AGING, ALZHEIMER'S DISEASE AND PROTEIN CROSSLINKING

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ABSTRACT

A hypothesis is presented that certain diseases like Alzheimer's dementia may be a result of disturbances in crosslinking of body fluids and cytoplasmic proteins. These disturbances in turn may be caused by a change, probably a decrease, of the fluid pH. Dropping of pH may be a result of different organismal and environmental factors such as trauma, a genetic defect, a slow virus infection, a toxin, a deficiency of calcium and/or magnesium, or hypoventilation. The influence of acidosis caused by a hypoventilation may be a main cause of diseases due to normal aging processes. The fact that living systems operate in a state far from thermodynamic equilibrium and are probably critically crosslinked makes them especially sensitive to even minor changes of some parameters, namely changes that act as information interactions. The hypothesis may indicate new lines of research as well as new lines of thinking about implications of some environmental factors such as of weak electromagnetic fields.

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SYSTEMS FAR FROM THERMODYNAMIC EQUILIBRIUM

It is well known that living systems are far from thermo-
dynamic equilibrium but only in recent years have non-equil-
ibrium models of living systems drawn greater attention. Owing
to the Prigogine's Brussel School works (1) it has become more
and more evident that chemical systems far from thermodynamic
equilibrium show phenomena that have long been reserved for
oscillations (2).

In our previous paper (3) we presented the hypothesis that
some protein macromolecules, most probably ones with short turn-
over half-time, are synthesized and perform their functions in a
living organism while being in a meta-stable non-equilibrium
state.

In more complicated systems, like a living cell or a
tissue, non-equilibrium phenomena are much more common. For
example, local changes of cytoplasmic viscoelasticity are very
probably due to auto-oscillatory sol-gel transitions (Figure 1)
of cytosol proteins like actin or tubulin (4), and are of a
crucial importance for any living cell (5). Different patholo-
gies may be connected with different possible disturbances of
these dissipative structures, such as changing of period and/or
amplitude, changing of a characteristic spatial wave length,
switching to damped oscillation and to non-oscillatory regime,
going to chaos.

Changing of aggregation state of a system's components in
general and sol-gel transition in particular are due to forming
cross-linkages between (macro)molecules in the system.   Di-
sulphide bridges are the best known types of covalent crosslinks
found in biological systems. Recently another type of crosslink
present in proteins, γ-glutamyl-ε-lysine side chain bridges
formed by calcium-dependent transglutaminase was reported (6).
Also, lipids and saccharides (especially mucopolysaccharides)
may be involved in protein crosslinking. Possible perturbations
of crosslinking processes and therefore of protein aggregation
may lead to serious pathological changes such as atherosclerosis
(7).

Systems far from thermodynamic equilibrium are sensitive to
small changes in internal parameters and external conditions.
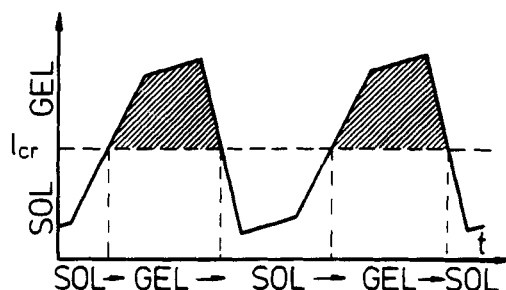Systems operating near transition points are extremely

FIGURE 1

Hypothetical sol-gel oscillating system (4).

sensitive.   Critically branched macromolecular systems such as
those near the sol-gel transition point (cytoplasm of a living
cell may be an example of such a system)(8).   Because proteins
are polyions, even a small change of pH or ionic strength of the
fluid in which they are embedded (cytoplasm, blood plasma,
cerebrospinal fluid), or the imposition of an electromagnetic
field may drastically change the state of protein aggregation,
leading to pathological states.   Similar changes may result from
small modifications of protein primary structure by detaching or
attaching a small molecule or a molecular fragment, most often
in an enzymatically catalyzed process.

