our discussion.

BACKGROUND

Plaques and Tangles

The 39- to 43-amino-acid amyloid β -peptides ($A\beta$) are found as aggregates in three locations in AD brains

¹ To whom correspondence should be addressed. Fax: (213) 740-0853.

² Current address: Laboratory of Cerebral Metabolism, Building 36, Room 1A-07, National Institutes of Health, Bethesda, MD 20892. Fax: (301) 480-1668.

(176, 180): (i) $A\beta$ in the senile (dense core or neuritic) plaques, which are a pathologic characteristic of AD and are most prevalent in regions of the brain that show extensive neurodegeneration during AD. (ii) $A\beta$ aggregates are also found in diffuse deposits of amyloid that accumulate more generally during aging and are found in regions of AD brains with little or no neurodegeneration. (iii) Cerebral blood vessels also accumulate $A\beta$ aggregates in AD brains, but also during normal aging. The source of the $A\beta$ in these different locations is not known and could be produced by local brain neurons, astrocytes, or microglia/macrophages. Alternatively, monomeric $A\beta$, which is present in the cerebrospinal fluid and blood, could be imported into the brain. The $A\beta$ peptides extracted from AD brains can range in size from 39 to 43 amino acids, although $A\beta_{1-42}$ is the main component in the two types of extracellular amyloid deposits of senile plaques (104, 162). The origins of $A\beta$ peptides in the aggregates remain a major puzzle in AD. In addition to $A\beta$ peptides, SP and diffuse amyloid deposits contain numerous other proteins typical of inflammatory processes, such as cytokines, acute phase proteins, and complement system proteins and regulators (38, 117, 201). Neurofibrillary tangles (NFT), another pathological hallmark of AD, occur within neurons, where they are associated with abnormal morphology of the cell body and processes, and loss of synapses (198). The extent of neurodegeneration can be correlated with the density of both NFT and SP (22, 198).

The schedule for formation of SP and NFT during the prolonged course of AD is not known. Biopsy and postmortem studies generally show both SP and NFT at early clinical stages of AD (73, 108). However, a recent study of very early dementia found extensive amyloid deposits in the hippocampus, whereas the density of neuritic plaques or NFT in the hippocampus was not notably different from that in age-matched controls (125). Thus, amyloid accumulations may precede NFT formation in AD pathogenesis. While the numbers of NFT increase with the severity of clinical symptoms (73, 108, 125), there may be little further change in the amount of aggregated $A\beta$ or senile plaques ("amyloid burden") during the later course of AD (73).

A Chemical Clock, the Racemization of $A\beta_{1-42}$

The $A\beta_{1-42}$ in senile plaques appears at postmortem to be chronologically older than that in cerebrovascular amyloid, as judged by the accumulation of spontaneously racemized amino acids, a thermodynamically driven process. The accumulation of D-aspartyl residues can be used a biomarker of age in long-lived proteins that turn over slowly or not at all. In tooth dentin, D-aspartate accumulates as a linear function of age from birth onward (113). In AD brains, there are

three-fold more D-aspartyl and isoaspartyl residues in $A\beta_{1-42}$ from senile plaques than that from cerebral vessels, which suggests that $A\beta_{1-42}$ turns over more slowly in senile plaques than in cerebral vessels (104, 162). Thus, $A\beta_{1-42}$ in SP appears to be older than in the cerebral vessels of AD brains, which predicts that SP amyloid is at greater risk than that in the cerebral vasculature for accumulating AGEs and other oxidative changes.

Glycation

The oxidation of long-lived proteins through nonenzymatic reactions of glucose and other reducing sugars with free amino groups, particularly the ϵ -amino of lysine, is a slow inexorable process throughout the lifespan (182, 190, 206, 218). These changes are thermodynamically driven, like racemization, but are distinguished from those of racemization by the major role of glucose in the microenvironment of a protein that reacts nonenzymatically. The initial adduct of glucose with lysine forms a Schiff base, which is reversible, but then slowly converts to a stable ketoamine through the Amadori rearrangement during a time course of days. Glycated hemoglobin in erythrocytes (HbA_{1c}) is widely used in clinical chemistry as a marker for unregulated hyperglycemia during diabetes, because the extent of glycation as HbA_{1c} is a direct measure of the integrated exposure of erythrocyte hemoglobin to blood glucose (83, 206).

The initial Amadori product is subject to slow oxidation and complex condensation reactions to form Maillard products that are also referred to as advanced glycation or glycoxidation endproducts, over longer times that span weeks or months (10, 207). The glycoxidation product pentosidine, for example, can form covalent links with side-chain nitrogens of both lysine and arginine that can result in the cross-linking of adjacent peptide chains and great increases in insolubility. In many cases, these reactions proceed to form brown fluorescent compounds. In the eye, there is a life-long linear accumulation of LM-1 (lens Maillard product-1), a blue fluorescent adduct of crystallins (133). Model reactions *in vitro* indicate that ribose or ascorbate are more likely sources of LM-1 than glucose. Moreover, glucose-6-phosphate and glyceraldehyde-3-phosphate (G3P) are even more reactive than glucose in promoting glycoxidation, as discussed later.

Glycation and Diabetes

Control of plasma glucose levels within normal bounds is often effective in reducing the complications of diabetes. Studies of 1059 middle-aged patients (97) and 229 elderly patients (88) with type II diabetes (non-insulin-dependent diabetes mellitus) found that metabolic control of plasma glucose (as assessed by glycosylated

hemoglobin levels) was a strong predictor of stroke. In the Diabetes Control and Complications Trial (DCCT), a clinical study of 1441 patients with type I diabetes (insulin-dependent diabetes mellitus), it was found that strict control of plasma glucose concentrations diminished long-term complications including diabetic retinopathy, neuropathy, and kidney lesions (29). Related to these complications is the increased leakage of plasma proteins from increased vascular permeability in retina, kidney, and nerve (155). During a 2- to 4-week administration of AGEs (as AGE-modified albumin), rats developed increased vascular permeability at normoglycemia (207). AGEs may therefore contribute directly to the long-term complications of diabetes.

In type II diabetics, the levels of pentosidine in the skin in general correlate with the severity of diabetic complications (134). However, glycoxidative processes may differ importantly between tissues for a given level of blood glucose. For example, in dogs that had varying degrees of sustained hyperglycemia for 5 years, collagen in the dura mater of brain was more glycosylated at lower blood HbA_{1c} (integrated glucose exposure) glucose than the crystallins of the eye lens; note that pentosidine in crystallins is formed by glycation from glucose, whereas another adduct cited above, LM-1, may be formed from other sugars (133, 134). Such 5-year studies are unusual because their prolonged duration revealed outcomes of different grades of hyperglycemia that might be difficult to resolve in the much shorter-lived diabetic rats. These authors proposed that there are tissue differences in thresholds for the formation of pentosidine.

A further complexity is the absence of correlations between HbA_{1c} and two properties of dura collagen, digestibility by pepsin and fluorescence (134), which suggest yet other chemical or cellular processes. Moreover, there is no general relationship among species of mammals between blood glucose concentration and the rate of AGE accumulation in collagen and other proteins (183). The growing list of reactive sugars that drive the formation of AGEs led us to anticipate diverse outcomes in the biochemistry of aging, with differences between tissues according to local metabolic characteristics that yield variations in particular reducing sugars.

AGEP and Normal Aging

During normal aging, long-lived proteins in many tissues show a slow accumulation of AGEs, e.g., collagen in human skin and brain dura mater (182, 183). The extent of AGE accumulation in normal brains during aging is unclear, because results are sensitive to the particular immunoreagents. On one hand, two reports using polyclonal antisera to glycosylated poly-L-lysine detected AGEs in granules in the perikarya of large neurons of normal brains. Li *et al.* (103) found AGEs in the same hippocampal and cortical

neuron fields of normal human brains that acquire NFTs in AD; few were found in the brains of young individuals (<25 years). Moreover, these same reagents applied to rodents and several domestic mammals were immunoreactive in neuronal nuclei, not only in the older specimens, but also in calves (102). In contrast, using antisera to pentosidine-KLH conjugates, Smith *et al.* (190) detected very few AGEs in normal aged human brains except in the occasional SP or NFT found in controls for their studies. Nonetheless, cerebral blood vessels of both controls and AD had AGEs (190). The presence of AGEs in cerebral blood vessels of older individuals is consistent with the progressive age-related accumulation of AGEs in connective tissues throughout the body (183). Further studies are needed to define precisely the subcellular entities that were immunoreactive in the studies of Li *et al.* (102, 103).

Many other oxidative processes are important to aging besides those associated with glycation and the formation of AGEs. Gafni showed that muscle glyceraldehyde-3-phosphate dehydrogenase (G3PDH) becomes less catalytically active in aging rats, because a significant fraction of the enzyme molecules become oxidized at the catalytically active Cys-139 near the active site (31, 44). A contributing factor may be the nearly twofold age-related increase in the ratio of oxidized:reduced glutathione (GSSG:GSH) in skeletal muscle (142). The phenomenon of decreased enzyme activity because of age-related oxidation is well established, and examples can be found in many tissues, including brain, that are independent of glycation (100, 193).

Glycosylated SP and NFT May Promote Further Oxidative Damage

Both SPs and NFTs display AGEs. In AD brains, a monoclonal antibody to AGE conjugated to serum albumin was reactive with senile plaques (81). Similarly, pentosidine groups were detected by immunohistochemistry within the matrix of SP and NFT in postmortem samples from individuals at end stages of AD (190, 203, 214). The antisera to glycosylated poly-L-lysine also detected AGEs in the perikarya of NFT-bearing neurons of AD brains (103), which suggests that this immunoreagent detects a different AGE than found by other immunoreagents (190). Data are lacking on the rate of accumulation of AGEs during AD, but might be obtained from existing biopsy specimens. AGEs are of rapidly growing interest as mechanisms in the formation of highly aggregated proteins in the SP and NFT of AD brains for several reasons. As noted above, AGEs can covalently cross-link adjacent polypeptide chains and thus could promote the formation of highly insoluble protein aggregates. Moreover, AGEs can propagate free-radical reactions that may catalyze further damage to proteins, lipids, or DNA

(86); e.g., the AGEPs formed by experimentally glycated recombinant human tau produce oxygen free radicals (215). Furthermore, experimentally glycated A β peptides promote the aggregation of A β , which might be an early factor in the development of SPs (203). Similarly, glycated tau readily forms large aggregates that might promote the formation of NFT (214).

At the cellular level, AGEPs show activities pertinent to neurodegeneration. AGEPs can stimulate macrophages to produce reactive oxygen species (ROS) (206), a response that merits investigation for brain microglia/macrophages. Peripheral macrophages recognize AGEPs and other oxidized substrates by scavenger receptors (136, 205, 214). Fibrillar A β (a component of SPs) adheres to microglial surfaces by interactions with the scavenger receptor (34). Receptors for AGEPs ("RAGE") bind to amyloid β peptide *in vitro*, causing oxidative stress in neurons and endothelial cells, as well as activation of microglia (cytokine production and chemotaxis) (216). We speculate that if cellular surface density of RAGE increases with AGEPs, then tissue sensitivity to toxicity of β -amyloid may also increase, to thereby couple cellular levels of AGEPs to the pathology of AD. AGEPs can also be chemoattractants for cells that produce ROS (82). Furthermore, AGEPs after lipofection into cultured neurons induced lipid peroxidation, IL-6 expression, and activation of NF- κ B, a transcription factor associated with oxidative stress (215). Moreover, the lipofected neurons increased the release of an A β monomer (not characterized for amino acid residues).

Changes in Myelin during AD and Aging

Myelin thinning (myelin pallor) is found in brains during "normal aging" (80, 181). Vascular damage can lead to focal demyelination (lacunar infarcts), which appears to have a different pathogenesis than myelin pallor (30, 197). It is also possible that some of these specimens from early studies included early stage AD. Demyelinating conditions can also arise during diabetes, in which glycation appears to have a role in attracting macrophages (207).

Apolipoprotein E

Apolipoprotein E (ApoE) is found with A β in senile plaques of AD brains (135). The ApoE allele ϵ 4 and the protein isoform E4 are associated with a higher risk of AD in elderly who are less than 80 years old (20, 153) and in late onset familial AD (195). ApoE allele polymorphisms are associated with quantitative variations in blood lipid levels that could influence vascular disease; e.g., the ϵ 4 allele is associated with higher, and ϵ 2 with lower, LDL cholesterol (25, 33). The E2 isoform has lower affinity for the LDL receptor, which reduces the uptake of VLDL by macrophages. Although diabetics also show these associations of ApoE isoforms with

serum levels of LDL cholesterol, ApoE allele frequencies for diabetics resemble those in Caucasian populations (33, 138, 168). However, it is unknown if ApoE isoforms influence the uptake of oxidized lipids or proteins by macrophage scavenger receptors.

AGE, BLOOD GLUCOSE, AND AD

We next consider the complex and controversial relationships among age, blood glucose, AD, and type II diabetes. In peripheral tissues, AGEPs are accumulated during normal aging, a process which is accelerated by diabetes, as discussed above. We also address physiological and biochemical factors in aging that are usually not considered in AD. This discussion does not assume that SP and NFT formation are the only processes important to AD.

Normal Trends for Increases of Blood Glucose during Human Aging

Rigorous and extensive studies during the past 30 years have established age-related trends for gradual increases of blood glucose, particularly after 65 years, an age when the prevalence of AD begins to increase exponentially. Data from 15,000 individuals, aged 20–74 years were collected by the Second National Health and Nutrition Survey (NHANES II) (58). In subjects with no medical history of diabetes, the mean plasma glucose after a 10-h fast was about 10% higher in an older group aged 65–74 years than in a younger group aged 20–44 years. After ingesting 75 g of glucose, mean plasma glucose was 30% higher in older subjects than in younger subjects, both at 1 and 2 h postglucose. This huge study confirmed other findings with fewer subjects; e.g., Davidson (24) showed after oral glucose that 1-h glucose levels increased an average of 9 mg/dl per decade.

How then do these trends relate to the incidence of maturity-onset diabetes or non-insulin-dependent diabetes, which is also referred to as type II diabetes. In the NHANES II survey (58), diabetes was defined by the National Diabetes Data Group (NDDG) by fasting plasma glucose >140 mg/dl and mean 1- and 2-h plasma glucose levels >200 mg/dl. Overt diabetes in 65- to 74-year-old group was eightfold more prevalent than among those 20–44 years old (17.7% vs 2.2%). The lesser condition of impaired glucose tolerance (IGT) was defined by the NDDG by fasting plasma glucose <140 mg/dl, 1-h levels >200 mg/dl, and in the range 140–199 mg/dl after 2 h. IGT increased fourfold, from 2.1% in the 20- to 44-year group to 9.2% in the 65- to 74-year group. By the criterion for impaired glucose tolerance of the World Health Organization (WHO), which does not include a required level of plasma glucose at 1-h postconsumption of the 75 g oral glucose, the prevalence of IGT increases more than threefold

(6.4%, 20–44 years; 22.8%, 65–74 years). By either criterion, a sizable proportion of those aged ≥ 65 years either have overt diabetes or show impaired glucose tolerance: NDDG (27%), WHO (41%). As Harris (59) points out, a huge number of older adults have cryptic diabetes that is not diagnosed by fasting blood glucose.

