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Aging and Cell Division

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One of the most impressive features of aging in diploid fibroblasts in vitro is the decline in saturation density expressed by curves representing the suvival of cell lines with a finite lifespan. This parameter is common to cell populations of different species, though they may otherwise differ in regard to their aging process.

This decline in saturation density must be due in part to an increase in cell size which is always associated with aging in vitro. The decrease in maximal cell density, however, cannot only be due to the increase in cell diameter, since in chick fibroblasts the first change in size occurs well before the saturation density starts declining.

An alternative explanation is that old cells are more sensitive to cell crowding. Several experimental facts support this idea:

1. When one compares cells obtained from adults with cell populations obtained from embryos one finds that adult cells in phase II have a pattern of DNA synthesis and a saturation density comparable to embryonic cells in Phase III; 2. Young human fibroblasts have a prolonged G_2 period only when the cultures become crowded; a prolongation of the G_2 period however, is already found before crowding in phase III human fibroblasts as well as in phase II adult fibroblasts; 3. Ionizing radiation which accelerates aging in vitro, has an immediate effect in decreasing the saturation density; 4. cortisone, which prolongs the lifespan of human fibroblasts, increases the saturation density.

If old cells are more sensitive to cell crowding, the mechanisms responsible for the arrest of cell division when cells become crowded would also be those that are disturbed during aging and which cause the loss of the division potential. Several authors have shown that it is a decrease in the synthesis of ribosomal RNA which causes the arrest of cell division when cultures reach saturation density. Hence, if our hypothesis is correct, it is a decreased efficiency in the synthesis of ribosomal RNA which is responsible for the loss in division potential.

Several experiments indicate that aging of fibroblasts in vitro is associated with changes in RNA synthesis. CRISTOFALO[1] has shown that the total RNA increases in cultures of human embryonic fibroblasts when they enter phase III. Our results have shown that the rate of RNA synthesis and transfer of RNA from the nucleus to the cytoplasm is decreased in cultures of human embryonic fibroblasts entering the late phase of their lifespan in vitro. We have also measured RNA synthesis during aging in chick fibroblasts and found a significant decrease in the total RNA synthesis which coincides with a significant fall in saturation density. We also measured RNA synthesis in human fibroblasts carried in the presence of cortisone, and found that they synthesize more ribosomal RNA than the control cultures. We suggest that this action by the hormone on ribosomal RNA synthesis results in an increased production of protein which would compensate to a certain extent for the accumulation of faulty molecules.

If aging in vitro is due to a disturbed synthesis of ribosoma RNA, it would agree with our previous data which suggested that the mechanisms responsible for the decreased growth potential of human fibroblasts are located in the periods preceding DNA synthesis and mitosis rather than in these periods themselves.

[1] V. J. CRISTOFALO, *Aging in Cell and Tissue Culture* p. 83–119 (Plenum Press, New York 1970), p. 83.
[2] L. HAYFLICK, Expl. Cell Res. *37*, 614 (1965).
[3] A. MACIEIRA-COELHO, Eur. J. clin. Biol. Res. *17*, 925 (1972).
[4] A. MACIEIRA-COELHO, Experientia *22*, 390 (1966).
[5] A. MACIEIRA-COELHO, p. 121–132 in *Aging in Cell* and Tissue Culture (Plenum Press, New York 1970), p. 121.
[6] A. MACIEIRA-COELHO and L. LIMA, Mech. Age. Devl. *2*, 13 (1973).
[7] A. MACIEIRA-COELHO, J. PONTEN and L. PHILIPSON, Expl Cell Res. *42*, 673 (1966).

