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H A L F J A A R L I J K S

Our journal urges all the people of the world to send any ideas, suggestions and recommendations (of any form they choose) concerning the way of possible artificial influencing the aging processes and the way of the achieving of the rapid and effective solving the problem of the artificial postponing of the natural (specific) limits of the onset of senility and death and of an appropriate life-proiongation of humans.

All suggestions received are to be analysed from the point of view of their applicability. The most perspective ones will be published in the section of the Journal «Recommendations» and (or) be delivered to investigators for an experimental approval.

Paying the authors of such recommendations is not provided for.

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IN THE YEAR 2000 :

"Elderly People will be young and stay so, till their breath of life snaps. One has to realise that a comparison between the elderly people of tomorrow and the old people of today will become impossible. Not only because one will live for a 100 years, or even more, but because being old will be completely different from now :

Grandmothers and grandfathers, the old age, the gray haired people will, also in name, belong to the past. Teenagers and twens will in their following life-phases continue to feel and behave as they did before.

(Prof. Polak in "The future of growing old", Agon Elseviers, Brussels-Amsterdam).

'First a new theory is attacked as absurd. Then it is admitted to be true but insignificant. Finally it is seen to be so important that its adversaries claim that they themselves discovered it.'

(William James)

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When this journal speaks, the world listens.

AGING, CROSSLINKING AND ALZHEIMER'S DISEASE

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ABSTRACT

Random crosslinking of macromolecules is a significant factor in aging. It has been shown that the degree of crosslinking is correlated with the amounts of freezable bound and non-freezable bound water associated with the macromolecule. Such water can be determined by differential scanning calorimetry.

We studied the insoluble fraction of proteinaceous macromolecules (Mn > 1 000 000) in young brains, (17-30 years), old (67-70 years), all normal, and in 63-69 year Alzheimer brains (autopsy material, grey matter, frontal lobe). The results are presented in Figure 2. The insoluble material from Alzheimer brains show not only the presence of similar high crosslinkage types of proteinaceous macromolecules present in brains of the same age, but in addition a large portion of macromolecular substance of low crosslinkage level otherwise found only in the young.

It appears that in the Alzheimer samples there has taken place a recent synthesis of still substantially non-crosslinked macromolecular matter. This may be of recent date, possibly from the onset of the disease.

Some possible interpretations are suggested.

INTRODUCTION

It has long been recognized that the physical and chemical properties of cell constituents undergo characteristic changes during the process of aging. These changes are particularly evident in macromolecules such as collagen which is a major component of skin, bone, cartilage and the cardiovascular system. Aging manifests itself e.g. in loss of elasticity of the collagen and has been shown to be due to, at least in part, the formation of covalent crosslinks in the collagen *(1)*. Although collagen is the only macromolecular protein in the body which has been clearly shown to undergo crosslinking reactions during the aging process in man and animals, it is generally agreed that similar crosslinking reactions are involved in many processes of maturing and aging. The crosslinking theory of aging has been thoroughly reviewed by Björksten *(2)*, who suggested that random, unwanted crosslinkages are important factors in aging. The crosslinkage theory of aging has also been discussed by Verzar *(3)*, King *(4)*, Carpenter *(5)* and Tanzer *(6)*. In view of this it seemed of importance to study other high molecular weight proteins in the body than collagen. The characterization of insoluble high molecular weight postmortem human brain proteins may be useful in investigating molecular defects, crosslinks and conformational changes connected with normal aging and wich certain neurologic diseases. We therefore studied the psycical-chemical properties of postmortem human brain proteinaceous material from gray matter of the frontal cortex. High molecular weight, virtually insoluble protein was isolated from nine autopsy samples. Three of these were from posthumously diagnosed Alzheimer diseased cases with no other evident cause of death, the other six were from non-neurologic cases in various age groups. The samples have been analyzed by microcalorimetry. Artificial aging has been caused by chemical crosslinking hin protein samples from young brains. All the samples have been tested for content of strongly bound aluminum. The results are preliminary and the tests continue.

RESULTS

Yield of protein. The yields of postmortem human brain proteinaceous material after extensive extraction with dimethylsulfoxide are collected in Table 1.

Table 1. Yields of insoluble protein in wt-% of original tissue.

Age	Yield	Age	Yield
67	70	30	13
68	12	28	12
63 Alzheimer	11	25	15
69 Alzheimer	17	17	9
69 Alzheimer	16		

Swelling tests. Protein samples were swollen in water for a week. The swelling ratio Q = (wt of swollen sample) / (wt of dry protein) as a function of age is plotted in Figure 1. The swelling ratio is highest in old age samples, young sapmles and Alzheimer samples are similar and swell much less.