The hyperglycemia of aging may be partly ameliorated by diet and exercise (16, 76, 77, 158). However, such effects are controversial, because declining glucose tolerance by 60 years cannot be solely attributed to age-related changes in diet or exercise. One study (793 subjects, 17–92 years) found differences in plasma glucose 2 h after glucose ingestion between those aged >60 years vs younger, even after adjusting for distribution of body fat, and physical fitness (186). The elderly group showed an 18% increase in 2-h plasma glucose above the younger group.

Special techniques are needed to assess the effect of aging on the efficacy of insulin to stimulate glucose uptake, because both plasma insulin and plasma glucose are elevated in aging. To study subjects with the same initial blood glucose, blood levels are controlled by the “glucose clamp technique,” in which insulin is administered iv at a constant rate and glucose is administered at a variable rate that maintains the plasma glucose at a set level, from which the rate of glucose utilization is calculated. Several studies show 30% less stimulation of glucose uptake by insulin (decreased insulin action, increased insulin resistance) in older vs younger individuals (28, 39, 40, 165). During a euglycemic clamp with a low insulin infusion rate, the steady state level of insulin was 1.8-fold higher in the elderly, whereas the rate of uptake of glucose was 40% less than in the young (39); this implies that the elderly have decreased insulin efficiency, i.e., the activity of insulin to increase glucose uptake. An alternative estimate of the effectiveness of insulin in stimulating glucose uptake (insulin sensitivity) developed by Bergman is given by a mathematical model of glucose and insulin dynamics (3, 4). In conjunction with an assay of C-peptide, which is secreted from the pancreas with insulin in equimolar amounts, this approach showed that β -cell function becomes increasingly insufficient with age in men (15). These findings were confirmed by others (53, 76, 77).

The blood–brain barrier does not protect the cerebral tissue from hyperglycemia. In humans, glucose in the cerebrospinal fluid is about 60% of that in the blood (41, 184). By NMR spectroscopy in healthy adolescents, brain glucose concentrations were only 25% of plasma glucose, even under hyperglycemia (52). At 5.7 mM blood glucose (normoglycemia), about 50% of the glucose transporters of the blood–brain barrier are occupied, whereas at 12.2 mM (hyperglycemia), 71% are occupied, implying that the transport capacity is not saturated. Other analyses are consistent with the

exposure of the brain during the hyperglycemia of diabetes to elevated levels of glucose (149, 177). Because the CSF glucose changes lag 2–4 h behind those in the blood (41), the brain may be exposed to even more net glucose during aging than peripheral tissues, if calculated on an integrated 24-h basis. These studies need to be extended to older normal and demented groups.

In sum, there are strong age-related trends for increased blood glucose in the same age group, 65–85 years, that experiences an accelerating prevalence of AD (79). In this regard, we are stuck by the observation that the accumulation of pentosidine in skin collagen accelerates exponentially with aging in humans (183). This nonlinear increase of AGEs is consistent with the model of self-catalyzing oxidative damage as AGEs accumulate, as discussed above (21, 86).

Cognitive Functions, Aging, and Blood Glucose

Impaired systemic glucose regulation has been associated in older humans and rats with memory impairments. Two studies of nondemented older subjects showed significant inverse correlations of cognitive performance with glucose intolerance. As measured by blood glucose after oral glucose loading, higher blood glucose was associated with poorer memory performance on a subsequent test day (55, 109). These findings may measure different aspects of brain function than detected in the enhancement of memory functions in normal elderly by manipulations of glucose or insulin (21, 109). Old rats showed similar inverse correlations between memory acquisition and glucose intolerance (194). Because old rodents lack SPs or NFTs, the basis for age-related cognitive declines of rodents cannot be identical with that of AD. Nonetheless, there is evidence for age-related increase in glycosylated epitopes (102), reviewed above. While patients with type II diabetes show decreased cognitive functions, e.g., Reaven *et al.* (159), follow-up studies are needed to show if these individuals were in early stages of AD or had cryptic vascular dementia.

An Unresolved Issue: The Presence or Absence of Associations of Diabetes and AD

Studies of the association of type II diabetes and AD give conflicting and inconsistent results, which we summarize in three groups: no statistically significant associations, positive or negative (five studies; Table 1A); negative (inverse) associations, i.e., disproportionately low prevalence of type II diabetes in AD (six studies; Table 1B); positive associations (two studies; Table 1C). The table summarizes the methods of evaluation for diabetes and AD, and subject pool sizes ($n < 50$ to >8000). We state the statistical significance level as reported.

TABLE 1
Summary of Studies on the Coincidence of Diabetes and Alzheimer's Disease

Authors	Design and method	Subjects (Male/Female)	Association of diabetes and AD	Diagnosis of diabetes	Diagnosis of AD
A. No statistically significant association (in chronological order)					
Heyman <i>et al.</i> , 1984 (Ref. 65)	Case-control: AD patients of Duke University survey; community-living controls	AD: 12/28 control: 24/56	No	History taken from spouse or close relative	"Rigorous clinical, laboratory and psychological criteria . . . at initial evaluation & during follow-up" (64)
Broe <i>et al.</i> , 1990 (Ref. 7)	Case-control dementia patients of Sydney, Australia community-living controls	AD: 64/106 control: 64/106	No	Interview (self-report)	NINCDS-ADRDA criteria for probable/possible AD
Kokmen <i>et al.</i> , 1991 (Ref. 85)	Case-control; retrospective review of newly diagnosed AD at the Mayo Clinic (1960-1974)	AD: 415 total control: 415, with same ratio of M/F	No	Review of community health records	NINCDS-ADRDA criteria, but "psychometric data were not available for each AD case"
Thorpe <i>et al.</i> , 1994 (Ref. 199)	Case-control; retrospective review of hospital records at Washington University St. Louis				
Autopsy series		AD: 130/83 control: 291/181	No	Glucose tolerance test and glycosuria	Multiple SP and NFT, particularly in the hippocampus
Hospital series		AD: 56/109 control: 83/83	No (diabetes was 50% more prevalent in AD patients; not significant)	Glucose tolerance test and glycosuria	Clinical evidence of dementia, including psychological evaluation
Curb <i>et al.</i> , 1996 (Ref. 23)	Honolulu Heart Program; prospective cohort study	total: 3774 M AD: 77 M	No (diabetes tested in 1965-1966; AD in 1991-1993)	Nonfasting, 1-h oral glucose tolerance	CERAD clinical evaluation and DSM-III-R criteria
B. Negative associations (in chronological order)					
Bucht <i>et al.</i> , 1983 (Ref. 11)	Case-series, post hoc review of records at Umeå University hospital	839 dementia patients (sex ratio not given) 317 AD, 457 MID, 65 confusional states; 60 cases of diabetes	Yes no diabetics in AD group	Oral glucose tolerance test, NDDG guidelines	History of insidious onset, slowly progressive dementia (global dementia); no hypertension, transitory ischemia, or stroke
	Case-control of elderly	AD: 22/23 control, hospital: 15/15; community-living: 16/15	Yes higher insulin in AD vs healthy community controls ($P < 0.05$), 90 min after oral glucose; blood glucose normal-low; no indication of diabetes	Oral glucose tolerance test, NDDG guidelines	
Wolf-Klein <i>et al.</i> , 1988 (Ref. 213)	Post hoc, case-series study of charts in geriatric outpatients at Hyde Park, NY Jewish Inst. Geriatric Care	AD: 28/47 normal mental status: 85/58 other (abnormal mental status): 60/70	Yes diabetes (1 case) was less in AD (3.5%) vs cognitively normal elderly (16.5%) $P < 0.01$	Medical records, no glucose test	DSM-III criteria
Ferini-Strambi <i>et al.</i> , 1990 (Ref. 37)	Case-control, University of Milan, Italy	AD: 25/38 controls, community-living: 25/38	Yes diabetes in AD (3%) vs normal controls (12%), $P < 0.05$	Interview with patient and close family member, no glucose test	NINCDS-ADRDA criteria
Landin <i>et al.</i> , 1993 (Ref. 95)	Case-series study, St. Jörrens Hospital, Hisings Backa, SWE	AD: 14/24 VaD: 8/6 other dementia: 11/8	Yes fasting glucose lower in AD, $P < 0.05$; no diabetes in AD; VaD 36%, other dementia 21%	Fasting glucose; medical history	NINCDS-ADRDA criteria; VaD, transient ischemic attacks and/or severe vascular disease
Mortel <i>et al.</i> , 1993 (Ref. 128)	Case-series study, VA Medical Center, Baylor University, Houston, TX	AD: 98/85 VaD: 84/53	Yes diabetes in AD (6%) vs VaD (23%), $P < 0.0001$	Case histories and interviews with family members; no glucose test	NINCDS-ADRDA criteria; VaD: California ADDTC criteria for probable ischemic VaD
Nielsen <i>et al.</i> , 1996 (Ref. 138)	Cross-sectional study of patients in outpatient dementia center, University of California at Irvine	AD: 47/76 VaD: 24/27 mixed: 21/36 other: 20/14	Yes diabetes in AD (1%) vs VaD (12%) vs mixed dementia (9%); χ^2 , $P < 0.05$	NDDG guidelines from patient records	NINCDS-ADRDA criteria for AD VaD: California ADDTC criteria for probable ischemic VaD; mixed: both AD and VaD
C. Positive associations					
Ott <i>et al.</i> , 1996 (Ref. 147)	Prospective cohort study; the Rotterdam Study	cohort: 7983 AD: 4% type II diabetes 12%	Yes type II diabetics had higher risk for dementia or VaD ($P = 0.05$); diabetics on insulin therapy had greater risk for AD than nondiabetics ($P = 0.05$)	Use of antidiabetic medication; randomly sampled with respect to meals, or postload serum glucose > 11 mM	NINCDS-ADRDA criteria
Leibson <i>et al.</i> , in press (Ref. 98)	Retrospective cohort study; Mayo Clinic records, 1970-1984	cohort: 1455 AD: 30/47 non-AD dementia: 41/60	Yes, M only M diabetics vs nondiabetics, risk of AD is > 2 -fold higher ($P < 0.05$); F diabetics, 37% higher (not significant)	NDDG criteria; and > 20 years old when diagnosed with diabetes	AD: DSM-III-R criteria, adapted for retrospective record review; dementia, insidious onset, slow progression and exclusion of other causes of dementia

Some of these studies must be viewed cautiously because more rigorous criteria for diagnosis of diabetes or AD have been developed (59, 60). Patient selection can also introduce bias; e.g., the recruitment of relatively healthy AD outpatients would tend to bias against those with AD who suffer complications of diabetes, e.g., leg or foot amputation, or peripheral neuropathy (see next section), which would impair local travel. Moreover, a major confound must be considered. The current diagnosis of AD by the NINCDS-ADRDA criteria (118) and the CERAD criteria (121, 126) excludes strokes. Because type II diabetes is associated with a two- to fivefold higher risk of stroke (2, 97, 110), it is possible that an individual with diabetes also developed early AD, but subsequently suffered stroke. In fact, stroke is a very common cause of dementia in the elderly and may be classified as multi-infarct dementia (MID), mixed dementia, or vascular dementia (17, 197). Estimates of vascular dementia range from 25 to 50% of dementias in the elderly (78, 143). The density and regional distribution of NFTs and SP in vascular dementia are also less well characterized than in AD. Thus, the presence of diabetes in MID or mixed dementia warrants much more emphasis in clinical evaluation. To an unknown extent that varies between clinical evaluators, there is a discrimination against even a provisional diagnosis of AD if a subject shows evidence of diabetes. Virtually any evidence of vascular impairment may suffice to disqualify the designation of "pure AD" and classify the individual as mixed or vascular dementia. Because of this conservative bias against the diagnosis of AD, some vascular and mixed dementia subjects with diabetes, in fact, might be reclassified as AD. As noted above, diabetes may be grossly underestimated in the general population. A glucose challenge or glucose-insulin tolerance test is needed and was not included in most studies. We first emphasize three community-based studies that offer the largest sample sizes, but that have divergent conclusions. Only one of these has been submitted as a full paper.

No significant AD-diabetes associations were found in five studies (Table 1A). In the fourth examination of the Honolulu Heart Program, Curb *et al.* (23; abstract) evaluated the cognitive status and fasting glucose of survivors of a very large cohort of men ($n = 8006$) resident in Honolulu who, almost 30 years before, had been tested for diabetes. There was no association of AD in 1991-1993 with diabetes, as evaluated in 1965-1966 by a glucose tolerance test (blood glucose 1 h after a glucose load) or in 1991-1993 by fasting blood glucose. Because 47% of the original cohort (1) are represented in the subgroup that survived until the recent evaluation for cognitive status, the analysis may be biased by death of nonhealthy members of the cohort who had diabetes and AD, both of which are mortality risk factors. Other studies of relatively smaller subject

groups using case-control designs found no significant associations, positive or negative, of diabetes among patients with AD (Table 1A): Heyman *et al.* (65), Broe *et al.* (7), Kokmen *et al.* (85), and Thorpe *et al.* (199).

Negative associations, or a disproportionately low prevalence, of diabetes among AD subjects met tests for statistical significance in five other studies of AD that used normal elderly controls or vascular dementia for comparison, Table 1B: Wolf-Klein *et al.* (213); Ferini-Strambi *et al.* (37), Landin *et al.* (95); Mortel *et al.* (128); and Nielson *et al.* (138). Moreover, Bucht *et al.* (11) did not find any overt diabetes in their large AD sample ($n = 317$), consistent with later reports; diabetes was mostly associated with multi-infarct dementia (12% incidence in this group).

On the other hand, two studies of large groups found positive associations of diabetes with risk of AD. Leibson *et al.* (98) from the Mayo Clinic analyzed 1455 men and women resident in Rochester, Minnesota who were diagnosed with diabetes in the years 1970-1984 and followed until 1985. For male diabetics, the odds ratio for AD was 2.27-fold higher than that of male nondiabetics ($P < 0.05$). For female diabetics, the odds ratio of 1.37 was not significant. There was no effect of subject age on the relationship between diabetes and the risk of AD, which argues against the importance of age-related increases in blood glucose with respect to AD in older diabetics. In the Rotterdam Study ($n = 7983$ residents), Ott *et al.* (147; abstract) report associations of AD and diabetes in individuals on insulin therapy (odds ratio, 2.7) and even higher significance for vascular dementia (odds ratio 3.1). Irrespective of insulin use, diabetics had higher odds for vascular dementia (3.1) than for AD (1.3). Moreover, others showed greater insulin resistance in AD, as judged by serum insulin after a glucose load (11) or after fasting (120). By itself, this degree of insulin elevation would not establish type II diabetes.

Confounds and other problems in analysis may be found in each type of study. Cross-sectional studies exclude diabetics in the population who would have developed AD had they lived long enough. Retrospective studies based on case histories often have insufficient quality control for diagnosis of AD or of diabetes. We do not know of data on HbA1c in AD that might indicate the extent of undiagnosed diabetes or of subtle chronic hyperglycemia. While Nielson *et al.* (138) did not find differences in fasting blood glucose between AD patients and other dementias (<50% of patients were tested for fasting blood glucose), some of the other dementias as diagnosed may have included diabetics because of the presence of stroke. Moreover, it is possible that some individuals who were diagnosed with mixed AD or vascular dementia were initially AD, but subsequently suffered strokes. This possibility may

arise with apoE ϵ 4 alleles, which predisposes to both stroke and AD.

