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.

Aging of connective tissues

by L. Robert

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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 Verzar. 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 Verzar 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 Verzar 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 Verzar.

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. Everything happens as if there were 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. 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 and those concerning the action of diabetes on the biosynthesis of collagen 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. 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. 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.

Figure 1. Decrease with age of the rate of incorporation of [14C]lysine and of [14C]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, in the presence of 20 μCi [14C]lysine or [14C]glucosamine. After incubation and washing the slices were extracted sequentially in 1 M CaCl2-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 collagen, 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.