high-altitude hypoxia (hypobaric hypoxia), asphyxic anoxia etc.

Similar models have been widely used for many years in the development of drugs for the treatment of cerebrovascular diseases[26-28].

At the present time the greatest progress in the therapy of cerebrovascular insufficiency has been achieved with haemodilution, osmotherapy, platelet aggregation inhibitors and cardiovascular treatment. Drug treatment of senile dementia is still in a preliminary state. Recent findings of fundamental pathogenetic mechanisms of dementia have increased our knowledge to the point where the experimental pharmacologist can make a start by devising suitable models for the development of effective drugs.

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Aging of connective tissues

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It is a particularly sad but fascinating task to write about the aging of connective tissues for this memorial issue devoted to Professor Verzár. His historical experiments on the aging of rat tail tendon opened up an important new research area on the molecular and cellular mechanisms of the aging of connective tissues. I had the privilege of meeting Professor Verzár several times during the last twenty years and these conversations and contacts were the prime incentive for our own work in the aging of connective tissues. Having been born in the same country as Professor Verzár and having left it at about fourty years later, my experimental approach may have been inspired by a similar basic biological-medical culture. I wish therefore to consider my work in general, and this article in particular, as a special tribute to the memory of Professor Verzár.

The state of the art in 1980

Our knowledge about the aging of connective tissues can schematically be divided in two distinct areas. The first one concerns the regulation of the biosynthesis of matrix macromolecules such as the collagens of different genetic types, the proteoglycans, elastin and of structural glycoproteins. The macromolecules belonging to these four families of intercellular matrix substances are synthesized in well-defined proportions by the differentiated mesenchymal cells and associate in specific patterns which can be recognized in every differentiated tissue.

The second aspect of aging research in the connective tissue area concerns the post-translational modifications as well as the catabolism of these macromolecules. These modifications are related to the age-

dependent modifications of their structure and function. This second aspect was studied in much greater detail as a result of Verzár's demonstration of a conspicuous change in the physicochemical properties of collagen with aging.

a) *The biosynthesis of matrix macromolecules as a function of age*

What is well established today as a result of experiments carried out in vitro on cell cultures or in vivo with animals of different ages or ex vivo using tissue culture conditions for freshly excised tissues surviving in vitro is that the relative rates of biosynthesis of matrix macromolecules change with age[1,2]. Everything happens as if there would be a well-defined program of biosynthesis concerning every type of macromolecule, this program being different for every differentiated mesenchymal cell type and varying also with the exact place this cell occupies in the organism.

These regulatory mechanisms concern a relatively large number of macromolecules such as the different collagen types, all the different proteoglycans, elastin, and structural glycoproteins such as fibronectin, laminin and others[3]. In order to account for all the different collagen types, proteoglycans and glycoproteins, we have to assume the existence of about 30 different structural genes or more, coding for the matrix macromolecule peptide chains and probably as many regulatory genes if not more, which determine the hormonal and genetically dictated relay mechanisms regulating the relative rates of the expression of these structural genes. For this part of the regulatory mechanisms, workable hypotheses can be elaborated and experiments are actively being carried out in several laboratories to test them. Among such experiments are those of Crystal et al. on the role of cAMP-levels on collagen biosynthesis[4] and those concerning the action of diabetes on the biosynthesis of collagen[5,6] to mention only a few.

There is however the age dependence of the expression of these genes, the so-called 'program of biosynthesis' where we still do not have good experimental models which could guide us in the elaboration of workable hypotheses. The change of the relative rates of the expression of these structural genes with age in the differentiated mesenchymal cells is well demonstrated but its mechanism is still unknown[7]. None of the so-called 'aging theories' sheds any specific light on this important problem.

b) *Degradation of matrix macromolecules, effect of age*

Much more is known about the catabolism of matrix macromolecules than about the regulation of their rate of synthesis with age. It has been shown in particular that the fibrous proteins such as collagen and elastin can undergo degradative processes in certain tissues which are age-dependent. This was particularly well demonstrated in the case of elastic fibers of the arterial wall and in the skin[8-10]. It could be shown using biopsy techniques with specific staining procedures and radioactive labeling methods that the rate of synthesis of cross-linked elastin decreases with age[1,2,7,10].