Young and Old Rats. ATP, Alkaline Phosphatase, Cholesterol and Protein Levels in the Blood; DNA and RNA Contents of the Liver. Regulation by an Aqueous Thymus Extract

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It is well-known that the involution of the thymus goes along with aging; since this involution also entails a general decline of immunocompetence, and different authors have suggested that aging could depend on an immunological disorder, PANTALOURIS[1] has postulated the hypothesis of a direct correlation between the thymus and senescence. These observations have led us to study the action of an aqueous thymus extract[2], on a few hematic parameters (ATP, cholesterol, protein levels and alkaline phosphatase) and on the nucleic acid contents of the liver of old rats (26 months). In addition the incorporation of H^3-uridine in the nucleic acids of the liver has been studied.

The determinations conducted on blood samples demonstrated in the old rats a greater total protein and cholesterol content and a smaller ATP content as compared to young rats (2 months) which confirms previously published data; the administration of aqueous thymus extract to old rats causes a decrease in proteins and cholesterol and an increase in ATP bringing the relative levels within the range of those observed in the young rats.

In old rats the aqueous thymus extract causes a decrease in the level of activity of serum alkaline phosphatase as compared to untreated animals of the same age; this decrease can be compared to the data reported by others demonstrating an increase of alkaline phosphatase in senescence, with particular reference to pre-tumor conditions, although a direct relationship between the level of alkaline phosphatase und age was not demonstrated.

As to what concerns the nucleic acids of the liver, in old rats as compared to the young, a smaller content of DNA extract-

able at the polymerized state with sodium lauryl sulphate[3], was found. This fact, which has also been confirmed by a different method of extraction in NaCl, is not be referred to a decrease of the total DNA content of the liver with aging, but rather, as demonstrated by comparison with results obtained with the methods of SWINDLEHURST[3] and of SCHNEIDER, to a decrease of extractability, probably as a consequence of a different physical-chemical state of the DNA-protein complex. The aqueous thymus extract administered to old rats increases the hepatic DNA content extractable at the polymerized state, bringing it to the levels observed in the young rats. No significant variation of the hepatic RNA content was observed between old treated and untreated rats.

The experiments concerning the incorporation of H^3-uridine in the RNA and DNA of the liver of old rats, demonstrate that the aqueous thymus extract causes a considerable and significant decrease of incorporation values in DNA and a statistically insignificant decrease of incorporation values in RNA. The data regarding DNA radioactivity can be correlated to the results obtained by PRICE[4] according to which the template activity of DNA for DNA polymerase increases with age; this is interpreted by the author as an accumulation of defective chains of DNA with aging, as demonstrated also by PELC[5]. It is interesting to note that the values of incorporation into DNA demonstrate a detectable activity of reduction of the ribonucleotides in the liver of old animals.

The preceeding data provide evidence of activity of the aqueous thymus extract expressed mainly in the control of DNA synthesis in the liver of old rats and in the increase of DNA extractibility, probably related to modifications of interactions in the DNA-protein complex. This regulatory activity exerted by the aqueous thymus extract on the nucleic acids of the liver is in agreement with our previous results concerning the effect of the same extracts on the metabolic activities of *E. coli*[6].

The results obtained in this work appear to bring biochemical data in support of the hypothesis of a direct correlation between thymus and senescence.

1 E. M. PANTALOURIS, Expl Geront. *7*, 73 (1972).
2 D. AMICI and G. L. GIANFRANCESCHI, Ital. J. Biochem., in press.
3 M. SWINDLEHURST, S. J. BERRY and W. FIRSHEIN, Biochim. biophys. Acta *228*, 313 (1971).
4 G. B. PRICE, S. P. MODAK and T. MAKINODAN, Science *171*, 917 (1971).
5 S. R. PELC, Expl Geront. *5*, 217 (1970).
6 G. L. GIANFRANCESCHI and D. AMICI, Ital. J. Biochem., in press.