ENERGETIC AND INFORMATIONAL INTERACTIONS

One may use a mechanical analogy to better understand the
issue.   A pack of cards lying on a table-top is in equilibrium.
Its potential energy shows a deep minimum, and a substantial
force like a gust of wind is necessary to scatter the cards.
The same pack, if arranged into a house of cards (the potential
energy being much higher) becomes extremely sensitive to very
small forces, whether acting on the whole system or even on a
single card.   Building such a non-equilibrium construction
requires the application of forces and an energy input much

higher than those needed to scatter the cards. But the response time of the system becomes extremely short, and system sensitivity extremely high.

The interaction (force) that so dramatically changes the state of a system far from equilibrium may be completely different than that used to build or maintain the system in that state. For example, if one of the cards bears a small magnet, the whole house of cards may become extremely sensitive to changes in the local electromagnetic field -- changes that would have no influence if the card with a magnet was inside a pack of cards lying on a surface. The interaction of electromagnetic waves with the receiving antenna of a television furnishes another example. The electromagnetic waves change the state of the television which is maintained far from equilibrium (by pumping with electric energy from an electric outlet) in such a way that pictures may be seen on the screen. Thus it is clear that changing the state of a system in equilibrium requires a significant force (an energetic interaction), whereas the state of a system far from equilibrium may be changed by a small force (informational interaction).

PROTEIN CROSSLINKING AND ALZHEIMER'S DISEASE

Starting with a single cell, an organism is created by a highly organized processes of differentiation and development. Mechanisms determining how a cell differentiates are thought to involve, apart from the processing of information contained within the cell genome, different informational interactions with other cells and the environment. These interactions include hormones, growth factors and neurotransmitters which interact with specific receptors, as well as weak electrical effects, small gradients of ionic strength, cell-to-cell contacts. Different informational processes and interactions are often interrelated. Hormone receptors, for example, are located on the cell membrane and within the cytoplasm but also they exist as a part of the chromatin structure (9).

The maintenance of proper differentiated, far from equilibrium states requires energy to stabilize them. Of course, some states are more energy-requiring than others.

Aging is considered to be the random dysdifferentiation of a
mature living system, the time-dependent drifting away of cells,
cell organization and homeostatic control from their optimum
state of function.   Compensational, renewal and repairing
processes help to keep a mature organism alive for a certain
period of time (9).

The same processes which lead to normal aging and to the
organism's death are also the main cause of different diseases,
especially brain diseases. The aging of the brain is likely to
involve the slow dysfunction of most of its cells, although some
cell types may degenerate selectively.   For example, in
Alzheimer's disease and senile dementia one observes a loss of
neurons producing a neurotransmitter acetylcholine, which are
situated mainly in the nucleus basalis of Meynert, the band of
Broca, and the medial septal nuclei (10).   Why are these
neurons selectively lost?   As suggested by Cutler (9) one
possible answer is that perhaps most of the cells making up the
brain are highly interdependent for their proper state of
differentiation on the proper differentiated state of other
cells making up the brain. Thus, a small drift of one type of
cell from its optimum output, say of a given neurotransmitter
product or growth factor, could conceivably cause something like
a dysdifferentiative catastrophe or cascade through positive
feedback mechanisms.   For example, small changes beginning in
the nucleus basalis of Meynert could affect the function of
cells in the cortex, which in turn might further alter the cells
in the nucleus basalis of Meynert through such by-products of
neurotransmitter degradation as free radicals and hydrogen
peroxidase (9). Similarly, in a multicomponent system far from
thermodynamic equilibrium with several interdependent reactions
going on, some species may be almost completely transformed
(e.g. oxygenated, reduced or split), whereas the net
concentration of other species essentially remains constant in
spite of their participation in several reactions.

Alzheimer's disease is a chronic, progressive neuro-
psychiatric disorder, leading to serious cognitive disturbances
(11).   It afflicts 1.5-2.0 million Americans, and kills more
than 100,000 each year (12). An autopsy of brains of people
with Alzheimer's disease shows characteristic pathological

changes (13). Accumulations of twisted filaments (neurofibril-
lary tangles) within neurons and amorphous aggregates of protein
(amyloid) adjacent to and within vessels are found, as well as
scattered focuses of cellular debris and amyloid called neuritic
plaques. There is also a significant loss of specific neurons
in certain regions of the brain (10). Biochemical and
immunological investigations show a reduction in the amount of
some neurotransmitters (e.g. acetylcholine), and of
neurotransmitter receptors (somatostatin, for example) (14,15).