In sum, although it is not yet possible to resolve the differences between the above studies on the association of diabetes and AD, we believe that the question of AD–diabetes coincidence merits further inquiry. An open question is the possibility of changes in glucose and insulin metabolism during the progression of AD (120). Craft *et al.* (21) suggest that hyperinsulinemia is more characteristic of early AD than later stages of AD and that insulin levels after hyperglycemia could indicate the stage of AD. Furthermore, Halter (57) suggests that AD could protect against type II diabetes as a consequence of the weight loss that is common during clinically diagnosed AD. At any age, weight loss would tend to diminish obesity-related insulin resistance and the presentation of hyperglycemia in tests of glucose tolerance (see below). Thus, food restriction is one of the recognized strategies in treating type II diabetes (59). Expanding on Halter's suggestion from above, we hypothesize that the hyperglycemia of aging could promote the onset of AD pathogenesis, e.g., through the glycooxidation of nascent amyloid deposits (see below), but could diminish subsequently when neurodegenerative changes lead to cachexia and weight loss. This prediction is consistent with the observed normal to low blood glucose in overt AD (11, 95). Bucht *et al.* (11) suggested how neurotransmitter deficiencies in AD could influence the secretion of metabolic hormones and the possible relationship of lower glucose to abnormal neuronal electrical activity. These questions may only be resolved by longitudinal studies of large subject pools that are aging with and without type II diabetes. Future studies may also have access to information on the long-sought genetic risk factors for type II diabetes, which could interact in important ways with AD gene risk factors.

Peripheral Neuropathy in AD?

Peripheral neuropathy is a common complication of diabetes that might be an indirect marker for chronic hyperglycemia in AD. Diabetics often suffer decreased sensitivity of cutaneous reflexes and slowed conduction in peripheral nerves (6, 32). The basis could be partial demyelination and shrinkage of axonal caliber; either would slow nerve conduction. AGEPs are implicated in several mechanisms of peripheral neuropathy (204, 207, 212). AGEPs on myelin are hypothesized by Vlassara *et al.* (204, 207) to promote endocytosis of myelin through macrophages that are attracted by AGEp receptors. AGEPs are also present on the vaso nervorum, which typically shows vascular wall thickening that may cause ischemia and subsequent neuronal dysfunctions (197). Accumulations of AGEPs on tubulin

and other cytoskeletal proteins (166, 212) could be a factor in axonal atrophy, because the neurocytoskeleton mediates the retrograde transport of neurotrophins.

There is no recognized evidence for peripheral neuropathy in AD—the topic is not mentioned in recent monographs and reviews. However, there are indications of slowed peripheral conduction velocity in AD. Levy *et al.* (101) characterized 28 senile dementia patients of average age 77; those with the most cognitive impairments had 25% slower conduction in the ulnar nerve than age-matched controls. When retested 1 year later, there was a further 15% decrease in conduction rate of the most demented group. This is provisional evidence for peripheral neural dysfunctions in AD, but does not establish the contribution of demyelination or the role of blood glucose.

HYPOTHESES ABOUT THE ACCUMULATION OF AGEPS DURING AD

We consider two hypotheses about AGEp in AD, (I) linear accumulation and (II) accelerated accumulation. Most parsimonious is hypothesis I, that AGEPs in AD brains accumulate as a linear function of time, after the initial formation of SP and NFT. As noted above, SP are present early in the clinical stages of AD and do not appear to accumulate further, whereas NFTs show progressive increases in numbers as cognitive functions deteriorate over many years. The hypothetical linear accumulation of AGEPs in either SP or NFT would be a passive process from exposure of slowly turning over proteins in the SP and NFT that are chemical targets of glucose in the interstitial fluid of the brain or of intracellular sugars. Because SP and NFT occur in early stages of AD, it is not surprising that AGEPs are detected in SP and NFT at the end stages of AD. AGEPs are also found in cerebral vessels of age-matched brains used as controls for AD (190). One may anticipate the detection of AGEPs in many other long-lived proteins throughout the brain, e.g., in basement membranes and extracellular matrix. In this regard, the accumulation of AGEPs in NFT, SP, and cerebrovasculature might be record the time-dependent exposure to blood glucose. Similarly, Mattson *et al.* (115) argued that AGEPs can be viewed as a “tombstone in Alzheimer's.” Recall from above another spontaneous age (time)-dependent change, the accumulation of racemized aspartyl groups in A β .

An alternative is hypothesis II, that the accumulation of AGEp accelerates during AD as a consequence of age-related increases in blood glucose. As reviewed above, a sizable and increasing proportion of those >65 years have some degree of hyperglycemia in the same age groups showing an accelerated accumulation of AGEPs in skin collagen during aging. Even modest

age-related increases in blood glucose can be predicted to enhance glycation of proteins, which is consistent with hypothesis II. A tissue-specific threshold of glycemia for formation of specific AGEs has been observed in experimentally induced (alloxan) diabetes in dogs (134). Evidence that clinically normal older subjects and rats show inverse correlations of cognitive performance with glucose intolerance (see above) is more consistent with hypothesis II than hypothesis I, since increased hyperglycemia would favor increased formation of AGEs in those individuals. Hypothesis II predicts even greater accumulations of AGEs in the SP and NFT of type II diabetics with mixed vascular dementia and AD.

The resolution of hypothesis I vs II might be achieved by comparisons of glycation in SP and NFT in biopsies with postmortem status. During the next decade, such longitudinal data may be obtained from biopsies taken during the implantation of shunts for infusion of neurotrophins in ongoing studies, as well as the previous studies of Bethanecol. Glycated or oxidized sites may, in the future, be recognized by imaging reagents.

HYPERADRENOCORTICISM, GLUCOSE METABOLISM, AND AGING

We now discuss the possible role of glucocorticoids in brain aging processes, because of evidence for elevation of glucocorticoids during aging and AD and because glucocorticoids interact with insulin and glucose metabolism. Moreover, some age-related changes in rat brain are subject to hormonal and metabolic manipulations, as shown by glucocorticoid treatments, food restriction, and hypophysectomy.

Cognitive Dysfunctions Associated with Elevated Glucocorticoids

Plasma cortisol tends to rise during aging in subgroups of humans. In a small sample of healthy (nondemented) elderly, longitudinal assessment of cognitive functions showed a marked decline of memory in individuals with progressive elevations of plasma cortisol (105, 106). In AD as well as in the normal elderly, more severe cognitive impairments are associated with elevations of cortisol (105, 178; Seeman *et al.*, in preparation). In laboratory rats, glucocorticoid elevations are common at later ages (94, 171) and are also found in individual animals with impaired spatial memory (74, 92). A possible mechanism is that elevated glucocorticoids tend to enhance glucose production by the liver, but to inhibit cellular glucose uptake in the hippocampus and other brain regions (129, 171). Glucocorticoid elevations impair glucose uptake by neurons, as well as astrocytes (202).

Neuroanatomical Correlations of Elevated Glucocorticoids

About 20 years ago, the pioneering studies of Landfield, Lynch, and Waymire showed that astrocytes became hypertrophied in the hippocampus of 2-year-old rats and that the degree of hypertrophy was positively correlated with blood corticosterone (90–94). Many subsequent studies demonstrate that glucocorticoid elevations can enhance experimental neurodegeneration in the hippocampus of young rodents and primates, as in the extensive studies of Sapolsky and colleagues (119, 169–171). However, the primary mechanism in the age-related changes in the hippocampus is unclear and could be direct, through glucocorticoid receptors on neurons or astrocytes, or indirect. The inhibition of cellular glucose uptake by glucocorticoids in the brain would be predicted to increase the glucose in the extracellular fluid that directly contacts SP and to increase their state of glycooxidation. Thus, elevated glucocorticoids might potentiate the formation of AGEs.

Adrenalectomy and Hypophysectomy

The converse effects of lowering glucocorticoids are consistent with the above studies. Rats that were adrenalectomized when young and maintained on low-dose corticosterone replacements showed reduced astrocyte hyperactivity during aging (93). The effects of hypophysectomy are also pertinent to these questions, since this even more drastic manipulation will lower adrenal and gonadal steroids. Another neurological change of aging is slowed by hypophysectomy, the degeneration of peripheral myelinated spinal roots (“radiculoneuropathy”) (35), which is common during aging in laboratory rats (8). Gonadal steroids could be involved in these age-related changes in astrocytes, because astrocyte activities are subject to sex steroids in the hypothalamus and hippocampus of rats (27, 96, 173, 174). There are few data on how hypophysectomy alters brain aging. The effects of hypophysectomy might extend beyond those of the adrenal and gonadal axis because of influences on oxidative metabolism through thyroid functions.

Food Restriction and Neural Functions in Aging Rats

Food restriction to 30–40% *ad libitum* and maintaining micronutrient requirements is one of the best established manipulations of aging in rodents. More than 50 studies since 1917 have consistently shown >30% increase in lifespan that resulted from slower mortality rate accelerations and that generally parallel the slowing of diverse pathophysiological changes in many organs (66, 187, 209). Food restriction also pro-

fects against some age-related changes in gene expression, e.g., in the inducibility of HSP70 in liver (63).

Food restriction has not been regarded as an important manipulation of brain aging by some neuroscientists, in part, because of technically sound reports that it has no effects at later ages at or beyond the average life span, e.g., in striatal dopamine receptor type D₂ (99, 163), in hippocampal neuron density, or retinal degeneration (145, 146). However, when chronic food-restricted rats are observed at middle-age, food restriction appears to slow pyramidal neuron loss, as evaluated by the greater neuronal density in the pyramidal layer of diet-restricted rats. The absence of an effect at later ages on pyramidal neuron density may indicate that diet-restricted rats had a delayed loss of neurons (146). At a functional level, several reports indicate that food restriction preserves LTP (long term potentiation) (67) and several measures of memory (152). Moreover, restricted rats have smaller age-related increases of an astrocyte mRNA that codes for glial fibrillary acidic protein (GFAP) (107, 137). GFAP is an intermediate filament that influences the shapes of astrocytes and astrocyte–neuronal interactions (84, 96). The activation of brain microglia is also attenuated by food restriction (T. Morgan *et al.*, in preparation). Moreover, a peripheral neuropathy of aging in rats (see above) is blocked by food restriction, as well as by hypophysectomy (35).

The slowing of these diverse neural age-related changes in food-restricted aging rats is paradoxical in terms of the glucocorticoid hypothesis, because food-restricted rats have elevated plasma corticosterone (167). A resolution of this puzzle may be that food restriction reduces blood glucose (and insulin) (112). Because food-restricted rats have less accumulation of glycated collagen in peripheral tissues (160), it is plausible that fewer glycated proteins accumulate in basement membranes and other locations in the brain. As alternatives to the glycooxidation mechanism, for example, food restriction modifies cytokine production (5), which could indirectly influence brain glia (96, 124).

In addition to astrocyte activation during aging, microglia become conspicuously activated in brains of all mammals examined. The microglial activation is found in the white matter of aging rodents (48, 151), which could represent reactions of microglia to AGEPs in the local myelin. Exactly the same result occurs during the peripheral neuropathies of diabetes (207). Because macrophages and microglia have scavenger receptors that recognize AGEPs (see above), the smaller microglial reactivity in food-restricted rats could reflect a diminished local accumulation of AGEPs. Because both food restriction and hypophysectomy lower blood glucose (161), we hypothesize that the lowered blood glucose reduces the amount of AGEPs that are formed, in both the peripheral and the central nervous systems.

Food restriction is also used to treat type II diabetes (59).

ENZYME INACTIVATION DURING AD

We next consider the consequences of oxidative damage during aging and AD to two enzymes of intermediary metabolism: glutamine synthetase and glyceraldehyde-3-phosphate dehydrogenase. These enzymes were selected to illustrate how initial oxidative modifications by glycation or another cause can lead to further neurodegenerative cascades. The following discussion, while frontally speculative, draws on strong examples from aging in nonneural tissues.

Glutamine Synthetase Impairment in AD and the Ammonia Stress Hypothesis

During normal aging, rodent and human brains show a loss of activity of glutamine synthetase, an enzyme found in the brain almost exclusively in astrocytes (139). In gerbils, glutamine synthetase activity decreased by about 35% between 3 and 15 months, which is middle age (14). Similarly, in humans, glutamine synthetase activity in frontal cerebral cortex was decreased by 45% between young (average 29 years) and older (average 70 years) adults (189). AD brains showed a further 40% reduction below normal elderly. The loss of enzyme activity during aging was paralleled by increases in brain carbonyl content in protein from gerbils and humans.

A working hypothesis is that the reduced activity of glutamine synthetase results from oxidative damage. Glutamine synthetase is readily inactivated by oxidative stress, e.g., as observed during reperfusion following ischemia which increases brain carbonyl content (144). Moreover, the opposing age-related changes in glutamine synthetase and carbonyl content were reversed in gerbils by ingestion of a free radical trapping compound (PBN) (14). Oxidation of glutamine synthetase in AD may come from excess production of free radicals and/or reduced protection from oxidative stress. The role of glycation has not been examined. The deposits of A β that accumulate during normal aging as diffuse amyloid and the additional accumulations in the senile plaques of AD could also generate local oxidation. For example, synthetic fragments of aggregated A β can produce free radicals that inactivate glutamine synthetase *in vitro* (61).

Ammonia toxicity could be consequent to the inactivation of glutamine synthetase that may provoke further neurodegeneration in aging and AD. Because glutamine synthetase consumes 98% of the ammonia entering the brain from the blood or cerebrospinal fluid (18), deficits of this key enzyme could lead to excess ammonia levels. In rat brain, chronic exposure to elevated levels of ammonia decreased the activity of glutamine

synthetase in cerebellum (−29%), hippocampus (−25%), and cerebral cortex (−14%) (46). The absence of decreases of enzyme activity in whole brain (19) implies that decreases are regionally specific. Increased brain ammonia has been proposed as a factor in AD (70, 179). In AD, increased net production of cerebral ammonia is indicated by arterio-venous difference measurements (71). Hoyer (69) hypothesizes that during incipient early-onset AD, the brain shifts from its normal status as an organ of net ammonia consumption to one of net ammonia production. In AD, blood ammonia is increased, according to some reports, but not others (70, 179). The activity of monoamine oxidase B (MAO-B) increases during normal aging in several brain regions (49, 192a), which should intensify ammonia production and oxidative stress as well, because the products of the reaction catalyzed by this enzyme include both ammonia and H₂O₂. Because acute ammonia toxicity also decreases cerebral glucose metabolism/g brain by 20% in rats (75), elevated brain ammonia could be a factor in the decreased cerebral glucose utilization in AD (150, 157). However, unlike AD, neuron involution is not conspicuous in acute liver failure (140) or in portal-systemic encephalopathy resulting from chronic liver disease (13). Among the consequences of ammonia toxicity to brain function during hepatic disease is a prominent astrocyte hypertrophy (140) that recalls the increase of activated astrocytes in AD (9, 47, 50, 130). More prolonged ammonia elevations might prove to be neurotoxic.

The production of amyloidogenic fragments of A β from APP could also be influenced by increased ammonia production. *In vitro* cell studies indicate that the production of soluble A β fragments depends on processing of APP through an acidic subcellular compartment (180). The possible increased production of ammonia, which tends to increase pH, thus might counteract the production of amyloidogenic fragments *in vivo* (188). While the direction of this effect would appear to be opposite to the amyloid toxicity hypothesis of AD, the subjects studied for ammonia production were already in clinical stages when the amyloid burden appears to have reached its maximum (73), as discussed earlier. Alternatively and more indirectly, increased ammonia may contribute to amyloid deposition by inhibiting oxidative metabolism (see below), thereby interfering with the processing of β -APP and generating amyloidogenic fragments (43).