Chronic Lathyrism, an Experimental Model of the Ageing of Human Connective Tissue

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Rats, submitted to a prolonged β-amino-propionitrile poisoning for 12 weeks, showed lesions of the connective tissue and of the fibroblasts. In the skin, collagen tissue was dislocated and broken into fragments, the elastic tissue disappeared, the fibroblast was vacuolized and presented evidence of injury. These lesions were comparable to those observed in human skin during ageing. In the aorta, this chronic lathyric poisoning brought about a dislocation of the elastic framework, an increase of the interstitial tissue and a lack of differentiation of the myocyts. These lesions correspond to the changes observed during human arteriosclerosis[1].

1 M. JULIAN, M. TH. FABRE and H. BOUISSON, Path. Biol. *19*, 389 (1971).

Histone Phosphorylation after Partial Hepatectomy in Young and Old Rats

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It has been seen that phosphorylation (like methylation and acetylation) of nuclear basic proteins is related with the control of gene expression. The diminished positive charge of phospho-histones could weaken their association with DNA, facilitating its accessibility. Phosphorylation could also play a role in the transport of histones from the cytoplasmic sites of synthesis into the nucleus. As a model of some sort of an in vivo synchronized culture of mammalian cells, we have chosen the regenerating liver after partial hepatectomy[1], investigating eventual changes of phosphorylation of the histones during aging.

Rats were injected with ^{32}P at various times after hepatectomy and sacrificed 1 h later. Histones were extracted from isolated liver nuclei[2,3], fractionated by disc-electrophoresis on polyacrylamide gels into F1, F3, F2B + F2A2, F2A1[4], and counted in a liquid scintillator counter using the Čerenkov effect.

A direct comparison of the results from young and old rats at various time after hepatectomy is shown in the Table. The

Histone fractions		Time after hepatectomy (hr) 4,5[a]		6,5		9,0	18,5		21,1	24,5	
F1	young	50		100		76	160		330	126	
	Old	15	−70%[b]	25	−75%		44	−72%		44	−65%
F3	y	54		74		30	88		130	72	
	o	50	− 7%	115	+55%		49	−43%		62	−14%
F2B	y	84		154		10	150		210	80	
F2A2	o	75	−10%	185	+20%		87	−42%		157	+96%
F2A1	y	35		108		18	100		170	76	
	o	12	−67%	55	−49%		40	−60%		42	−45%

[a] Specific activity in cpm/mg.
[b] Difference in percent of the specific activity from young to old rats.

data from young animals show 2 distinct peaks for all 4 histone fractions, one around 6 h, another around 20 h after hepatectomy; the second one being coincident with the major rate of DNA synthesis. The first peak could be interpreted as a preliminary decrease in the histone net charge allowing the DNA double helix to loosen up and duplicate.

Once the DNA is duplicated, it must be covered by newly formed histones arranged in a suitable position along the helices. Because of the strong positive charge of histones, incorrect interaction difficulty to reverse might occur[5]; therefore diminished charge is very important in the formation of the correct histone-DNA complex (second peak).

Preliminary data from old animals show a similar trend, but all fractions, except F2B + F2A2, have specific activities less than those of young animals. The less expressed phosphorylating rate of the histones in old animals could be the consequence of less efficient regulatory factors, thus causing first a reduced accessibility of the DNA template and second an eventually disturbed rearrangement of the histone-DNA complex.

The different behavior of the various histone fractions, particularly F2B + F2A2, could be explained as an expression of the different role which to each of the histone fractions is ascribed in binding to the DNA.

[1] G. M. Higgins and R. M. Anderson, Arch. Path. *12*, 186 (1931).
[2] G. Blobel and V. R. Potter, Science *154*, 166 (1966).
[3] E. W. Johns, Biochem. J. *92*, 55 (1964).
[4] S. Panyim and R. Chalkley, Arch. Biochem. Biophys. *130*, 337 (1969).
[5] A. J. Louie and G. H. Dixon, Proc. natn. Acad. Sci., USA *69*, 1975 (1972).