Based on symptoms and pathological findings, at least six
conceptual models of Alzheimer's disease have been proposed:
that the sickness is caused by faulty genes, by abnormal
accumulation of proteins, by an infectious agent (virus or
prion), by an environmental toxin, by inadequate blood flow and
energy metabolism, or by selective loss of cholinergic neurons
(ones that release acetylcholine). Each model is supported by
some observational or experimental evidence and contradicted by
other evidence (13).

All the factors listed above may be, however, either a
cause or a direct result of disturbances in crosslinking and
aggregation of proteins forming the cytoskeleton of neurons.
The cytoskeleton is formed of three ultrastructurally distinct
elements made of fibrous macromolecules: microtubules 24 nm in
diameter, intermediate filament 10 nm in diameter, and micro-
filaments about 5 nm in diameter and composed of polymerized
actin. The neuronal intermediate filaments, called neuro-
filaments, are antigenically distinct from ones found in other
cells, and they have long been thought to be involved in axonal
transport, apart from being a structural support system (16).
The synchronous movement of neurofilament component proteins in
slow axoplasmic transport has been demonstrated (17).

It has been shown that normal neurofilaments can alter
their size and shape in vitro with changes in the ionic
structure of the medium in which they are suspended, and their
solubility may alter with their configuration (17). Cross-
linkages between neurofilaments and other elements of cyto-
skeleton, especially microtubules, have also been demonstrated
(18). This may interfere with axonal transport by causing some-
times dramatic changes in axoplasm properties. An impaired

axonal transport of neurofilaments may result in severe pathological disorders, Alzheimer's disease being very probably one of those.

But similar pathomorphological and immunopathological changes like the ones observed in Alzheimer's brains are also observed in several other diseases. Amyloid plaques are also observed in Down's syndrome, Pick's disease, Creutzfeld-Jakob disease, kuru, and scrapie (16). Appearance of serum autoantibodies that are specific for the component proteins of 10 nm axonal neurofilaments has been observed in many neurodegenerative diseases, including parkinsonism with dementia and amyotrophic lateral sclerosis. These autoantibodies also react energy metabolism, or by selective loss of cholinergic neurons (ones that release acetylcholine). Each model is supported by some observational or experimental evidence and contradicted by other evidence (13).

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VITAL IMPORTANCE OF pH DISTURBANCES

The appearance of neurofibrillary tangles, neuritic plaques and amyloid plaques in Alzheimer's disease brains may be a consequence of impaired axonal transport of neurofilament, which may have many different causes, such as trauma, a genetic effect, a toxin, calcium and magnesium deficiency, slow virus infection (16). Impaired neurofilament transport may be also a basic mechanism of pathogenesis of other neurodegenerative diseases. Very probably it is the accumulation of neurofilament in the perikaryon, proximal to the axon, which causes cell death, especially in neurons with high transport requirement, such as motor neurons (21).

Impaired axonal transport is itself probably due to disturbances of protein crosslinking. As we have already discussed, crosslinking may dramatically change properties of the system. For example, age-dependent increase in cellular membrane viscosity (decrease in memrane fluidity)(9) may be caused by increasing the degree of crosslinking of membrane proteins. It is well known that viscosity becomes almost infinite at the sol-gel transition point. Since living systems operate far from thermodynamic equilibrium, and probably in a
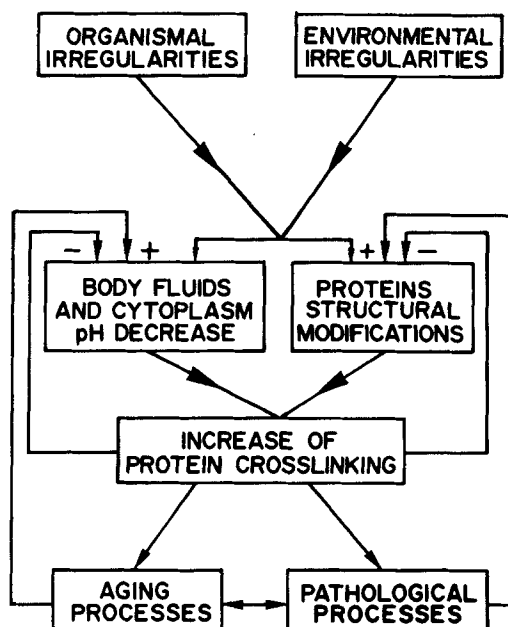
FIGURE 2