Particular aspects of intermediary metabolism in neurons and astrocytes may be altered by inhibition of glutamine synthetase, with possible consequences to excitatory amino acids. Normal brain functions depend on balanced metabolic traffic between the intermediates of the classical citric acid cycle, glutamate and glutamine in astrocytes and neurons (Fig. 1) (62, 200). Neuronal glutamate release requires an equivalent

influx of carbon atoms for metabolic conversion to glutamate (17a). However, neuronal metabolism of glucose, pyruvate, or lactate cannot supply carbon atoms for net synthesis of glutamate, because neurons lack both pyruvate carboxylase and (cytosolic) malic enzyme (the “anapleurotic” enzymes) through which carbons from pyruvate or lactate replenish citric acid cycle pools and that of glutamate or GABA (12, 42, 87, 185). Astrocytes possess both enzymes, whose products further in the citric acid cycle yield the glutamine released into the extracellular space that is then taken up by neurons. Besides glutamine, astrocytes also release lactate, malate, α -ketoglutarate, and citrate (192, 210, 211). Neurons take up glutamine, malate, and α -ketoglutarate, possibly lactate (200), but apparently not citrate (211).

The evidence for reduced fixation of ammonium by impaired glutamine synthetase in aging and AD predicts a glutamine deficit with further metabolic consequences. Magnetic resonance spectroscopic measurements of patients with probable AD indicate a modest (−20%) deficit of cerebral glutamate + glutamine (122), which is consistent with this prediction. Neuronal uptake of malate, α -ketoglutarate, or other possible citric acid cycle metabolites might increase in proportion to the putative glutamine deficit (17a) (Fig. 2). The activity of pyruvate carboxylase is decreased in rat brain during ammonia treatment *in vitro* (−20%) and *in vivo* during hyperammonemia (−53%) (36). Infusion of [¹⁴C]NaHCO₃ with and without ammonium acetate into the cat carotid artery showed that ammonia increases the amount of CO₂ fixation (via pyruvate carboxylase), detected as [¹⁴C]glutamine (208). However, the [¹⁴C]glutamine content reflects the citric acid cycle activity as well as the fixation of carbon dioxide by pyruvate carboxylase (208).

Postmortem studies of the AD cerebral cortex show >50% decrease in α -ketoglutarate dehydrogenase activity and >25% loss of cytochrome oxidase activity (114, 131). The citric acid cycle *in vivo* by NMR spectroscopy is being studied (164), but no data are available for AD. Portacaval shunted rats exhibited increased cerebral NO synthase (NOS) activities (141, 156), suggesting that elevated ammonia levels (such as observed in portacaval shunting) may stimulate NO production. Possible effects of increased production of NO are discussed below. We mention a study of CSF that detected glutamine synthetase in most (38/39) patients with AD, but only occasionally in (1/44) neurological controls or normal patients (54); the cell source of glutamine synthetase could include activated astrocytes.

Alternatively, decreased release of glutamate from glutamatergic neurons could compensate for the putative glutamine deficit. This putative homeostatic loop might be a factor in a deficit of cerebral cortical and hippocampal glutamate found in AD (154, 172), although these deficits are usually attributed to degenera-

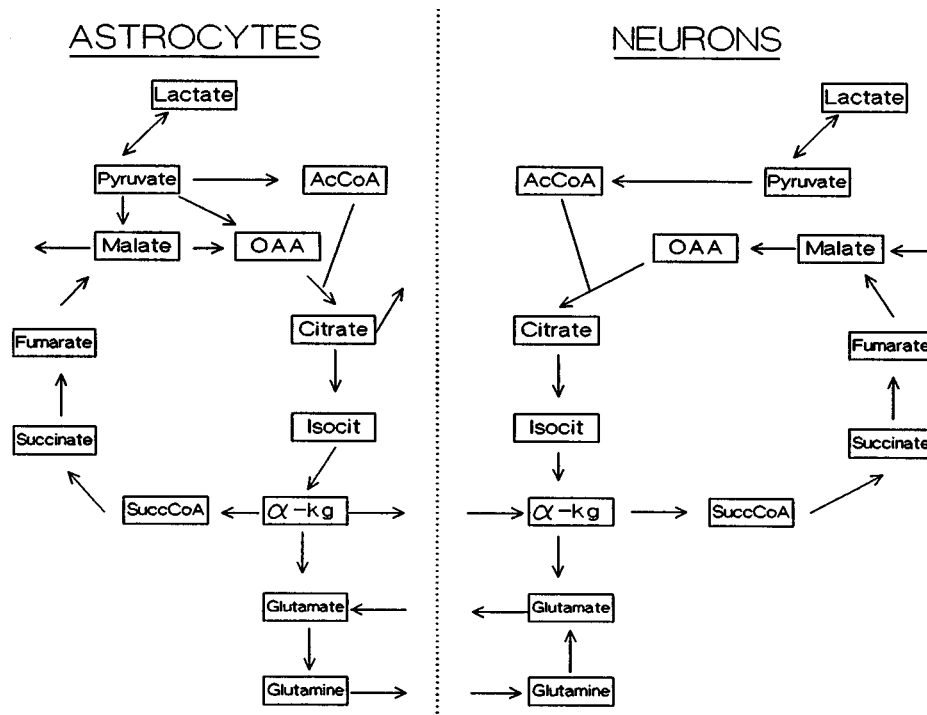


FIG. 1. Normal metabolic flow and intercellular traffic between intermediates of the citric acid cycle in astrocytes and glutamatergic neurons. Arrows indicate the direction of movement of carbon skeletons (metabolic flow) and of molecules (intercellular traffic). Neurons convert pyruvate to acetyl CoA and CO_2 , a reaction catalyzed by pyruvate dehydrogenase. Acetyl CoA is required by citrate synthase and is sufficient for the maintenance of the citric acid cycle, by replacing the two carbon atoms lost as CO_2 during each traversal of the cycle, provided that intermediates are at steady state. It is remarkable that neurons lack enzymes that convert pyruvate to any intermediates of the citric acid cycle, e.g., oxaloacetate or malate. Therefore, pyruvate produced from glucose by glycolysis cannot augment intermediates of the citric acid cycle in neurons. To replenish the carbons of neurotransmitter glutamate (without affecting the rate of oxidative metabolism), neurons must import glutamine or citric acid cycle intermediates. *In vitro*, neurons take up α -ketoglutarate and malate as well as glutamine from the extracellular fluid. Unlike neurons, astrocytes possess enzymes (see text) that convert pyruvate directly to citric acid cycle intermediates, e.g., malate and oxaloacetate. Astrocytic glycolysis can therefore be coordinated with (astrocytic) conversion of pyruvate to a molecule (e.g., malate) which serves a dual function: constituent of the citric acid cycle and means of replenishing the intermediates of the neuronal citric acid cycle (after release into the extracellular space and uptake by neurons). Astrocytes in culture release malate, citrate, α -ketoglutarate, and glutamine.

tion of the glutamatergic perforant path (132, 148). Because glutamine is normally produced in excess of metabolic needs (51), the effects of gradual reduction of glutamine synthetase would be slight until the excess production of glutamine is challenged. Inhibition of glutamine synthetase, in addition to decreasing the fixation of nitrogen, also diverts glutamate in astrocytes to α -ketoglutarate through glutamate dehydrogenase, which produces ammonia. Thus, inhibition of glutamine synthetase could cause further production of ammonia. The ammonia could be trapped by the actions of glutamate dehydrogenase in neurons working in the direction of net formation of glutamate (Fig. 2), or it could diffuse into neighboring cells (inhibiting enzymes of the citric acid cycle) and then into the CSF. Ammonia directly inhibits α -ketoglutarate dehydrogenase (89). In agreement with these predictions, recall that the activity of α -ketoglutarate dehydrogenase is reduced by >50% in cerebral cortical samples from AD patients (114). These and other predictions can be

evaluated with more detailed information on citric acid cycle metabolites in AD.

Prediction: Inactivation of Glyceraldehyde-3-phosphate Dehydrogenase

As a further example of metabolic interactions during AD that promote oxidative damage, we consider relationships among $\text{A}\beta$, nitric oxide (NO), and G3PDH, which metabolizes a substrate that can glycate proteins. The glycating ability of G3P exceeds that of glucose (45). G3PDH catalyzes the phosphorylation of G3P to 1,3-bisphosphoglycerate. As noted earlier, aging rats show decreased activity of skeletal muscle G3PDH (31, 44, 142). In muscle, the age-related oxidative change is partly reversible, which implicates a general shift in cell redox (100), rather than advanced glycation endproducts. Furthermore, the deposits of $\text{A}\beta$ that accumulate during aging and AD might promote the formation of AGEs, by the following mechanism. A

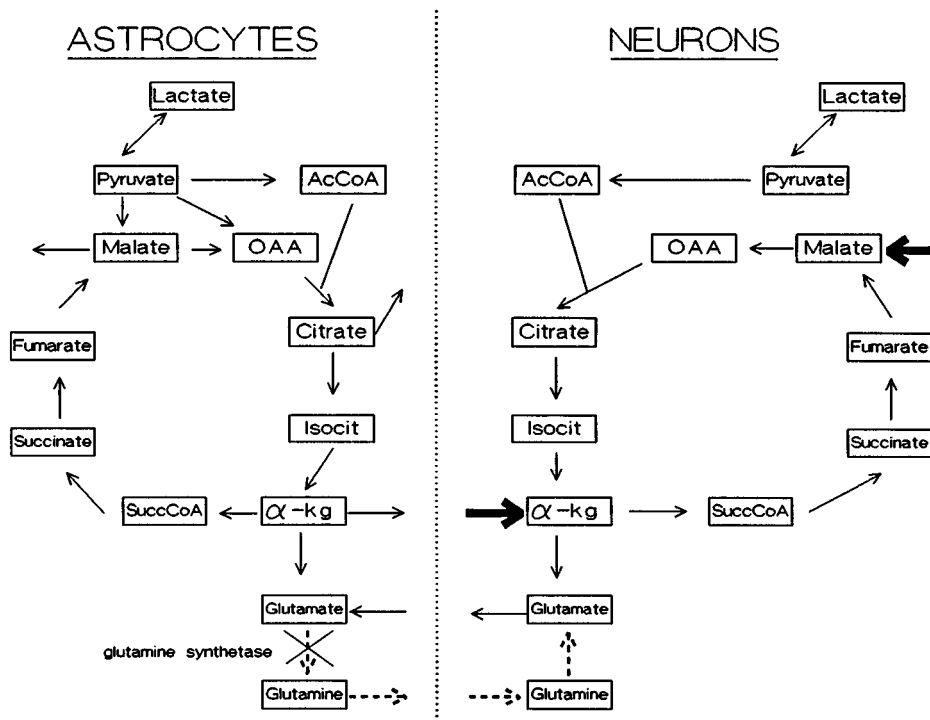


FIG. 2. Minimal consequences of inactivation of glutamine synthetase (hypothesis). Dotted arrows indicate decreased metabolic flux; thick arrows indicate increased flux. Secondary to the decrease of rate of conversion of glutamate to glutamine (predominantly in glia), an increase in the concentration of ammonia is predicted. (This is similar but not identical to the infusion of ammonium salt, which increases the concentration of ammonia but would not decrease the glutamine synthetase flux.) The decrease of glutamine synthesis in astrocytes should be coincident with a decreased release of glutamine into the extracellular fluid, and eventually a decreased rate of uptake of glutamine into neurons. We assume that the extracellular pool is not a sufficient source of glutamine for neurons and that to maintain the same rate of release of glutamate from glutamatergic neurons, other carbon sources are needed. Thus, the increased import of malate and/or α -ketoglutarate into neurons may offset the deficit of carbon. We predict that astrocytic glycolysis and oxidation of pyruvate will increase as the neuronal demand for glutamine in the face of inactivation of glutamine synthetase gradually exhausts the supply of glutamine available from the extracellular fluid. Concomitant with the increase in glial pyruvate oxidation will be an increased release by astrocytes into the extracellular fluid of intermediates of the citric acid cycle for uptake by the neurons.

fragment of A β can activate NO synthase (72), and at least one of the redox forms of NO inhibits G3PDH in brain (26, 116, 217). Slight inhibition of G3PDH will lead to increases in G3P, more glycation of G3PDH, and thus further inhibition of G3PDH. The combination of NO and superoxide anion generates peroxynitrite, a potent oxidizing agent implicated in inhibition of G3PDH by NO (123). Increased ammonia stress in AD (see above) might contribute to peroxynitrite formation by stimulating production of NO (141, 156).

We hypothesize that G3PDH is inhibited in AD, which should increase the intracellular pool of G3P and might then gradually increase the small flux from glucose-6-phosphate into the pentose phosphate pathway (PPP) (Fig. 3). However, the PPP produces G3P from each mole of glucose-6-phosphate, which would compound the problem of excessive amounts of G3P (as a dead-end product). Alternatively, the PPP together with fructose-1,6-bisphosphatase can in principle function as a cycle; 1 mol of glucose-6-phosphate can be completely oxidized to 6 mol of carbon dioxide, with the

generation of 12 mol of NADPH (196, p. 432). A functional gluconeogenic pathway (including fructose-1,6-bisphosphatase) has been demonstrated in astrocytic cultures (175). Thus astrocytes *in vivo* may compensate for gradual inhibition of G3PDH by gradually increasing the nonoxidative metabolism of glucose (via the PPP and a gluconeogenic enzyme). This particular pathway for glucose oxidation does not produce ATP, however, but does produce ample NADPH. Owing to reduced levels of antioxidant enzymes in brain, generation of NADPH is essential for the reduction of oxidized glutathione produced by the action of glutathione peroxidase (68). Peroxidase is necessary to dispose of the H₂O₂ produced from the actions of superoxide dismutase and monoamine oxidase (56). Thus in our speculative model, the inhibition of G3PDH causes an increase in the astrocytic defense against free radicals at the expense of generating ATP. Consistent with this prediction, AD brains show increased activity of glucose-6-phosphate dehydrogenase (G6PDH), the initial enzyme of the oxidative branch of the PPP (111). Elevation of

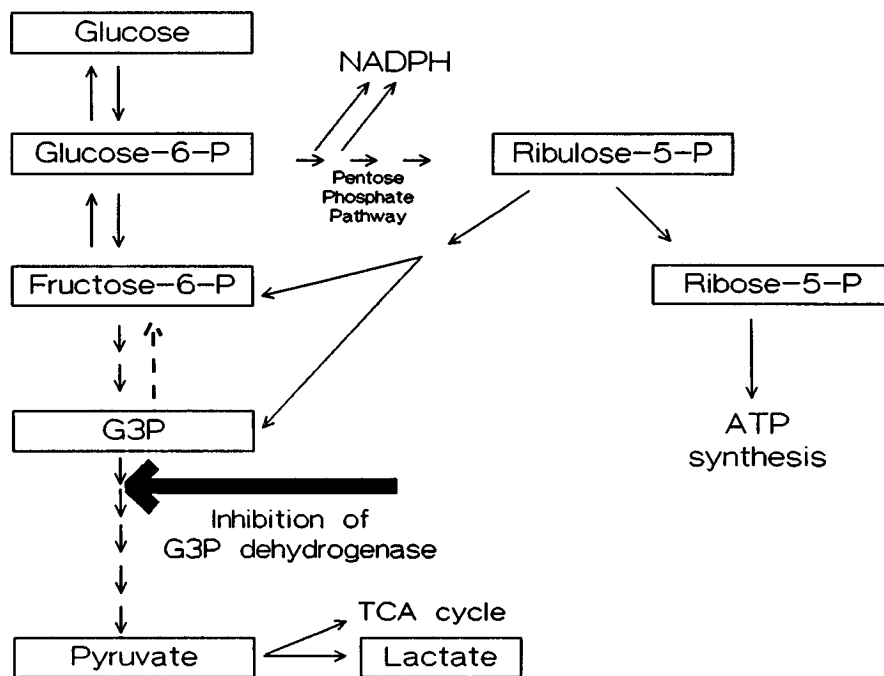


FIG. 3. Possible consequences of the inhibition of glyceraldehyde-3-phosphate dehydrogenase (G3PDH). We hypothesize that an immediate effect is to increase glyceraldehyde-3-phosphate (G3P), which will gradually increase the flow into the pentose phosphate pathway (PPP). However, operation of the PPP itself produces G3P for each G6P entering the pathway. Instead, we propose that the PPP together with fructose-1,6-bisphosphatase (dotted line) might function as an oxidative cycle (196, p. 432), producing 6 molecules of CO_2 and 12 molecules of NADPH for every molecule of G6P consumed. NADPH is important in maintaining the glutathione redox cycle, which protects against reactive oxygen species, including H_2O_2 . According to this model, the inhibition of G3PDH forces astrocytes and perhaps neurons to adapt to decreasing rates of ATP production (diminished rates of glycolysis and of provision of pyruvate and acetyl CoA for the citric acid cycle). By utilizing the PPP and a gluconeogenic enzyme (fructose-1,6-bisphosphatase), the cell increases the NADPH/NADP⁺ ratio and its resistance to oxidative stress.

the rate of the PPP in AD brains might also result from increased oxidative stress, e.g., from A β fragments (see above).