Recent Developments on Protein Synthesis in Ageing Cells

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A brief survey will be given on some recent developments in the study of age-related alterations in the mechanism of protein synthesis. The Orgel hypothesis has provided the stimulus for several groups of investigators to study the appearance of the postulated altered, biologically inactive proteins in different biological systems. Evidence has been obtained that with increasing chronological age, random errors are introduced, probably at the translational level as postulated by Orgel, resulting in an increasing accumulation of non-functional enzyme molecules. The altered proteins have been detected by immunochemical and biophysical methods. The use of aminoacid analogues has permitted the experimental production of 'aged' error-containing proteins, resulting in an acceleration of the ageing process in vivo. Further research now centers on the isolation, purification and sequence analysis of biologically inactivated enzyme proteins from various animal systems. In this context, the use of cell cultures of animal and human origin has proved a very valuable tool. A new aspect has been introduced by the use of nematodes, lower animals with short lifespan and entirely composed of postmitotic cells. These can easily and inexpensively be cultured in large numbers permitting biochemical work. An ageing process with the appearance of biologically inactive enzyme molecules has been demonstrated in these animals, and preparation and analysis of these altered enzymes is now in progress in two research groups. This is hopeful new departure for experimental gerontological research.

H. Gershon and D. Gershon, Proc. natn. Acad. Sci., USA *70*, 909 (1973).
H. P. von Hahn, Adv. Geront. Res. *3*, 1 (1971).
H. P. von Hahn, Proc. 9th Intern. Congr. Gerontol., Kiev 1972, Vol. 1, p. 33.
B. J. Harrison and R. Holliday, Nature Lond. *213*, 990 (1967).
R. Holliday and G. M. Tarrant, Nature Lond. *238*, 26 (1972).
C. M. Lewis and G. M. Tarrant, Nature, Lond. *239*, 316 (1972).
L. E. Orgel, Proc. natn. Acad. Sci., USA *59*, 517 (1963).

Influences on the Structural Stability of Collagen

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By extraction of water from tendon fibres with hypertonic salt solutions, as 5 *M* $NaClO_4$, one increases the tension, as can be registered by isometric tensimetry[1]. This reaction, which was formerly called 'chemical contraction', is reversible[2]. It is now explained as an influence of interhelical water molecules which act as a 'clutch' on the tension produced by crosslinks. The tension by $NaClO_4$ disappears if the fibre is immersed in (physiological) 0.85% NaCl and can immediately be produced again by 5 *M* $NaClO_4$.

The present explanation of crosslinks[3] is that they are 'attributable to the presence of two aldehyde-containing amino acids', which reach with other amino acids in collagen, as with lysinonorleucine of a reduced Schiff base product of lysine with some aldehyde. The tension of the fibre is increased by 0.035 *M* formaldehyde and also this can be reversed. In old animals' tendons, this is possible several hundred times.

Methionin, the amino acid which contains SH groups in 0.5–0.8% in collagen[4] inhibits the tension of the fibre and this cannot be produced with hypertonic salt solution again. Young animals' fibres are inhibited by 0.0007 *M* solutions. Old animals' tendons reach with somewhat higher concentrations. Cystein has a similar action. Methionin is known for its 'active methylene transfer' in different metabolic processes. It seemes possible that it interfers with aldehyde-groups and Schiff base is produced and the crosslink is destroyed. The water solubility of collagen is increased and in old fibres the larger percentage of insoluble collagen is diminished by methionin.

In vivo experiments, which are still being continued, showed that injection into living tendons decreases tension, which, however, is corrected by an antagonistic tension production.

[1] F. Verzár, Int. Rev. connect. Tissue Res. *2* (1964).
[2] F. Verzár, Z. physiol. Chem. *335*, 38 (1963).
[3] M. L. Tanzer, Science *180*, 561 (1973).
[4] K. A. Piez, E. A. Eigner and M. S. Lewis, Biochemistry *2*, 58 (1963).