Effect of organismal and environmental irregularities on protein
crosslinking (the basic molecular mechanism of aging processes
and pathological changes) leads to aging and/or to different
diseases.   Negative (regulatory) feedbacks (denoted by "-")
enable the organism to maintain its homeostasis. But when some
changes become irreversible, positive (amplifying) feedbacks
(denoted by "+") are produced, leading to progressively greater
changes that often are pathological.

critically-crosslinked   state,   even   a   small   perturbation   of
system parameters may lead to dramatic changes of physics-
chemical properties.   Also environmental factors may play a
similar role (Figure 2).   For example, amyotrophic lateral
sclerosis, parkinsonism with dementia, and early appearance of
neurofibrillary   tangles   in   Alzheimer's   disease   are   three
diseases that neurologists consider to be non-related, yet each

shows a high incidence of calcium and magnesium deficiency as is
observed in certain populations in the Western Pacific (16).
Calcium and magnesium ions are important in both enzymatic and
nonenzymatic protein crosslinking, including actin-myosin cross-
linking, a protein system crucial in muscle contraction but also
present practically in most cells (4,22).

Disturbances of the pH of physiological fluids may be the
most important factor leading eventually to serious pathological
changes. It is well known that blood plasma, interstitial fluid
and cerebrospinal fluid are physiologically slightly alkaline
(pH = 7.4-7.6) whereas isoelectric points, pI's (i.e. pH for
which the molecule is electrically neutral) of blood proteins
are generally smaller: pI = 4.7 for albumin, 5.8 for fibrino-
gen, 6.9 for hemoglobin, 6.4-7.2 for -globulins (23). Thus, at
pH = 7.6, those proteins have a net negative charge and are
soluble. If, however, because of hypoxia or other causes, the
pH of physiological fluids drops down even slightly, the net
negative charge of proteins and peptides diminishes, and they
may stick together forming crosslinked aggregates and polymers
of higher molecular weights. In some cases an increase of pH,
(e.g., due to hyperventilation) also may cause proteins to stick
together.

Resulting protein precipitation may cause drastic changes
of physiochemical properties of the system. It has been
suggested by Lipinski (7) that a decrease of blood pH and
enhanced peroxidative crosslinking due to chronic hypoxia are
responsible for a gradual loss of negative charges in the
arterial walls, thus contributing to atherosclerosis. Little
information is available concerning the pH within individual
neurons and in axoplasm, but one may imagine formation of
neurofibrillar tangles and amyloid plaques being a result of a
similar mechanism, leading eventually to impairments and death
of neurons. It is also important to remember that all enzymes,
neurotransmitter and hormone receptors, as well as many hormones
are themselves proteins, often operating in a state far from
equilibrium (3). They therefore are often highly influenced by
even minor changes of pH and other parameters.

Acidosis (lowering blood plasma pH) may be of metabolic or

respiratory origin.   For example, in hypoglycemia the total
production of CO_2 in the brain tissue may increase to twice the
basal level, and the resulting acidosis leads to so-called hypo-
glycemic convulsions.   The large oxygen consumption by the
brain, about 25% of the total body at rest oxygen consumption,
accounts for the extreme sensitivity of the brain to hypoxia.
For example, it has been observed that alpinists often show
serious neurastenic behavior (alpine brain astenia) and even
permanent brain damage. Coma and irreversible damage, leading
to serious neurodegenerative diseases or even to death may occur
even after brief anoxia due, for example, to hypoventilation.