SYNOPSIS

This inquiry has considered linkages among aging, metabolism, and AD that draw on scattered areas of information. We anticipate useful insights from analyzing how age-related changes in glucose metabolism and metabolic hormones impact on brain aging. These changes may even trigger events leading to AD according to the following summary that assumes an inexorable accumulation of AGEs and other glycosylated macromolecules during aging. We suggested how the accumulation of glycosylated molecules could influence nitrogen and carbohydrate metabolism. At some threshold, the glycosylated substrates in the brain activate macrophage/microglial cells through scavenger receptors. The activation of macrophages causes the release of cytokines and reactive oxygen species that can damage local neurons. Thus, the slow activation of microglia and other cells by exposure to AGEs and oxidized proteins could further fan the fires of molecular oxidation and cell inflammatory responses. Myelin-

ated neurons could be a major target for AGE accumulation that would impair cognitive functions. The trend for elevations of blood glucose in late middle-age could accelerate this process and therefore could be a trigger for AD, even if subsequent weight loss at later stages of AD caused attenuation of hyperglycemia. Genetics may well come into this picture through the major ongoing efforts to identify allelic polymorphisms and rarer mutants that influence the risk of type II diabetes. We would not be surprised if some of these alleles interact with genetic risk factors for AD.

ACKNOWLEDGMENTS

This analysis was supported by the following grants from the N.I.H.: R01-AG 13499 and P50-AG05142 (C.E.F.); and DK-29867 (D.M.C.). We are grateful for critical comments and advice from Richard Bergman (USC), Jeffrey Halter (University of Michigan), Cynthia Leibson (Mayo Clinic), Lon Schneider (USC), Mary McKenna (University of Maryland), Joseph Rogers (Sun Health Research Institute).

REFERENCES

1. ABBOTT, R. D., R. P. DONAHUE, S. W. MACMAHON, D. M. REED, AND K. YANO. 1987. Diabetes and the risk of stroke—The Honolulu Heart Program. *J. Am. Med. Assoc.* **257**: 949–952.

2. BELL, D. S. H. 1994. Stroke in the diabetic patient. *Diabetes Care* **17**: 213–219.
3. BERGMAN, R. N. 1989. Toward physiological understanding of glucose tolerance: Minimal model approach. *Diabetes* **38**: 1512–1527.
4. BERGMAN, R. N. 1990. Quantitative approaches to pathogenesis of age-related metabolic conditions. In *Biomedical Advances in Aging* (A. L. Goldstein, Ed.), Plenum, New York.
5. BRADLEY, S. F., A. VIBHAGOO, S. L. KUNKEL, AND C. A. KAUFFMAN. 1989. Monokine secretion in aging and protein malnutrition. *J. Leukocyte Biol.* **45**: 510–514.
6. BREWSTER, W. J., P. FERNYHOUGH, L. T. DIEMEL, L. MOHIUDDIN, AND D. R. TOMLINSON. 1994. Diabetic neuropathy, nerve growth factor and other neurotropic factors. *Trends Neurosci.* **17**: 321–324.
7. BROE, G. A., A. S. HENDERSON, H. CREASEY, E. MCCUSKER, A. E. KORTEN, A. F. JORM, W. LONGLEY, AND J. C. ANTHONY. 1990. A case-control study of Alzheimer's disease in Australia. *Neurology* **40**: 1698–1707.
8. BRONSON, R. T. 1990. Rate of occurrence of lesions in 20 inbred and hybrid genotypes of rats and mice sacrificed at 6 month intervals during the first years of life. In *Genetic Effects on Aging II* (D. E. Harrison, Ed.), Telford Press, Caldwell, NJ.
9. BRUN, A., X. LIU, AND C. ERIKSON. 1995. Synapse loss and gliosis in the molecular layer of the cerebral cortex in Alzheimer's disease and in frontal lobe degeneration. *Neurodegeneration* **4**: 171–177.
10. BUCALA, R., A. CERAMI, AND H. VLASSARA. 1995. Advanced glycosylation end products in diabetic complications. *Diabetes Rev.* **3**: 258–268.
11. BUCHT, G., R. ADOLFSSON, F. LITHNER, AND B. WINBLAD. 1983. Changes in blood glucose and insulin secretion in patients with senile dementia of Alzheimer type. *Acta Med. Scand.* **213**: 387–392.
12. BUKATO, G., Z. KOCHAN, AND J. SWIERCZYNSKI. 1995. Different regulatory properties of the cytosolic and mitochondrial forms of malic enzyme isolated from human brain. *Int. J. Biochem. Cell Biol.* **27**: 1003–1008.
13. BUTTERWORTH, R. F. 1993. Portal-systemic encephalopathy: A disorder of neuron-astrocytic metabolic trafficking. *Dev. Neurosci.* **15**: 313–319.
14. CARNEY, J. M., P. E. STARKE-REED, C. N. OLIVER, R. W. LANDUM, M. S. CHENG, J. F. WU, AND R. A. FLOYD. 1991. Reversal of age-related increase in brain protein oxidation, decrease in enzyme activity, and loss in temporal and spatial memory by chronic administration of the spin-trapping compound *N*-tert-butyl-alpha-phenylnitron. *Proc. Natl. Acad. Sci. USA* **88**: 3633–3636.
15. CHEN, M., R. N. BERGMAN, G. PACINI, G., AND D. PORTE, JR. 1985. Pathogenesis of age-related glucose intolerance in man: Insulin resistance and decreased β -cell function. *J. Clin. Endocrinol. Metab.* **60**: 13–20.
16. CHEN, M., J. B. HALTER, AND D. PORTE, JR. 1987. The role of dietary carbohydrate in the decreased glucose tolerance of the elderly. *J. Am. Geriatr. Soc.* **35**: 417–424.
17. CHUI, H. C., J. I. VICTOROFF, D. MARGOLIN, W. JAGUST, R. SHANKLE, AND R. KATZMAN. 1992. Criteria for the diagnosis of ischemic vascular dementia proposed by the State of California Alzheimer's Disease Diagnostic and Treatment Centers. *Neurology* **42**: 473–478.
- 17a. COHEN, D. M. Inhibition of glutamine synthetase induces critical energy threshold for neuronal survival. In *Cerebrovascular Pathology in Alzheimers Disease*. Annals N.Y. Academy of Science, in press.
18. COOPER, A. J. L., AND F. PLUM. 1987. Biochemistry and physiology of brain ammonia. *Physiol. Rev.* **67**: 440–519.
19. COOPER, A. J. L., S. N. MORA, N. F. CRUZ, AND A. S. GELBARD. 1985. Cerebral ammonia metabolism in hyperammonemic rats. *J. Neurochem.* **44**: 1716–1723.
20. CORDER, E. H., A. M. SAUNDERS, W. J. STRITTMATTER, D. E. SCHMECHEL, P. C. GASKELL, G. W. SMALL, A. D. ROSES, J. L. HAINES, AND M. A. PERICAK-VANCE. 1993. Gene dose of apolipoprotein E type 4 allele and the risk of Alzheimer's disease in late onset families. *Science* **261**: 921–923.
21. CRAFT, S., J. NEWCOMER, S. KANNE, S. DAGOGO-JACK, P. CRYER, Y. SHELIN, J. LUBY, A. DAGOGO-JACK, AND A. ALDERSON. 1996. Memory improvement following induced hyperinsulinemia in Alzheimer's disease. *Neurobiol. Aging* **17**: 123–130.
22. CUMMINGS, B. J., E. HEAD, A. J. AFAGH, N. W. MILGRAM, AND C. W. COTMAN. β -Amyloid accumulation correlates with cognitive dysfunction in the aged canine. *Neurobiol. Learn. Mem.* **65**, in press.
23. CURB, J. D., B. L. RODRIQUEZ, H. PETROVICH, K. H. MASAKI, C. M. BURCHFIEL, W. ROSS, R. CHEN, T. HARRIS, AND L. R. WHITE. 1996. The relationship of diabetes and glucose tolerance to Alzheimer's disease and vascular dementia. *Neurobiol. Aging* **17**(4S): S122. [Abstract 488].
24. DAVIDSON, M. B. 1979. The effect of aging on carbohydrate metabolism: A review of the English literature and a practical approach to the diagnosis of diabetes mellitus in the elderly. *Metabolism* **28**: 688–705.
25. DAVIGNON, J., R. GREGG, AND C. F. SING. 1988. Apolipoprotein E polymorphism and atherosclerosis. *Arteriosclerosis* **8**: 1–21.
26. DAWSON, T. M., AND S. H. SNYDER. 1994. Gases as biological messengers: Nitric oxide and carbon monoxide in the brain. *J. Neurosci.* **14**: 5147–5159.
27. DAY, J. R., N. J. LAPING, M. LAMPERT-ETCHELLS, S. A. BROWN, J. P. O'CALLAGHAN, T. H. MCNEILL, AND C. E. FINCH. 1993. Gonadal steroids regulate the expression of GFAP in the adult male rat hippocampus. *Neuroscience* **55**: 435–443.
28. DEFONZO, R. A. 1979. Glucose intolerance in aging. Evidence for tissue insensitivity to glucose. *Diabetes* **28**: 1095–1101.
29. Diabetes Control and Complications Trial Research Group. 1993. The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. *N. Engl. J. Med.* **329**: 977–986.
30. DUARA, R. 1994. Neuroimaging with CT and MRI in Alzheimer disease. In *Alzheimer Disease* (R. D. Terry, R. Katzman, and K. L. Bick, Eds.), p. 175. Raven Press, New York.
31. DULIC, V., AND A. GAFNI. 1987. Mechanism of aging of rat muscle glyceraldehyde-3-phosphate dehydrogenase studied by selective enzyme-oxidation. *Mech. Age. Dev.* **40**: 289–306.
32. DYCK, P. J., K. M. KRATZ, W. J. LITCHY, R. KLEIN, J. M. PACH, D. M. WILTON, P. C. O'BRIEN, AND L. J. MELTON. 1993. The prevalence by staged severity of various types of diabetic neuropathy, retinopathy, and nephropathy in a population based cohort. *Neurology* **43**: 817–824.
33. EICHNER, J. E., R. E. FERRELL, M. I. KAMBOH, L. H. KULLER, D. J. BECKNER, AND T. J. ORCHARD. 1992. The impact of the apolipoprotein E polymorphism on the lipoprotein profile in insulin-dependent diabetes: The Pittsburgh Epidemiology of Diabetes Complications Study IX. *Metabolism* **41**: 347–351.
34. EL KHOURY, J., S. E. HICKMAN, C. A. THOMAS, L. CAO, S. C. SILVERSTEIN, AND J. D. LOIKE. 1996. Scavenger receptor-mediated adhesion of microglia to β -amyloid fibrils. *Nature* **382**: 716–719.
35. EVERITT, A. V., N. J. SEEDSMAN, AND F. JONES. 1980. The effects of hypophysectomy and continuous food restriction, begun at