Biogenesis, Maturation and Aging of Elastic Tissue

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The molecular mechanism of the biogenesis of elastic fibres and elastic laminae involves the coordinated synthesis of at least two types of macromolecules of the intercellular matrix: structural glycoproteins and proelastin. Morphologically the former kind was identified to microfibrils. The structural glycoprotein-microfibrils form the scaffolding onto which proelastin molecules seem to adhere by electrostatic forces. After this initial positioning of proelastin by microfibrils lysine oxydase transforms some of the ε-amino residues of lysine into δ-amino adipic semi-aldehyde. The crosslinking process

leads to the formation of lysinonorleucine, desmosine, isodesmosine. Using labelled precursors (^{14}C-lysine, ^{3}H-glucosamine etc), we investigated the rate of biosynthesis of macromolecular precursors of elastic tissue. The relative rates of synthesis of these two constituents (structural glycoproteins and proelastin) changes continuously from birth through maturation and aging. Effective elastogenesis requires well defined proportions of these macromolecules as well as an efficient crosslinking process. It appears possible that after maturation the proelastin synthesis is still going on but no efficient crosslinking exists. Therefore mature elastin appears not to have a measurable turnover. In vitro incorporation studies (4 h incubation or culture conditions up to 24 h incubation) supported these conclusions showing active incorporation of ^{14}C-lysine into elastin. In aging elastic tissue as well as in atheriosclerotic lesions, a relative increase in glycoprotein synthesis could be observed explaining the morphological findings reported by several pathologists in such conditions, as well as the change in the ratio of polar to apolar aminoacids in partially purified elastin preparations. At the same time and through the action of tissue elastases a partial degradation of elastic fibres occurs resulting in an increased extractibility of partially crosslinked elastin. This was demonstrated by the attack of such pathological elastin samples by trypsin to which normal elastin resists. The trypsin lysates did contain desmosine crosslinks.

These processes can explain the slow release of elastin peptides in the circulation entertaining an active autoimmunization process which can lead to the formation of autoantibodies to elastin. Their role in the pathogenesis of the atherosclerotic diseases was demonstrated by the creation of arteriosclerotic lesions by immunizing rabbits with elastin.

Isometric Tension and Solubility of Collagen in Dorsal and Ventral Tail Tendons of Rats

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STEGMAN'S method of hydroxyprolin estimation was used. Male rats of 1 month, 3 to 5 months and 15–16 months were compared. The results of mean values and standard differences were compared by the *t*-test, as the Table showes. The dorsal tendons have significantly higher values in all 3 age groups.

Tension measurements were then made on 30 ♂ rats of 6 month of age. The influence of 5 *M* $NaClO_4$ was tested by the method used in former paper. This was modified and graphically registered in g per mg tendon. Ventral tendons gave 10.13 g ($S\bar{x} = 0.24$ g) and dorsal tendons 9.31 g ($S\bar{x} = 0.20$ g), $p < 0.0025$. Both, the measurements of solubility and tension may be explaned so that dorsal tendons collagen has less crosslinks. However at present it remains undecided whether this is inborn or caused by differences of usage.

1. It is concluded that spontaneous activity cannot be generally used as 'age parameter' (ASCHOFF 1957). 2. Obvious age differences of motoric activity are seen by producing complex behaviour activities and are then useful as age parameters. 3. Psychomotoric stimulants also act differently at different ages and it may be useful to combine such tests with the former.

1 G. HOFECKER, P. MOSER and F. VERZÁR, Gerontologia *18*, 28 (1972).
2 F. VERZÁR, Z. physiol. Chem. *335*, 38 (1963).

A New Method to Evaluate the Arterial Elasticity

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The method which is described in detail[1] is based on the principle that every tissue has a characteristic frequency which depends mainly on the elastic behaviour of the material. With the measuring technique described, it was possible to determine the frequency of superficial arteries. The radialis artery is especially well suited. The results are reproducible and are not influenced by haemodynamic changes. The method is suitable to test the elastic behaviour of arterial walls and to provide conclusions as to the arterio-sclerotic changes.