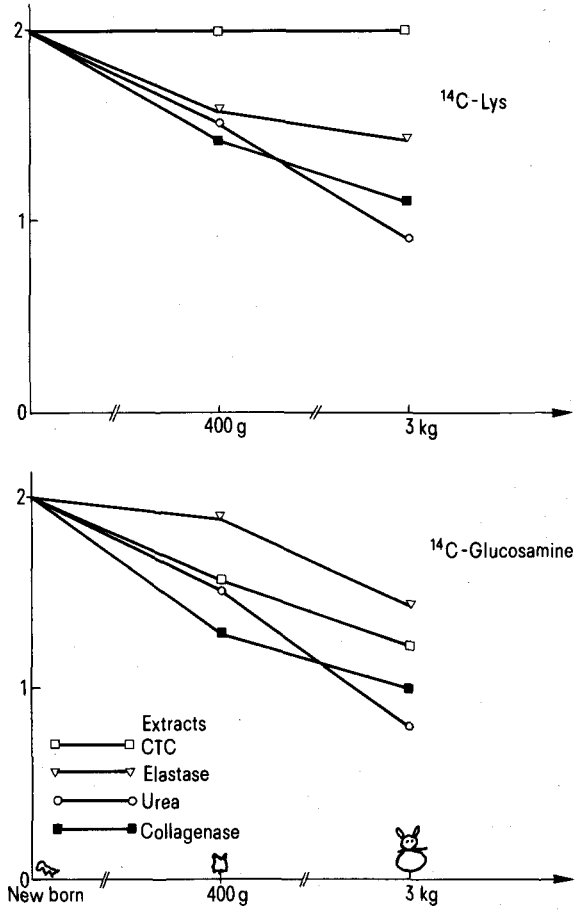

Figure 1. Decrease with age of the rate of incorporation of ^{14}C-lysine and of ^{14}C-glucosamine in the macromolecules of the rabbit aorta. Aorta slices from inborn rabbits (Strain Alfort; Prof. Théret media only) were incubated in organ culture conditions for 6 days in Petri-Greiner dishes in 5 ml MEM-medium supplemented with 10% foetal calf serum, streptomycin and penicillin, as described[1], in the presence of 20 µCi ^{14}C-lysine or ^{14}C-glucosamine. After incubation and washing the slices were extracted sequentially in 1 M $CaCl_2$-tris citrate (CTC-extract), with collagenase, then with 8 M urea - 0.1 M mercaptoethanol and the final residue was solubilized with cryst. Pancreatic elastase. Radioactivity was determined in the extracts and expressed as cpm/mg DNA. These values reflect the relative rates of incorporation of the above precursors in the macromolecules present in the extracts (CTC-extract: all soluble proteins and glycoproteins, collagenase-extract: the insoluble polymeric collagene, urea extract: structural glycoproteins and stroma bound proteoglycans; elastase extract: fibrous elastin. The figure represents on the ordinates the log percent of these incorporation values related to those obtained with the newborn aortas (= 100%) On the abscisse the age of the animals expressed as their weight (400 g young rabbits and 3-3.5 kg adult rabbits). Notice the different rates of decrease of the rate of incorporation for the different families of macromolecules. These curves reflect the age dependent 'program' of biosynthesis of matrix macromolecules[1,2,7].

Figure 1 shows the results of such an experiment carried out with rabbit aorta organ cultures. This experiment shows that not only for elastin but for all the matrix macromolecules there was a decreased rate of synthesis with age. This was accompanied by an increased rate of degradation with age of elastin. Some recent data are available on the mechanism of this increasing degradation of the elastic fibers with age. First of all, the decreased desmosine content can be the result either of a decreased rate of synthesis (for instance decreased activity of lysine-oxidase) or a decreased availability of tropoelastin and also of an increased rate of elastolysis. This latter case was demonstrated by isolating elastolytic proteases from the arterial wall itself[11,12] and by demonstrating that their activity increases with age[10].

It was shown by Bouissou and co-workers that the rate of disappearance of elastic fibers is parallel in the human aorta and in the sub-papillary layer of the skin[8,9]. The mechanism of this degradation of skin elastin was partially elucidated in our laboratory in the last few years in collaboration with W. Hornebeck, D. Brechemier, C. Frances and G. Bellon by isolating an elastolytic protease from fibroblasts cultured from human and rat skin and from human vulva[12,13]. Human vulva was shown to be rich in elastic fibers, and fibroblasts obtained from this tissue were shown to be especially rich in an elastolytic protease[13]. It appears therefore that the increased rate of elastolysis with age may be due to an increased rate of synthesis and secretion of the elastolytic proteases by several cells.

Another factor which appears to influence this process is the increasing rate of saturation of elastic fibers with lipids. It was shown in our laboratory and also by Franzblau, Kagan, Kramsch and Hollander in Boston, that the increased saturation with lipids of elastic fibers increases their susceptibility to elastolytic proteases and decreases their rheological efficiency[14,15]. Although similar detailed studies are not yet available for the collagenases and hydrolases attacking proteoglycans, it is probable that at least part of the age-dependent decrease of some of these proteins in several tissues could be attributed to a similar age-dependent increase of lytic enzyme activity. The fragility of lysosomes could depend at least in several tissues on age as well as on the hydrolases content.

c) *Post-translational modifications of connective tissue macromolecules as a function of age*

One of the well documented post-translational modifications of matrix macromolecules concerns the phenomenon demonstrated by Verzár decades ago. He showed that the appearent cross-linking of collagen increases with age; at least this hypothesis was forwarded by Verzár to explain the increasing rate of resistance of collagen fibrils to heat denaturation with age.