Thermodynamic analysis. The details of this method have been published elsewhere *(7,8)*. The amount of non-freezable bound water

in protein : water samples was calculated from differential scanning calorimetric measurements. Results from these determinations are plotted in Figure 2 as a function of protein concentration in the sample. The ages of the patients are indicated in the diagram. There are marked differences between proteinaceous material from the young and from the old age group.

The Alzheimer samples form intermediate cases. The differences are probably due to conformational differences between the samples and to differences in molecular weight distribution. The results support a hypothesis of irreversible accumulation of rigid crosslinked polymers during aging of human brain cells. It does not support the idea that the neuropathologic distinction between normal human brain aging and Alzheimer's disease is essentially quantitative *(9)*. Deficiencies in protein synthesis have been reported to occur in Alzheimer's disease *(10,11)* and our results seem to correlate well with these findings.

«Artificial aging test». In order to test whether chemical crosslinking alone can alter the properties of a protein sample from young brain to correspond to those of an old age sample, in analogy with the general concept by Björksten et al. *(2)*, we reacted protein from a 17 year old brain with potassium persulfate and glutaric aldehyde respectively. Both are well known as crosslinkers for proteins and model proteins *(7, 12)*. All tests showed a decrease in the amount of non-freezable bound water with increasing crosslinking. The reaction conditions for glutaric aldehyde were adjusted to synthesize a product physically as similar as possible to old age samples. The results of estimates of the amount of non-freezable bound water in such a sample are seen in Figure 3. On the other hand such «artificial aging tests» required application to the isolated protein, otherwise the presence of e.g. soluble proteins and lipids does deplete the reagent.

Analyses for aluminum. The role of aluminum in aging and in disease has been discussed in the literature *(9)*. It has been suggested that Alzheimer's disease may be the result of a neurotoxic effect of intranuclear aluminum *(14)*. It therefore seemed of interest to test the aluminum concentration in the isolated protein samples. This would reveal whether the aluminium becomes more strongly bound with age and/or in Alzheimer's disease. We could find no evidence for enhanced aluminum values in the original tissue samples compared to what has been reported in the literature. Brain tissue samples were extracted in three different solvent systems ; aqueous sodium chloride, ethanol and dimethylsulfoxide respectively. Results are collected in Table 2.

In all cases the extraction removes >> 99.5 % of the aluminum originally present in the tissue. Dimethylsulfoxide is the most efficient solvent for the aluminum compounds. We find no significant difference in aluminum concentration in the samples from normal brains and from our Alzheimer diseased cases. We confirm the findings of previous researchers that a larger portion of aluminum remains unextractable in the brain tissue in samples from the older age group. This is related to age rather than to disease. The aluminum was determined on samples dissolved in nitric acid using a atomic absorption spectrometer equipped with a graphite furnace. The method is decribed elsewhere *(15)*.

Table 2. Aluminum concentration in postmortem human brain samples extracted with aqueous sodium chloride and dimethylsulfoxide respectively.

Controls extrated with		Age	Mean Al content, ng/g tissue
water		67	5.25
DMSO		67	3.08
water		68	7.32
DMSO		68	2.59
water		30	3.71
DMSO		30	1.30
water		28	2.65
DMSO		28	1.56
water		25	3.05
DMSO		25	1.24
water		48	3,53
DMSO		48	0.95
water	Alzheimer	69	3.97
DMSO	Alzheimer	69	3.47
water	Alzheimer	63	7.25
DMSO	Alzheimer	63	1.99
water	Alzheimer	69	3.65
DMSO	Alzheimer	69	1.07

DISCUSSION

Differential scanning calorimetry provides a convenient method to estimate the extent of crosslinkage in macromolecules ; applicable to insoluble proteinaceous material from human brain. Assuming a constant increase in random, «accidental» crosslinkages as time progresses, it is possible to estimate the approximate age of the insoluble deposits. The insoluble macromolecular material from Alzheimer brains thus contains a large portion of more recent material than the age matched controls. This portion possibly dates from the onset of the disease.

One possible explanation may be as follows : A neuron requires a protein Px. Due to a flaw in the synthesizing organelle this produces a slightly different, useless, protein Py. The nucleus of the neuron only understands that it did not get the Px, ordered and so repeats the call for «Px». The synthesizing organelle does not understand that its process is now faulty and so produces more and more Py in response to increasingly frantic calls for Px. This continues until the neuron is choked with useless Py.

If this should indeed be the true mechanism involved it should be possible to isolate enough Py to determine its difference from the Px and perhaps advance our knowledge to the point where either the cure or the prevention becomes apparent.