The mean values of vascular frequencies at different ages show a rise with aging. The correlation coefficient, $r = 0.83$ is highly significant. The vascular frequency of physically inactive persons is significantly higher than that of persons of the same age who have kept up athletic activity from youth to old age[2].

1 E. LANG, R. WEINZIERL, D. SCHILLING, M. HUBMANN and A. HULLARD, Acta geront. *1*, 17 (1972).
2 E. LANG, M. HUBMANN, A. HULLARD and W. FLÖGEL, Acta geront. *3*, 159 (1973).

Effect of Lymphocytes or Hormones on Ageing in Hypophysary Dwarf Mice[1]

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The influence of the lymphoid system on the ageing processes has been investigated by using as experimental animals 2 strains of hypopituitary dwarf mice (Snell and Ames). Congenital hypopituitarism causes in these mice reduced body growth, underdevelopment of the thymus and of thymus dependent functions and shortens the life-span of the animals to 4–5 months[2]. Death is preceeded in dwarf mice by certain signs, which normally are considered as aspects of ageing, such as greying and loss of hairs, cutaneous and subcutaneous atrophy, wrinkles and cataracts. The evaluations of some experimental parameters of ageing, such as ^{3}H-thymidine uptake in vivo, outgrowth of tissues explants and percentage of mitosis in tissue cultures, strongly suggest that the process of ageing in dwarf mice is precocious.

Percentage of soluble collagen

Age	1 month		3,5 months		15–16 months	
	Dorsal	Ventral	Dorsal	Ventral	Dorsal	Ventral
$\bar{x}$ (%)	63.69	59.27	45.20	39.60	16.63	12.34
$s\bar{x}$	2.41	2.16	1.91	1.52	1.70	1.41

Treatment of dwarf mice with 1 injection of lymphnode cells from normal littermates induces a delay in the appearance of ageing related symptoms and prolongs their life-span to 12–18 months[3]. Similar effects have been achieved by treating dwarf mice with 30 daily injections of growth hormone and thyroxine during the post-weaning period[3]. These data suggest that hormones are needed for the maturation and/or proliferation of some lymphoid cells, which can control the process of ageing.

[1] N. Fabris, W. Pierpaoli and E. Sorkin, Nature, Lond. *240*, 557 (1972).
[2] N. Fabris, W. Pierpaoli and E. Sorkin, Clin. exp. Immunol. *9*, 209 (1971).
[3] N. Fabris, W. Pierpaoli and E. Sorkin, Clin. exp. Immunol. *9*, 227 (1971).

A Biological Aging Test for the Central Regulation of the Estrous Cycle in the Rat. Its First Applications

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The hypothalamic sensitivity to estrogen increases with age in the castrated, previously cyclic, rat (Aschheim[1]). The same is true for the intact cyclic rat: its aptitude for pseudopregnancy (PP), triggered by the injection of estradiol benzoate at estrus, increases with age. This is the basis for a biological aging test of the central command of the estrous cycle. The curve which shows, as a function of age, the increasing proportion of PP triggered by the same amount of estrogen is S-shaped, like other aging curves. The increasing facility with age to release prolactin in response to an estrogenic stimulation manifests from puberty on. So, the rat can be tested during its entire period of cyclicity. The non-reproductibility of the test is about 6% and does not change with age. Sensitivity differs with strains. In the oldest age group, the injection of estrogen induces, in addition to PP, a second type of response: an estrus persisting for 10 to 30 days. On the basis of 1000 trials, this biological aging test for adaptability seems to be reliable. It is easy to perform in strictly physiological conditions, without mutilation of the animal. Its first applications to problems of experimentally modified aging of the hypothalamic regulation of the cyclic gonadotropic function are discussed. They concern hemicastration, multiparity and chronic treatment with oral contraceptives.