More recent studies, carried out mainly by Bailey and co-workers in Great-Britain, show that this is due probably to a change in the quality more than in the quantity of cross-links (mainly the reducible cross-links) of collagen fibers[16]. This would not exclude the possibility of an increased reticulation of certain collagen fibers although this point has not yet been documented in a conclusive fashion.

It has been suggested by several authors that a similar increase in non-natural cross-links may be produced by lipid peroxidation in collagen and elastic fibers. The increase with age of the fluorescence of elastic fibers (fig. 2) may well be due to such a phenomenon. Although detailed knowledge is still lacking on this point, recent results obtained by Banga et al. and by

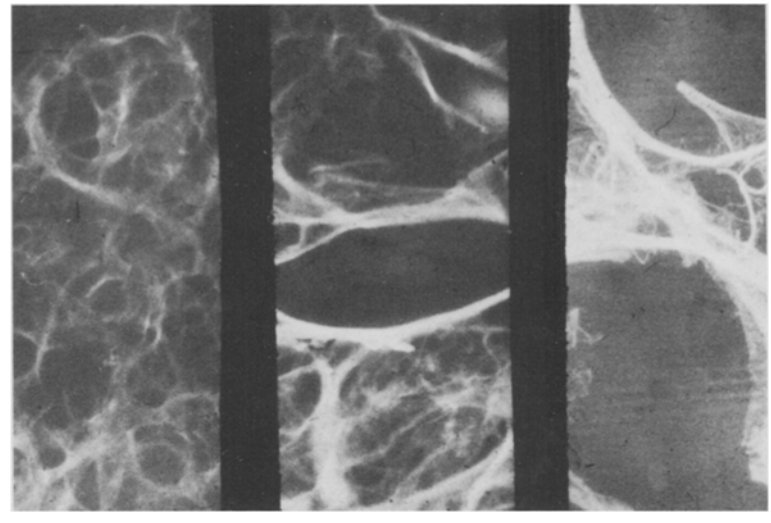

Figure 2. Increase with age of the autofluorescence of lung elastin, (photo due to the courtesy of Dr. Clara Szemenyei, IId., Dept. of Pathology University of Budapest). Elastin in the human lung of a new born (left), adult (middle), and old (70 years) individual.

Deyl et al. point to the probability of such a process[17,18].

The possible post-translational modifications which may occur in the biosynthesis of proteoglycans are less well documented. It appears that the size of the proteoglycan aggregates may change with age and this may be due either to a decrease of hyaluronic acid or possibly to the modification of the proteoglycan core protein itself.

It is hoped that more information will be available in the near future on these important matters.

Conclusion

The brief summary of our present knowledge on the aging of intercellular matrix macromolecules shows the progress which has been made since the original important discovery of Verzár, but it also points out the very considerable gaps which still have to be filled by continued research efforts in this theoretically and practically important area. It is no secret to anyone that most of the disabling and killing diseases of advanced societies concern connective tissues: arteriosclerosis, diabetes, pulmonary obstructive lung diseases, osteoarticular diseases and cancer itself are all age-dependent, so-called aging diseases. The interaction between intercellular matrix and cancer cells plays an important role in the spreading of the tumors. For these other major diseases, the direct involvement of intercellular matrix is well documented. It is therefore hoped that a better grasp of the basic mechanisms involved in these diseases will help us to understand the difference between pathology and aging per se.

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Conclusion: What is the future of experimental gerontology?

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Fritz Verzár's first published articles on aging research were abstracts of three papers presented at the Third International Congress of Gerontology in London, in 1954[1-3]. They concerned adaptation in old age, using as examples compensatory hypertrophy of the kidney and adaptation to low oxygen pressure in the rat. In 1955, his last full year as Professor of Physiology at Basel University, when he was 69, a brief communication in Experientia[4] on 'thermoelastic contraction of skin and nerves' marks the beginning of an astounding period of another 20 years of scientific productivity in an entirely new field, at an age when most of us are or hope to be in retirement. Both of these major topics, loss of adaptation at the physiological and cellular levels, and structural aging of connective tissue, are still central issues of experimental aging research today, as the six reports presented here demonstrate.

Verzár always insisted that only a multi-disciplinary approach would be adequate in attacking the problem of why and how we (and animals, and plants) age. In each of the present reports this point is also evident and insisted upon by the authors. Thus, aging of the immune system (Fabris[5]) cannot be discussed without a detailed understanding of neuroendocrine functions. Neuroendocrinology is also the bridge between the biology of the aging brain and the regulation of cellular genetics (Frolkis[6]). The aging of cells in vitro