Aluminum is not a factor in the isolates from any of the Alzheimer cases we have studied. Its increase with age, though slow, allows us to extrapolate that aluminum will be a serious threat to those who have survived all other hazards.

Figure 3 shows that the addition of either one of two very different types of crosslinking agent (glutaric aldehyde and potassium persulfate) to the isolated macromolecular proteinaceous material from a young person, changes the curve to match the corresponding curve for an old person. This confirms that the differential scanning calorimetry is useful as a test for the crosslinkage density in proteinaceous macromolecules.

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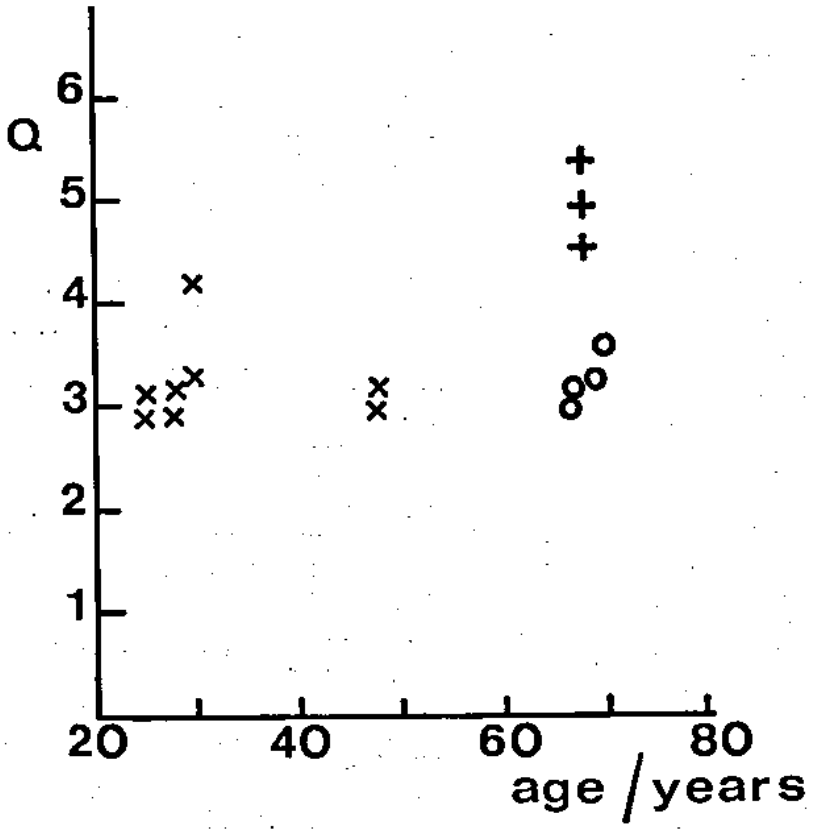

CAPTIONS TO FIGURES

Figure 1. The swelling ratio Q as a function of age in isolated postmortem human brain protein. × and + normal cases, and o Alzheimer diseased cases.