- ages of 70 and 400 days on collagen aging, proteinuria, incidence of pathology, and longevity in the male rat. *Mech. Age. Dev.* **12**: 161–172.
36. FAFF-MICHALAK, L., AND J. ALBRECHT. 1991. Aspartate aminotransferase, malate dehydrogenase, and pyruvate carboxylase activities in rat cerebral synaptic and nonsynaptic mitochondria: Effects of in vitro treatment with ammonia, hyperammonemia, and hepatic encephalopathy. *Metab. Brain Dis.* **6**: 187–197.
 37. FERINI-STRAMBI, L., S. SMIRNE, P. GARANCINI, P. PINTO, AND M. FRANCESCHI. 1990. Clinical and epidemiological aspects of Alzheimer's disease with presenile onset: A case control study. *Neuroepidemiology* **9**: 39–49.
 38. FINCH, C. E., AND J. MARCHALONIS. 1996. An evolutionary perspective on amyloid and inflammatory features of Alzheimer disease. *Neurobiol. Aging* **17**: 809–815.
 39. FINK, R. I., O. G. KOLTERMAN, J. GRIFFIN, AND J. M. OLEFSKY. 1983. Mechanisms of insulin resistance in aging. *J. Clin. Invest.* **71**: 1523–1535.
 40. FINK, R. I., P. WALLACE, AND J. M. OLEFSKY. 1986. Effects of aging on glucose-mediated glucose disposal and glucose transport. *J. Clin. Invest.* **77**: 2034–2041.
 41. FISHMAN, R. A. 1992. *Cerebrospinal Fluid in Diseases of the Nervous System*, 2nd ed., pp. 217–220. W. B. Saunders Co. Harcourt, Brace, Jovanovich, Inc., Philadelphia, PA.
 42. FRENKEL, R. 1972. Isolation and some properties of a cytosol and a mitochondrial malic enzyme from bovine brain. *Arch. Biochem. Biophys.* **152**: 136–143.
 43. GABUZDA, D., J. BUSCIGLIO, L. B. CHEN, P. MATSUDAIRA, AND B. A. YANKER. 1994. Inhibition of energy metabolism alters the processing of amyloid precursor protein and induces a potentially amyloidogenic derivative. *J. Biol. Chem.* **269**: 13623–13628.
 44. GAFNI, A., AND N. NOY. 1984. Age-related effects in enzyme catalysis. *Mol. Cell. Biochem.* **59**: 113–129.
 45. GIARDINO, I., D. EDELSTEIN, AND M. BROWNLEE. 1994. Nongenzytic glycosylation in vitro and in bovine endothelial cells alters basic fibroblast growth factor activity. *J. Clin. Invest.* **94**: 110–117.
 46. GIRARD, G., J. F. GIGUERE, AND R. F. BUTTERWORTH. 1993. Region-selective reductions in activities of glutamine synthetase in rat brain following portocaval anastomosis. *Metab. Brain Dis.* **8**: 207–215.
 47. GOODISON, K. L., I. M. PARHAD, C. L. WHITE, III, A. A. F. SIMA, AND A. W. CLARK. 1993. Neuronal and glial gene expression in neocortex of Down's syndrome and Alzheimer's disease. *J. Neuropathol. Exp. Neurol.* **52**: 192–198.
 48. GORDON, M. N., L. A. HOLCOMB, W. A. SCHREIER, AND D. G. MORGAN. 1994. MHC class II antigen expression by microglial after deafferentation in aged rats. *Soc. Neurosci. Abstr.* **20**: 1033.
 49. GOTTFRIES, C. G., AND B. E. ROOS. 1976. Monoamine metabolites in cerebrospinal fluid (CSF) in patients with organic presenile and senile dementias. *Akt. Geront.* **6**: 37–42.
 50. GRIFFIN, W. S. T., L. C. STANLEY, C. LING, L. WHITE, V. MACLEOD, L. J. PERROT, C. L. WHITE, III, AND C. ARAOZ. 1989. Brain interleukin 1 and S-100 immunoreactivity are elevated in Down syndrome and Alzheimer disease. *Proc. Natl. Acad. Sci. USA* **86**: 7611–7615.
 51. GRILL, V., O. BJORKMAN, M. GUTNIAK, AND M. LINDQVIST. 1992. Brain uptake and release of amino acids in nondiabetic and insulin-dependent diabetic subjects: Important role of glutamine release for nitrogen balance. *Metabolism* **41**: 28–32.
 52. GRUETTER, R., E. J. NOVOTNY, S. D. BOULWARE, D. L. ROTHMAN, G. F. MASON, G. I. SHULMAN, R. G. SHULMAN, AND W. V. TAMBORLANE. 1992. Direct measurement of brain glucose concentrations in humans by ¹³C NMR spectroscopy. *Proc. Natl. Acad. Sci. USA* **89**: 1109–1112, 1992; erratum, *ibid.*, **89**: 12208.
 53. GUMBINER, B., K. S. POLONSKY, W. F. BELTZ, P. WALLACE, G. BRECHTEL, AND R. I. FINK. 1989. Effects of aging on insulin secretion. *Diabetes* **38**: 1549–1556.
 54. GUNNERSEN, D., AND B. HALEY. 1992. Detection of glutamine synthetase in the cerebrospinal fluid of Alzheimer diseased patients: A potential diagnostic biochemical marker. *Proc. Natl. Acad. Sci. USA* **89**: 11949–11953.
 55. HALL, J. L., L. A. GONDER-FREDERICK, W. W. CHEWNING, J. SILVEIRA, AND P. E. GOLD. 1989. Glucose enhancement of performance on memory tests in young and aged humans. *Neuropsychologia* **9**: 1129–1138.
 56. HALLIWELL, B. 1992. Reactive oxygen species and the central nervous system. *J. Neurochem.* **59**: 1609–1623.
 57. HALTER, J. B. 1996. Alzheimer's disease and non-insulin-dependent diabetes mellitus: Common features do not make common bedfellows. *J. Am. Geriatr. Soc.* **44**: 992–993. [Editorial].
 58. HARRIS, M. I., W. C. HADDEN, W. C. KNOWLER, AND P. H. BENNETT. 1987. Prevalence of Diabetes and impaired glucose tolerance and plasma glucose levels in U.S. population aged 20–74 yr. *Diabetes* **36**: 523–534.
 59. HARRIS, M. I. 1993. Undiagnosed NIDDM: Clinical and public health issues. *Diabetes Care* **16**: 642–652.
 60. HARRIS, M. I. 1995. Epidemiologic studies on the pathogenesis of non-insulin-dependent diabetes mellitus (NIDDM). *Clin. Invest. Med.* **18**: 231–239.
 61. HENSLEY, K., J. M. CARNEY, M. P. MATTSON, M. AKSENOVA, M. HARRIS, J. F. WU, R. A. FLOYD, AND D. A. BUTTERFIELD. 1994. A model for beta-amyloid aggregation and neurotoxicity based on free radical generation by the peptide: Relevance to Alzheimer disease. *Proc. Natl. Acad. Sci.* **91**: 3270–3274.
 62. HERTZ, L. 1993. Metabolic interactions between neurons and astrocytes. In *Biology and Pathology of Astrocyte-Neuron Interactions* (S. Fedoroff, B. H. J. Juurlink, and R. Doucette, Eds.), Plenum, New York.
 63. HEYDARI, A. R., B. WU, R. Y. TAKAHASHI, R. STRONG, AND A. RICHARDSON. 1993. Expression of heat shock protein 70 is altered by age and diet at the level of transcription. *Mol. Cell. Biol.* **13**: 2909–2918.
 64. HEYMAN, A., W. E. WILKINSON, B. J. HURWITZ, D. SCHMECHEL, A. H. SIGMON, T. WEINBERG, M. J. HELMS, AND M. SWIFT. 1983. Alzheimer's disease: Genetic aspects and associated clinical disorders. *Ann. Neurol.* **14**: 507–515.
 65. HEYMAN, A., W. E. WILKINSON, J. A. STAFFORD, M. J. HELMS, A. H. SIGMON, AND T. WEINBERG. 1984. Alzheimer's disease: A study of epidemiological aspects. *Ann. Neurol.* **15**: 335–341.
 66. HOPKIN, K. 1995. Aging in focus: Caloric restriction may put the brakes on aging. *J. NIH Res.* **7**: 47–50.
 67. HORI, N., I. HIROTSU, P. J. DAVIS, AND D. O. CARPENTER. 1992. Long-term potentiation is lost in aged rats but preserved by calorie restriction. *NeuroReport* **3**: 1085–1088.
 68. HOTHERSALL, J. S., A. L. GREENBAUM, AND P. MCLEAN. 1982. The functional significance of the pentose phosphate pathway in synaptosomes: Protection against peroxidative damage by catecholamines and oxidants. *J. Neurochem.* **39**: 1325–1332.
 69. HOYER, S. 1993. Intermediary metabolism disturbance in AD/SDAT and its relation to molecular events. *Prog. Neuro-Psychopharmacol. Biol. Psychiatr.* **17**: 199–228.
 70. HOYER, S. 1994. Possible role of ammonia in the brain in dementia of Alzheimer type. *Adv. Exp. Med. Biol.* **368**: 197–205.

71. HOYER, S., AND R. NITSCH. 1989. Cerebral excess release of neurotransmitter amino acids subsequent to reduced cerebral glucose metabolism in early-onset dementia of Alzheimer type. *J. Neural Transm.* **75**: 227-232.
72. HU, J. R., AND E. E. EL-FAKAHANY. 1993. Beta amyloid 25-35 activates nitric oxide synthase in a neuronal clone. *NeuroReport* **4**: 760-762.
73. HYMAN, B. T., K. MARZLOFF, AND P. V. ARRIAGADA. 1993. The lack of accumulation of senile plaques or amyloid burden in Alzheimer's disease suggests a dynamic balance between amyloid deposition and resolution. *J. Neuropathol. Exp. Neurol.* **52**: 594-600.
74. ISSA, A. M., W. ROWE, S. GAUTHIER, AND M. J. MEANEY. 1990. Hypothalamic-pituitary-adrenal activity in aged, cognitively impaired and cognitively unimpaired rats. *J. Neurosci.* **10**: 3247-3254.
75. JESSY, J., M. R. DEJOSEPH, AND R. A. HAWKINS. 1991. Hyperammonemia depresses glucose consumption throughout the brain. *Biochem. J.* **277**: 693-696.
76. KAHN, S. E., V. G. LARSON, J. C. BEARD, K. C. CAIN, G. W. FELLINGHAM, R. S. SCHWARTZ, R. C. VEITH, J. R. STRATTON, M. D. CERQUEIRA, AND I. B. ABRASS. 1990. Effect of exercise on insulin action, glucose tolerance, and insulin secretion in aging. *Am. J. Physiol.* **258**: E937-E943.
77. KAHN, S. E., V. G. LARSON, R. S. SCHWARTZ, J. C. BEARD, K. C. CAIN, G. W. FELLINGHAM, J. R. STRATTON, M. D. CERQUEIRA, AND I. B. ABRASS. 1992. Exercise training delineates the importance of B-cell dysfunction to the glucose intolerance of human aging. *J. Clin. Endocrinol. Metab.* **74**: 1336-1342.
78. KATZMAN, R. 1983. Vascular disease and dementia. In *H. Houston Merritt Memorial Volume* (M. D. Yahr, Ed.), pp. 153-176. Raven Press, New York.
79. KATZMAN, R., AND C. KAWAS. 1994. The epidemiology of dementia and Alzheimer disease. In *Alzheimer Disease* (R. D. Terry, R. Katzman and K. L. Bick, Eds.), p. 105. Raven Press, New York.
80. KEMPER, T. 1984. Neuroanatomical and neuropathological changes in normal aging and in dementia. In *Clinical Neurology of Aging* (M. L. Albert, Ed.), pp. 9-52. Oxford Univ. Press, New York.
81. KIMURA, T., J. TAKAMATSU, N. ARAKI, M. GOTO, A. KONDO, T. MIYAKAWA, AND S. HORIUCHI. 1995. Are advanced glycation end-products associated with amyloidosis in Alzheimer's disease. *NeuroReport* **6**: 866-868.
82. KIRSTEIN, M., J. BRETT, S. RADOFF, S. OGAWA, D. STERN, AND H. VLASSARA. 1990. Advanced protein glycosylation induces trans-endothelial human monocyte chemotaxis and secretion of platelet-derived growth factor: Role in vascular disease of diabetes and aging. *Proc. Natl. Acad. Sci. USA* **87**: 9010-9014.
83. KOENIG, R. J., C. M. PETERSON, R. L. JONES, C. SAUDEK, M. LEHRMAN, AND A. CERAMI. 1976. Correlation of glucose regulation and hemoglobin A1c in diabetes mellitus. *N. Engl. J. Med.* **295**: 17-420.
84. KOHAMA, S. G., J. R. GOSS, C. E. FINCH, AND T. H. MCNEILL. 1995. Increases of glial fibrillary acidic protein in the aging female mouse brain. *Neurobiol. Aging* **16**: 59-67.
85. KOKMEN, E., C. M. BEARD, V. CHANDRA, K. P. OFFORD, B. S. SCHOENBERG, AND D. J. BALLARD. 1991. Clinical risk factors for Alzheimer's disease: A population-based case-control study. *Neurology* **41**: 1393-1397.
86. KRISTAL, B. S., AND B. P. YU. 1992. An emerging hypothesis: Synergistic induction of aging by free radicals and Maillard reactions. *J. Gerontol. Biol. Sci.* **47**: B107-B114.
87. KURZ, G. M., H. WIESINGER, AND B. HAMPRECHT. 1993. Purification of cytosolic malic enzyme from bovine brain, generation of monoclonal antibodies, and immunocytochemical localization of the enzyme in glial cells of neural primary cultures. *J. Neurochem.* **60**: 1467-1474.
88. KUUSISTO, J., L. MYKKANEN, K. PYORALA, AND M. LAAKSO. 1994. Non-insulin-dependent diabetes and its metabolic control are important predictors of stroke in elderly patients. *Stroke* **25**: 1157-1164.
89. LAI, J. C. K., AND A. J. L. COOPER. 1986. Brain α -ketoglutarate dehydrogenase complex: Kinetic properties, regional distribution, and effects of inhibitors. *J. Neurochem.* **47**: 1376-1386.
90. LANDFIELD, P. W., G. ROSE, L. SANDLES, T. C. WOHLSTADTER, AND G. LYNCH. 1977. Patterns of astroglial hypertrophy and neuronal degeneration in the hippocampus of aged, memory-deficient rats. *J. Gerontol.* **32**: 3-12.
91. LANDFIELD, P. W., J. L. WAYMIRE, AND G. LYNCH. 1978. Hippocampal aging and adrenocorticoids: Quantitative correlations. *Science* **202**: 1098-1102.
92. LANDFIELD, P. W., R. W. BASKIN, AND T. A. PITLER. 1981. Brain-aging correlates: Retardation by hormonal-pharmacological treatments. *Science* **214**: 581-584.
93. LANDFIELD, P. W. 1987. Modulation of brain aging correlates by long-term alterations of adrenal steroids and neurally active peptides. *Prog. Brain Res.* **72**: 279-300.
94. LANDFIELD, P. W. 1994. The role of glucocorticoids in brain aging and Alzheimer's disease: An integrative physiological hypothesis (Nathan Shock Memorial Lecture 1990). *Exp. Gerontol.* **29**: 3-11.
95. LANDIN, K., K. BLENNOW, A. WALLIN, AND C.-G. GOTTFRIES. 1993. Low blood pressure and blood glucose levels in Alzheimer's disease: Evidence for a hypometabolic disorder? *J. Intern. Med.* **233**: 357-363.
96. LAPING, N. J., B. TETER, N. R. NICHOLS, J. ROZOVSKY, AND C. E. FINCH. 1994. Glial fibrillary acidic protein: Regulation by hormones, cytokines, and growth factors. *Brain Pathol.* **4**: 259-274.
97. LEHTO, S., T. RONNEMAA, K. PYORALA, AND M. LAAKSO. 1996. Predictors of stroke in middle-aged patients with non-insulin-dependent diabetes. *Stroke* **27**: 63-68.
98. LEIBSON, C. L., W. A. ROCCA, V. A. HANSON, R. CHA, E. KOKMEN, P. C. O'BRIEN, AND P. J. PALUMBO. The risk of dementia among persons with diabetes mellitus: A population based cohort study. *Am. J. Epidemiol.*, in press.
99. LEVIN, P., J. K. JANDA, J. A. JOSEPH, D. K. INGRAM, AND G. S. ROTH. 1981. Dietary restriction retards the age-associated loss of rat striatal dopaminergic receptors. *Science* **214**: 561-562.
100. LEVINE, R. L., AND E. STADTMAN. 1996. Protein modifications with aging. in *Handbook of the Biology of Aging*, (E. L. Schneider and J. W. Rowe, Eds.), 4th ed., pp. 184-197. Academic Press, San Diego.
101. LEVY, R., A. ISAACS, AND G. HAWKS. 1970. Neurophysiological correlates of senile dementia: I. motor and sensory nerve conduction velocity. *Psychol. Med.* **1**: 40-47.
102. LI, J. J., D. VOISIN, A.-L. QUIQUERIZ, AND C. BOURAS. 1994. Differential expression of advanced glycosylation end-products in neurons of different species. *Brain Res.* **641**: 285-288.
103. LI, J. L., M. SURINI, S. CATSICAS, E. KAWASHIMA, AND C. BOURAS. 1995. Age-dependent accumulation of advanced glycosylation end products in human neurons. *Neurobiol. Aging* **16**: 69-76.
104. LOWENSON, J. D., A. E. ROHER, AND S. CLARKE. 1994. Protein aging—Extracellular amyloid formation and intracellular repair. *Trends Cardiovasc. Med.* **4**: 3-8.
105. LUPIEN, S., A. R. LECOURS, I. LUSSIER, G. SCHWARTZ, N. P. V. NAIR, AND M. J. MEANEY. 1994. Basal cortisol levels of cognitive deficits in human aging. *J. Neurosci.* **14**: 2893-2903.