Low pH has a two-fold effect -- it facilitates protein
aggregation and increases generation of free radicals, which in
turn increase peroxidation (oxidative breakdown of fatty acids
in cellular membranes)(24).   Free radicals, such as hydroxy
(• OH) and possibly alkoxy (RO •) intermediates of lipid
peroxidation can fragment and crosslink protein (25).   In the
absence of molecular oxygen, •OH induces crosslinks in protein
which are often resistant to reduction, whereas in the presence
of oxygen the fragmentation, involving peptide-bond hydrolysis,
is much more pronounced (26).

Some products of radical peroxidation may then accumulate
within cells.   For example, lipofuscin (an aggregate of
peroxidized lipid and proteins) accumulates in lysosomes of aged
cells and in Alzheimer's disease brain cells (26).   An
accumulation of denatured protein may interfere with and finally
impair cell function.   Lack of cellular reductants and
antioxidants is a cause of serious pathological changes.

DISCUSSION

Body ventilation normally deteriorates with aging; the
maximum vital capacity at the age of 70 is only about 55% of
that at the age of 35 (9).   Disturbances of ventilation and
eventual impairment caused by them may show a cooperative
character -- a deterioration of ventilation causes initially a
slight hypoventilation which eventually causes impairing in
protein crosslinking leading to small changes in the brain but
also small sclerotic blood clots which, in turn, further

deteriorate ventilation, leading to still greater changes. The system switches from a normal state to a pathological one. Switching between two states is a well-known phenomenon in a bistable system far from equilibrium. It is a characteristic feature of such a switching process that there exists a certain lag-time (which may be fairly long) during which system properties change only slightly. Then in a short period of time the system switches to the other stationary state and system properties may change dramatically. In a crosslinking process a lag-time period corresponds to normal aggregation; aggregation may facilitate further crosslink forming (cooperativity effect) and finally the system comes to the critical point when gel is formed, dramatically changing the system properties. In eventual clinical symptoms it would correspond to chronic and acute states, respectively.

The fact that in Alzheimer's disease amyloid usually surrounds and invades cerebral blood vessels as well as accumulates in plaques replacing degenerating nerve terminals suggests that changes in the blood and in the nervous tissue may have the same primary cause which may be as simple as a small decrease of pH. Amyloid damages vessels causing a leakage into the brain tissue of other blood proteins, eventually activating an enzyme that converts neurofilaments into paired helical filaments and further damaging the neurons (13).

It has also been shown that dementia may in some patients result from multiple small strokes, and that the regions affected by the strokes are precisely those showing the glucose-consumption deficit (13). On the other hand blood flow and oxygen consumption continue to decline in elderly people, being about 30% lower in those with dementia. But brain blood supply is strictly afflicted by neurons controlling the dilation and contraction of brain arterioles. These neurons release specific neurotransmitters, especially dopamine, for which blood vessels have specific receptors (13,27). Brain supply may be also disturbed through blood-brain barrier changes (28). So, either the damage of some specific neurons, or deficiency of some neurotransmitters or their receptors may result in adiminished blood supply, hypoventilation and eventual pH decrease, increased protein crosslinking, and eventually to a further decline of brain blood supply.

Similarly, some genetic factors, some infectious agents (slow viruses), some toxins and metals (aluminum (29,30), manganese (31)) all may increase protein crosslinking, leading eventually to Alzheimer's and other diseases.

CONCLUDING REMARKS

Increased protein crosslinking, probably due to a decrease of body fluids pH, may be a common base of the different models of Alzheimer's disease that have been proposed, and probably of different neurodegenerative and other diseases.

Different regions of the brain do different jobs. A deficit in performance resulting from damage in one region is often quite unlike one resulting from damage to a different region. In reality, a given brain region or a given kind of neuron may be afflicted in a specific case (even if the underlying mechanisms is exactly the same in different cases) as a simple result of the fact that the primary damages were specifically localized, and then amplified through a feedback as we have discussed.

Our hypothesis says that normal aging processes, as well as Alzheimer's disease and other different diseases, may be caused by disturbances of protein crosslinking due itself to such a simple cause as a change (probably a decrease) of body fluids pH. The hypothesis is but a framework for thought, which however may indicate new lines of research, of diagnosis and of pharmacological treatment in different diseases, as well as show some important environmental implications of such factors as weak electromagnetic fields (32).

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