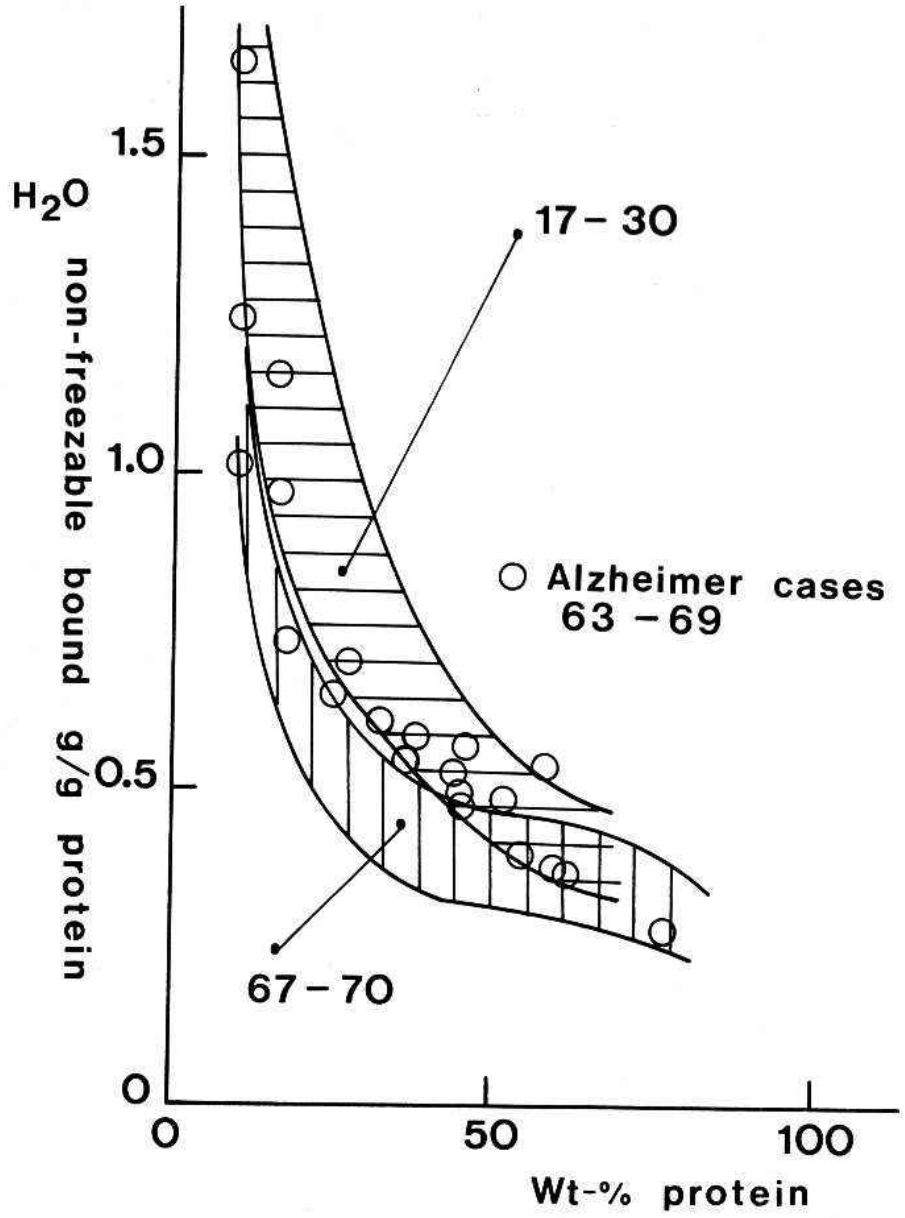

Figure 2. Amount of non-freezable bound water as a function of protein concentration in samples of postmortem human brain protein. The ages of the patients are indicated in the digram. Shaded areas are from non-neurologic samples, o from Alzheimer samples (8).

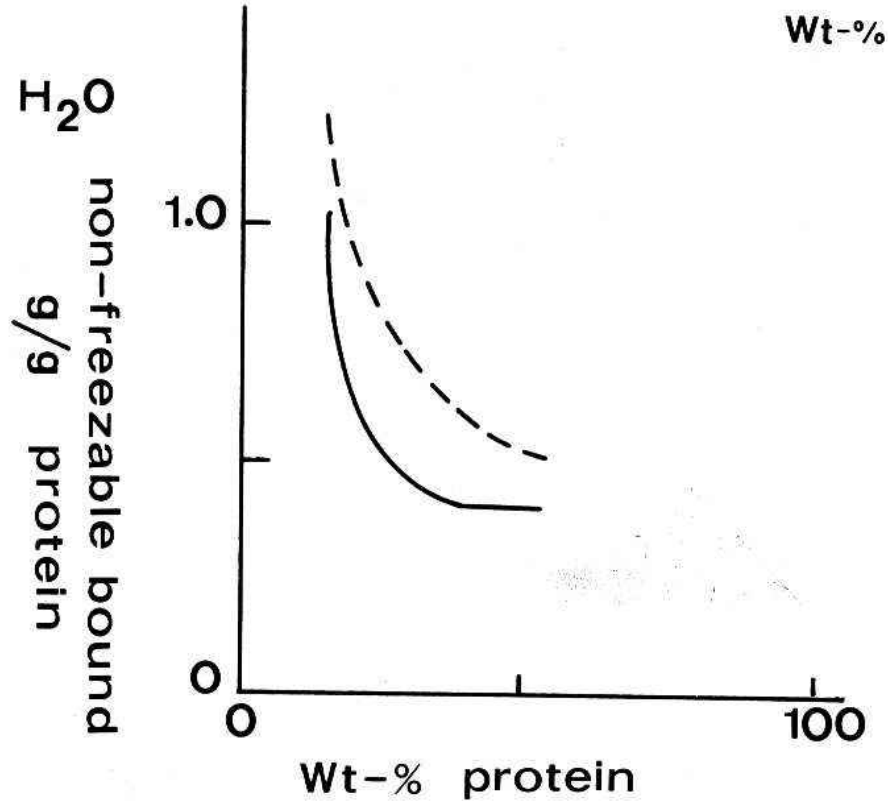

Figure 3. Amount of non-freezable bound water as a function of protein concentration in postmortem human brain from a 17 year old case, dotted line. Same sample after«artificial aging test», solid line.