106. LUPIEN, S., A. R. LECOURS, G. SCHWARTZ, S. SHARMA, R. L. HAUGER, M. J. MEANEY, N. P. V. NAIR. 1996. Longitudinal study of basal cortisol levels in health elderly subjects: Evidence for subgroups. *Neurobiol. Aging* **17**: 95–105.
107. MAJOR, D. E., J. P. KESSLAK, C. W. COTMAN, C. E. FINCH, J. R. DAY. Life-long dietary restriction attenuates age-related increases in glial fibrillary acidic protein (GFAP) mRNA in the rat hippocampus. *Brain Res.*, in press.
108. MANN, D. M. A., B. MARCZYNIUK, P. O. YATES, D. NEARY, AND J. S. SNOWDEN. 1988. The progression of the pathological changes of Alzheimer's disease in frontal and temporal neocortex examined both at biopsy and at autopsy. *Neuropathol. Appl. Neurol.* **14**: 177–195.
109. MANNING, C. A., J. L. HALL, AND P. E. GOLD. 1990. Glucose effects on memory and other neuropsychological tests in elderly humans. *Psych. Sci.* **1**: 307–311.
110. MANSON, J. E., G. A. COLDITZ, M. J. STAMPFER, W. C. WILLETT, A. S. KROLEWSKI, B. ROSNER, R. A. ARKY, F. E. SPEIZER, AND C. H. HENNEKENS. 1991. A prospective study of maturity-onset diabetes mellitus and risk of coronary heart disease and stroke in women. *Arch. Int. Med.* **151**: 1141–1147.
111. MARTINS, R. N., C. G. HARPER, G. B. STOKES, AND C. L. MASTERS. 1986. Increased cerebral glucose-6-phosphate dehydrogenase activity in Alzheimer's disease may reflect oxidative stress. *J. Neurochem.* **46**: 1042–1045.
112. MASORO, E. J., R. J. M. MCCARTER, M. S. KATZ, AND C. A. MCMAHAN. 1992. Dietary restriction alters characteristics of glucose fuel use. *J. Gerontol. Biol. Sci.* **47**: B202–B208.
113. MASTERS, P. M. 1983. Stereochemically altered noncollagenous protein from human dentin. *Calc. Tiss. Int.* **35**: 43–47.
114. MASTROGIACOMO, F., C. BERGERON, AND S. J. KISH. 1993. Brain alpha-ketoglutarate dehydrogenase complex activity in Alzheimer's disease. *J. Neurochem.* **61**: 2007–2014.
115. MATTSON, M. P., J. W. CARNEY, AND D. A. BUTTERFIELD. 1995. A tombstone in Alzheimer's? *Nature* **373**: 481. [Letter]
116. McDONALD, L. J., AND J. MOSS. 1993. Stimulation by nitric oxide of an NAD linkage to glyceraldehyde-3-phosphate dehydrogenase. *Proc. Natl. Acad. Sci. USA* **90**: 6238–6241.
117. MCGEER, P. L., AND E. G. MCGEER. 1992. Complement proteins and complement inhibitors in Alzheimer's disease. *Res. Immunol.* **143**: 621–623.
118. MCKHANN, G., D. DRACHMAN, M. FOLSTEIN, R. KATZMAN, D. PRICE, AND E. M. STADLAN. 1984. Clinical diagnosis of Alzheimer's disease: Report of the NINCDS-ADRDA Work Group under the auspices of Department of Health and Human Services Task Force on Alzheimer's Disease. *Neurology* **34**: 939–944.
119. MEANEY, M. J., D. H. AITKEN, S. BHATNAGAR, C. VAN BERKEL, AND R. M. SAPOLSKY. 1988. Postnatal handling attenuates neuroendocrine, anatomical, and cognitive impairments related to the aged hippocampus. *Science* **238**: 766–768.
120. MENEILLY, G. S., AND A. HILL. 1993. Alterations in glucose metabolism in patients with Alzheimer's disease. *J. Am. Geriatric Soc.* **41**: 710–714.
121. MIRRA, S. S., A. HEYMAN, D. MCKEEL, S. M. SUMI, B. J. CRAIN, L. M. BROWNLEE, F. S. VOGEL, J. P. HUGHES, G. VAN BELLE, L. BERG, AND Participating CERAD Neuropathologists. 1991. The Consortium to Establish a Registry for Alzheimer's Disease (CERAD). Part II. Standardization of the neuropathologic assessment of Alzheimer's disease. *Neurology* **41**: 479–486.
122. MOATS, R. A., T. ERNS, T. K. SHONK, AND B. D. ROSS. 1994. Abnormal cerebral metabolite concentrations in patients with probable Alzheimer disease. *Magn. Reson. Med.* **32**: 110–115.
123. MOHR, S., J. S. STAMLER, AND B. BRUNE. 1994. Mechanism of covalent modification of glyceraldehyde-3-phosphate dehydrogenase at its active site thiol by nitric oxide, peroxyxynitrite, and related nitrosating agents. *FEBS Lett.* **348**: 223–227.
124. MORGAN, T. E., N. J. LAPING, I. ROZOVSKY, T. ODA, H. HOGAN, C. E. FINCH, G. M. PASINETTI. 1995. Clusterin expression in astrocytes is influenced by TGF- β 1 and heterotypic cell interactions. *J. Neuroimmunol.* **58**: 101–110.
125. MORRIS, J. C., M. STORANDT, D. W. MCKEEL, E. H. RUBIN, J. L. PRICE, E. A. GRANT, AND L. BERG. 1996. Cerebral amyloid deposition and diffuse plaques in "normal" aging: Evidence for presymptomatic and very mild Alzheimer's disease. *Neurology* **46**: 707–719.
126. MORRIS, J. C., A. HEYMAN, R. C. MOHS, J. P. HUGHES, G. VAN BELLE, G. FILLENBAUM, E. D. MELLITS, C. CLARK, AND THE CERAD Investigators. 1989. The Consortium to Establish a Registry for Alzheimer's Disease (CERAD). Part I. Clinical and neuropsychological assessment of Alzheimer's disease. *Neurology* **39**: 1159–1165.
127. DELETED IN PROOF.
128. MORTEL, K. F., S. WOOD, M. A. PAVOL, J. S. MEYER, AND J. L. REXER. 1993. Analysis of familial and individual risk factors among patients with ischemic vascular dementia and Alzheimer's disease. *Angiology* **44**: 599–605.
129. MUNCK, A., P. GUYRE, AND N. HOLBROOK. 1984. Physiological functions of glucocorticoids during stress and their relation to pharmacological actions. *Endo. Rev.* **5**: 25–49.
130. MURPHY JR., G. M., W. G. ELLIS, Y-L. LEE, K. E. STULTZ, R. SHRIVASTAVA, J. R. TINKLEBERG, AND L. F. ENG. 1992. In *Progress in Brain Research*. (A. C. H. Yu, L. Hertz, M. D. Norenberg, E. Sykova and S. G. Waxman, Eds.), p. 475. Elsevier, New York.
131. MUTISYA, E. M., A. C. BOWLING, AND M. F. BEAL. 1994. Cortical cytochrome oxidase activity is reduced in Alzheimer's disease. *J. Neurochem.* **63**: 2179–2184.
132. MYHRER, T. 1993. Animal models of Alzheimer's disease: Glutamatergic denervation as an alternative approach to cholinergic denervation. *Neurosci. and Biobehavioral Reviews* **17**: 195–202.
133. NAGARAJ, R. H., AND V. M. MONNIER. 1992. Isolation and characterization of a blue fluorophore from human eye lens crystallins: In vitro formation from Maillard reaction with ascorbate and ribose. *Biochim. Biophys. Acta* **1116**: 34–42.
134. NAGARAJ, R. H., T. S. KERN, D. R. SELL, J. FOGARTY, R. L. ENGERMAN, AND V. M. MONNIER. 1996. Evidence of a glycemic threshold for the formation of pentosidine in diabetic dog lens but not in collagen. *Diabetes* **45**: 587–594.
135. NASLUND, J., J. THYBERG, L. O. TJERNBERG, C. WERNSTEDT, A. R. KARLSTROM, N. BOGDANOVIC, S. E. GANDY, L. LANNFELT, L. TERENIUS, AND C. NORDSTEDT. 1995. Characterization of stable complexes involving apolipoprotein E and the amyloid β peptide in Alzheimer's disease brain. *Neuron* **15**: 219–228.
136. NEEPER, M., A. M. SCHMIDT, J. BRETT, S. D. YAN, F. WANG, Y-C. E. PAN, K. ELLISTON, D. STERN, AND A. SHAW. 1992. Cloning and expression of a cell surface receptor for advanced glycosylation end products of proteins. *J. Biol. Chem.* **267**: 14998–15004.
137. NICHOLS, N. R., C. E. FINCH, AND J. F. NELSON. 1995. Food restriction delays the age-related increases of GFAP mRNA in rat hypothalamus. *Neurobiol. Aging* **16**: 105–110.
138. NIELSON, K. A., J. H. NOLAN, N. C. BERCHTOLD, C. A. SANDMAN, R. A. MULNARD, AND C. W. COTMAN. 1996. Apolipoprotein-E genotyping of diabetic dementia patients: Is diabetes rare in Alzheimer disease? *J. Am. Geriatr. Soc.* **44**: 1–8.
139. NORENBURG, M. D., AND A. MARTINEZ-HERNANDEZ. 1979. Fine structural localization of glutamine synthetase in astrocytes in rat brain. *Brain Res.* **161**: 303–310.

140. NORENBURG, M. D. 1987. The role of astrocytes in hepatic encephalopathy. *Neurochem. Pathol.* **6**: 13–33.
141. NORENBURG, M. D., AND Y. ITZHAK. 1995. Acute liver failure and hyperammonemia increase nitric oxide synthase in mouse brain. *Soc. Neurosci. Abstracts* **21**: 869. [No. 345.9]
142. NOY, N., H. SCHWARTZ, AND A. GAFNI. 1985. Age-related changes in the redox status of rat muscle cells and their role in enzyme-aging. *Mech. Age. Dev.* **29**: 63–69.
143. O'BRIEN, M. D. 1988. Vascular dementia is underdiagnosed. *Arch. Neurol.* **45**: 797–798.
144. OLIVER, C. N., P. E. STARKE-REED, E. R. STADTMAN, G. J. LIU, J. M. CARNEY, AND R. A. FLOYD. 1990. Oxidative damage to brain proteins, loss of glutamine synthetase activity, and production of free radicals during ischemia/reperfusion-induced injury to gerbil brain. *Proc. Natl. Acad. Sci. USA* **87**: 5144–5147.
145. O'STEEN, W. K., AND P. W. LANDFIELD. 1991. Dietary restriction does not alter retinal aging in the Fischer 344 rat. *Neurobiol. Aging* **12**: 455–462.
146. O'STEEN, W. K., L. B. CADWALLADER, S. VINSANT, AND P. W. LANDFIELD. 1991. Biomarkers of hippocampal and retinal aging are not altered by dietary restriction. *Soc. Neurosci. Abstr.* **16**: 1161. [No. 480.16]
147. OTT, A., R. P. STOLK, M. M. B. BRETELER, AND A. HOFMAN. 1996. The association between diabetes mellitus and dementia. *Neurology* **Feb.** [Abstract P05.084]
148. PALMER, A. M., AND S. GERSHON. 1990. Is the neuronal basis of Alzheimer's disease cholinergic or glutamatergic? *FASEB J.* **4**: 2745–2752.
149. PELLIGRINO, D. A., J. C. LAMANNA, R. B. DUCKROW, R. M. BRYAN, JR., AND S. I. HARIK. 1992. Hyperglycemia and blood-brain barrier glucose transport. *J. Cerebr. Blood Flow Metab.* **12**: 887–899.
150. PEPPARD, R. F., W. R. W. MARTIN, C. M. CLARK, G. D. CARR, P. L. MCGEER, AND D. B. CALNE. 1990. Cortical glucose metabolism in Parkinson's and Alzheimer's disease. *J. Neurosci. Res.* **27**: 561–568.
151. PERRY, V. H., M. K. MATYSZAK, AND S. FEARN. 1993. Altered antigen expression of microglia in the aged rodent CNS. *GLIA* **7**: 60–67.
152. PITSIKAS, N., AND S. ALGERI. 1992. Deterioration of spatial and nonspatial reference and working memory in aged rats: Protective effect of life-long calorie restriction. *Neurobiol. Aging* **13**: 369–373.
153. POIRIER, J., J. DAVIGNON, D. BOUTHILLIER, S. KOGAN, P. BERTRAND, AND S. GAUTHIER. 1993. Apolipoprotein E polymorphism and Alzheimer's disease. *Lancet* **342**: 697–699.
154. PROCTOR, A. W., A. M. PALMER, P. T. FRANCIS, S. L. LOWE, D. NEARY, E. MURPHY, R. DOSHI, AND D. M. BOWEN. 1988. Evidence of glutamatergic denervation and possible abnormal metabolism in Alzheimer's disease. *J. Neurochem.* **50**: 790–802.
155. PUGLIESE, G., R. G. TILTON, AND J. R. WILLIAMSON. 1991. Glucose-induced metabolic imbalances in the pathogenesis of diabetic vascular disease. *Diabetes Metab. Rev.* **7**: 35–59.
156. RAO, V. L. R., R. M. AUDET, AND R. F. BUTTERWORTH. 1995. Increased nitric oxide synthase activities and L-[³H]arginine uptake in brain following portacaval anastomosis. *J. Neurochem.* **65**: 677–681.
157. RAPOPORT, S. I., B. HORWITZ, C. L. GRADY, J. V. HAXBY, C. DECARLI, AND M. B. SCHAPIRO. 1991. Abnormal brain glucose metabolism in Alzheimer's disease, as measured by positron emission tomography. *Adv. Exp. Med. Biol.* **291**: 231–248.
158. REAVEN, G. M., AND E. P. REAVEN. 1985. Age, glucose intolerance, and non-insulin-dependent diabetes mellitus. *J. Am. Geriatr. Soc.* **33**: 286–290.
159. REAVEN, G. M., L. W. THOMPSON, D. NAHUM ET AL. 1990. Relationship between hyperglycemia and cognitive function in older NIDDM patients. *Diabetes Care* **13**: 16–21.
160. REISER, K. M. 1994. Influence of age and long-term dietary restriction on enzymatically mediated crosslinks and nonenzymatic glycation of collagen in mice. *J. Gerontol.* **49**: B71–B79.
161. RODGERS, B., A. LAU, C. NICOL. 1994. Hypophysectomy or adrenalectomy of rats with insulin-dependent diabetes mellitus partially restores responsiveness to GH. *Proc. Soc. Exp. Biol. Med.* **207**: 220–226.
162. ROHER, A. E., J. D. LOWENSON, S. CLARKE, A. S. WOODS, R. J. COTTER, E. GOWING, AND M. J. BALL. 1993. β -Amyloid-(1–42) is a major component of cerebrovascular amyloid deposits: Implications for the pathology of Alzheimer disease. *Proc. Nat. Acad. Sci. USA* **90**: 10836–10840.
163. ROTH, G. S., D. K. INGRAM, AND J. A. JOSEPH. 1984. Delayed loss of striatal dopamine receptors during aging of dietarily restricted rats. *Brain Res.* **300**: 27–32.
164. ROTHMAN, D. L., E. J. NOVOTNY, G. I. SHULMAN, A. M. HOWSEMAN, O. A. C. PETROFF, G. MASON, T. NIXON, C. C. HANSTOCK, J. W. PRICHARD, AND R. G. SHULMAN. 1992. ¹H-[¹³C] NMR measurements of [4-¹³C]glutamate turnover in human brain. *Proc. Natl. Acad. Sci. USA* **89**: 9603–9606.
165. ROWE, J. W., K. L. MINAKER, J. A. PALLOTTA, AND J. S. FLIER. 1983. Characterization of the insulin resistance of aging. *J. Clin. Invest.* **71**: 1581–1587.
166. RYLE, C., AND M. DONAGHY. 1995. Non-enzymatic glycation of peripheral nerve proteins in human diabetics. *J. Neurol. Sci.* **129**: 62–68.
167. SABATINO, F., E. J. MASORO, C. A. MCMAHAN, AND R. W. KUHN. 1991. Assessment of the role of glucocorticoid system in aging processes and in the action of food restriction. *J. Gerontol.* **46**: B171–B179.
168. SALO, M. K., R. RANTANEN, T. HUUPPONEN, T. LEHTIMAKI, H. JOKELA. 1993. Apolipoprotein E phenotypes and plasma lipids in diabetic children and adolescents. *Eur. J. Pediatr.* **152**: 564–588.
169. SAPOLSKY, R. M. 1985. A mechanism for glucocorticoid toxicity in the hippocampus: Increased neuronal vulnerability to metabolic insults. *J. Neurosci.* **5**: 1228–1232.
170. SAPOLSKY, R. M., H. UNO, C. S. REBERT, AND C. E. FINCH. 1990. Hippocampal damage associated with prolonged glucocorticoid exposure in primates. *J. Neurosci.* **10**: 2897–2902.
171. SAPOLSKY, R. M. 1992. *Stress, the Aging Brain, and the Mechanisms of Neuron Death*, MIT Press, Cambridge, MA.
172. SASAKI, H., O. MURAMOTO, I. KANAZAWA, H. ARAI, K. KOSAKA, AND R. IZUKA. 1986. Regional distribution of amino acid transmitters in postmortem brains of presenile and senile dementia of Alzheimer type. *Ann. Neurol.* **19**: 263–269.
173. SCHIPPER, H., J. R. BRAWER, J. F. NELSON, L. S. FELICIO, AND C. E. FINCH. 1981. Role of the gonads in the histologic aging of the hypothalamic arcuate nucleus. *Biol. Reprod.* **25**: 413–419.
174. SCHIPPER, H. M. 1996. Astrocytes, brain aging and neurodegeneration. *Neurobiol. Aging* **17**: 467–480.
175. SCHMOLL, D., E. FUHRMANN, R. GEBHARDT, AND B. HAMPRECHT. 1995. Significant amounts of glycogen are synthesized from 3-carbon compounds in astroglial primary cultures from mice with participation of the mitochondrial phosphoenolpyruvate carboxykinase isoenzyme. *Eur. J. Biochem.* **227**: 308–315.
176. SCHUBERT, D. 1994. *The Structure and Function of Alzheimer's Amyloid Beta Proteins*. R. G. Landes, Austin, TX.
177. SEAQUIST, E. R., K. UGURBIL, AND R. GRUETTER. 1996. Cerebral

- glucose concentrations in humans by ^1H MRS at 4 Tesla. *Proc. Int. Soc. Magnet. Resonance in Med.* **1**: 409 [Abstract]
178. SEEMAN, T. E., L. F. BERKMAN, D. BLAZER, AND J. W. ROWE. 1994. Social ties and support and neuroendocrine function: The MacArthur studies of successful aging. *Ann Behav. Med.* **16**: 95–106.
 179. SEILER, N. 1993. Is ammonia a pathogenetic factor in Alzheimer's disease? *Neurochem. Res.* **18**: 235–245.
 180. SELKOE, D. J. 1994. Normal and abnormal biology of the β -amyloid precursor protein. *Annu. Rev. Neurosci.* **17**: 489–517.
 181. SELKOE, D., AND K. KOSIK. 1984. Neurochemical changes with aging. In *Clinical Neurology of Aging* (M. L. Albert, Ed.), pp. 53–75. Oxford Univ. Press, New York.
 182. SELL, D. R., AND V. M. MONNIER. 1990. Structure elucidation of a senescence cross-link from human extracellular matrix. *J. Biol. Chem.* **266**: 21597–21602.
 183. SELL, D. R., M. A. LANE, W. A. JOHNSON, E. J. MASORO, O. B. MOCK, K. REISER, J. F. FOGARTY, R. G. CUTLER, D. K. INGRAM, G. S. ROTH, V. M. MONNIER. 1996. Longevity and the genetic determination of collagen glycoxidation kinetics in mammalian senescence. *Proc. Natl. Acad. Sci. USA* **93**: 485–490.
 184. SERVO, C., AND E. PITKANEN. 1975. Variation in polyol levels in cerebrospinal fluid and serum in diabetic patients. *Diabetologia* **11**: 575–580.
 185. SHANK, R. P., G. S. BENNETT, S. O. FREYTAG, AND G. CAMPBELL. 1985. Pyruvate carboxylase: an astrocytic-specific enzyme implicated in the replenishment of amino acid transmitter pools. *Brain Res.* **329**: 364–367.
 186. SHIMOKATA, H., D. C. MULLER, J. L. FLEG, J. SORKIN, A. W. ZIEMBA, AND R. ANDRES. 1991. Age as Independent Determinant of Glucose Tolerance. *Diabetes* **40**: 44–51.
 187. SHIMOKAWA, I., Y. HIGAMI, G. B. HUBBARD, C. A. MCMAHAN, E. J. MASORO, AND B. P. YU. 1993. Diet and the suitability of the male Fischer 344 rat as a model for aging research. *J. Gerontol.* **48**: B27–B32.
 188. SISODIA, S. S., AND D. L. PRICE. 1995. Role of the β -amyloid protein in Alzheimer's disease. *FASEB J.* **9**: 366–370.
 189. SMITH, C. D., J. M. CARNEY, P. E. STARKE-REED, C. N. OLIVER, E. R. STADTMAN, R. A. FLOYD, AND W. R. MARKESBERY. 1991. Excess brain protein oxidation and enzyme dysfunction in normal aging and in Alzheimer disease. *Proc. Natl. Acad. Sci. USA* **88**: 10540–10543.
 190. SMITH, M. A., S. TANEDA, P. L. RICHEY, S. MIYATA, S.-D. YAN, D. STERN, L. M. SAYRE, V. M. MONNIER, AND G. PERRY. 1994. Advanced Maillard reaction end products are associated with Alzheimer disease pathology. *Proc. Natl. Acad. Sci. USA* **91**: 5710–5714.
 191. SMITH, M. A., L. M. SAYRE, AND G. PERRY. 1996. Diabetes mellitus and Alzheimer's disease: Glycation as a biochemical link. *Diabetologia* **39**: 247.
 192. SONNEWALD, U., N. WESTERGAARD, S. B. PETERSEN, G. UNSGARD, AND A. SCHOUSBOE. 1993. Metabolism of $[\text{U-}^{13}\text{C}]$ glutamate in astrocytes studied by ^{13}C -NMR spectroscopy: Incorporation of more label into lactate than into glutamine demonstrates the importance of the tricarboxylic acid cycle. *J. Neurochem.* **61**: 1179–1182.
 - 192a. SPARKS, D. L., WOELTZ, V. M., AND MARKESBERY, W. R. 1991. Alterations in brain monoamine oxidase activity in aging, Alzheimer's disease, and Pick's disease. *Arch. Neurol.* **48**: 718–721.
 193. STADTMAN, E. R. 1992. Protein oxidation and aging. *Science* **257**: 1220–1224.
 194. STONE, W. S., G. L. WENK, D. S. OLTON, AND P. E. GOLD. 1990. Poor blood glucose regulation predicts sleep and memory deficits in normal aged rats. *J. Gerontol.* **45**: B169–B173.
 195. STRITTMATTER, W. J., A. M. SAUNDERS, D. SCHMECHEL, M. PERICAK-VANCE, J. ENGHILD, G. S. SALVESEN, AND A. D. ROSES. 1993. Apolipoprotein E: High-avidity binding to β -amyloid and increased frequency of type 4 allele in late-onset familial Alzheimer disease. *Proc. Natl. Acad. Sci. USA* **90**: 1977–1981.
 196. STRYER, L. 1988. *Biochemistry*, 3rd ed. W. H. Freeman, New York.
 197. TATEMACHI, T. K., N. SACKTOR, AND R. MAYEUX. 1994. Dementia associated with cerebrovascular disease, other degenerative diseases and metabolic disorders. In *Alzheimer Disease* (R. D. Terry, R. Katzman and K. L. Bick, Eds.), pp. 123 Raven Press, New York.
 198. TERRY, R. D., E. MASLIAH, AND L. A. HANSEN. 1994. Structural basis of the cognitive alterations in Alzheimer disease. In *Alzheimer Disease* (R. D. Terry, R. Katzman and K. L. Bick, Eds.), pp. 179–198. Raven Press, New York.
 199. THORPE, J., L. P. WIDMAN, A. WALLIN, J. BEISWANGER, AND H. T. BLUMENTHAL. 1994. Comorbidity of other chronic age-dependent diseases in dementia. *Aging Clin. Exp. Res.* **6**: 159–166.
 200. TSAKOPOULOS, M., AND P. J. MAGISTRETTI. 1996. Metabolic coupling between glia and neurons. *J. Neurosci.* **16**: 877–885.
 201. VEERHUIS, R., I. JANSSEN, C. E. HACK, AND P. EIKELENBOOM. 1995. Early complement components in Alzheimer's disease brains. *Acta Neuropathol.* **91**: 53–60.
 202. VIRGIN, C., T. HA, D. PACKAN, C. TOMBAUGH, S. YANG, H. HORNER, AND R. SAPOLSKY. 1991. Glucocorticoids inhibit glucose transport and glutamate uptake in hippocampal astrocytes: Implications for glucocorticoid neurotoxicity. *J. Neurochem.* **57**: 1422.
 203. VITEK, M. P., K. BJATTACHARYA, M. J. GLENDENING, E. STOPA, H. VLASSARA, R. BUCALA, K. MANOGUE, AND A. CERAMI. 1994. Advanced glycation end products contribute to amyloidosis in Alzheimer's disease. *Proc. Natl. Acad. Sci. USA* **91**: 4766–4770.
 204. VLASSARA, H., M. BROWNLEE, AND A. CERAMI. 1984. Accumulation of diabetic rat peripheral nerve myelin by macrophages increases with the presence of advanced glycosylation endproducts. *J. Exp. Med.* **160**: 197–207.
 205. VLASSARA, H., M. BROWNLEE, AND A. CERAMI. 1985. Recognition and uptake of human diabetic peripheral nerve myelin by macrophages. *Diabetes* **34**: 553–557.
 206. VLASSARA, H., H. FUH, Z. MAKITA, S. KRUNGKRAI, A. CERAMI, AND R. BUCALA. 1992. Exogenous advanced glycosylation end products induce complex vascular dysfunction in normal animals: A model for diabetic and aging complications. *Proc. Natl. Acad. Sci. USA* **89**: 12043–12047.
 207. VLASSARA, H., R. BUCALA, AND L. STRIKER. 1994. Biology of disease: Pathogenic effects of advanced glycosylation: Biochemical, biologic, and clinical implications for diabetes and aging. *Lab. Invest.* **70**: 138.
 208. WAELSCH, H., S. BERL, C. A. ROSSI, D. D. CLARKE, AND D. P. PURPURA. 1964. Quantitative aspects of CO_2 fixation in mammalian brain *in vivo*. *J. Neurochem.* **11**: 717–728.
 209. WEINDRUCH, R. H., AND R. L. WALFORD. 1988. *The Retardation of Aging and Disease by Dietary Restriction*. Springfield, IL.
 210. WESTERGAARD, N., U. SONNEWALD, A. SCHOUSBOE. 1994a. Release of α -ketoglutarate, malate and succinate from cultured astrocytes: Possible role in amino acid neurotransmitter homeostasis. *Neurosci. Letts.* **176**: 105–109.
 211. WESTERGAARD, N., U. SONNEWALD, G. UNSGARD, L. PENG, L. HERTZ, AND A. SCHOUSBOE. 1994b. Uptake, release, and metabolism of citrate in neurons and astrocytes in primary cultures. *J. Neurochem.* **62**: 1727–1733.

212. WILLIAMS, S. K., N. L. HOWARTH, J. J. DEVENNY, AND M. W. BITENSKY. 1982. Structural and functional consequences of increased tubulin glycosylation in diabetes mellitus. *Proc. Natl. Acad. Sci. USA* **79**: 6546–6550.
213. WOLF-KLEIN, G. P., F. A. SILVERSONE, M. S. BROD, A. LEVY, C. J. FOLEY, V. TERMOTTO, AND J. BREUER. 1988. Are Alzheimer patients healthier? *J. Am. Geriatr. Soc.* **36**: 219–224.
214. YAN, S.-D., X. CHEN, A.-M. SCHMIDT, J. BRETT, G. GODMAN, Y.-S., ZOU, C. W. SCOTT, C. CAPUTO, T. FRAPPIER, M. A. SMITH, G. PERRY, S.-H. YEN, AND D. STERN. 1994. Glycated tau protein in Alzheimer disease: A mechanism for induction of oxidant stress. *Proc. Natl. Acad. Sci.* **91**: 7787–7795.
215. YAN, S. D., S. F. YAN, X. CHEN, J. FU, M. CHEN, P. KUPPUSAMY, M. A. SMITH, G. PERRY, G. C. GODMAN, P. NAWROTH, J. L. ZWEIER, AND D. STERN. 1995. Non-enzymatically glycated tau in Alzheimer's disease induces neuronal oxidant stress resulting in cytokine gene expression and release of amyloid β -peptide. *Nature Med.* **1**: 693–699.
216. YAN, S. D., X. CHEN, J. FU, M. CHEN, H. ZHU, A. ROHER, T. SLATTERY, L. ZHAO, M. NAGASHIMA, J. MORSER, A. MIGHELLI, P. NAWROTH, D. STERN, AND A. M. SCHMIDT. 1996. RAGE and amyloid- β peptide neurotoxicity in Alzheimer's disease. *Nature* **382**: 685–691.
217. ZHANG, J., AND S. H. SNYDER. 1992. Nitric oxide stimulates auto-ADP-ribosylation of glyceraldehyde-3-phosphate dehydrogenase. *Proc. Natl. Acad. Sci. USA* **89**: 9382–9385.
218. ZYZAK, D. V., J. M. RICHARDSON, S. R. THORPE, AND J. W. BAYNES. 1995. Formation of reactive intermediates from Amadori compounds under physiological conditions. *Arch. Biochem. Biophys.* **316**: 547–554.