Long-term hemicastrated rats and multiparous rats are biologically older than their respective controls; rats treated during 4 months with ethinylestradiol, a synthetic progestin or the mixture of these 2 hormones are, after cevation of the treatments and the recurrence of estrous cycles, biologically younger than their controls[2,3].

[1] P. Aschheim, Colloques Nationaux du CNRS, No. 927: Neuroendocrinologie 363 (1970).
[2] P. Aschheim, Gen. comp. Endocr. *18*, 573 (1972).
[3] P. Aschheim, Annls Endocr. *32*, 264 (1971).

Hypophysectomy and Aging: Primary or Secondary Ovarian Senescence

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The characteristic aspect of the senile ovary could result from a primary deficiency in the ovary itself (the depletion of the oocyte stock, functional modifications of ovarian cells) or simply reflect a change in its central neuro-humoral command. The standard theory of primary ovarian aging is at variance with some facts observed after hypophysectomy which interrupts the central regulation and thus may allow primary ovarian senescence to become apparent.

In the course of our investigations, we worked with longterm hypophysectomized rats. The formation of testis-like tubules and of interstitial cords, characteristic structures of the senile ovary, which appear in the intact rat at the age of 24 months, can be observed in rats hypophysectomized when 26 days old, from the age of 5 months on and especially at the age of 11 months. It will be shown that this fact is due, not to a premature ovarian aging after hypophysectomy but to the resultant deficiency of gonadotropins.

Thus, interstitial cords and testis-like tubules indicate secondary ovarian senescence. Moreover, they must be withdrawn from the number of structures for which a primary ovarian senescence has been postulated.

[1] E. T. Engle, Cancer Res. *6*, 578 (1946).
[2] L. Loeb, Arch. Path. *46*, 401 (1948).
[3] P. J. Thung, in *Structural Aspects of Ageing* (Ed. H. G. Bourne, Pitman Publishing Corp., New York 1961), p. 113.

An Aqueous Thymus Extract Modifies DNA-Protein Interactions in the Liver of Old Rats. Spectrophotometrical Data

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Our previous research[1] demonstrated that the administration of an aqueous extract of calf thymus[2] modifies in the liver of 26-month old rats the extractability of DNA in the polymerized state. This result leads to the hypothesis of a different physicalchemical state of the deoxyribonucleoproteins in the animal treated with the aqueous thymus extract. The present study is intended to verify this possibility by a spectrophotometrical analysis of nucleoproteic fractions obtained from the liver of old rats treated with an aqueous thymus extract, as compared to untreated animals.

In the nucleoproteins of the treated rats as compared to the controls one can observe: 1. a lesser hyperchromaticity of DNA-protein complex after heating at 100 °C for 45 min and subsequent rapid cooling; 2. a smaller concentration of non-histone proteins whose interactions with DNA can be overcomed by deproteinization with chloroform in low ionic strength medium: this fact is in agreement with the results of Galubitskaja et al.[3] which demonstrate that senescence is accompanied by an increase (21%) of the non-histone proteins of rat liver nuclei; 3. a greater concentration of histone proteins which can be dissociated from the DNA by deproteinization with chloroform in the presence of 1 *M* $NaClO_4$; 4. a smaller contribution of DNA to the variations of absorbance at 300 nm which are evident in DNA-protein complexes when the pH is increased from 10 to 12.5[4]. The latter result confirms the point[3].

These results demonstrate that aqueous thymus extract modifies the interactions between DNA and proteins by increasing the associations between histone proteins and DNA. If interpreted in the light of results obtained by different authors, according to which the stabilization of the interactions between DNA and proteins increases with senescence, these data could appear in contradiction with the results described in our preceding work, according to which the thymus extract has a protective function towards modifications of biological parameters correlated with senescence. However, in our opinion, the action of the aqueous thymus extract can be correctly interpreted not as a stabilization but as an increase in the number of electrostatic interactions between DNA and

proteins, this action probably being correlated to the biological activity of the histone proteins which control the transcriptional activities of DNA. This hypothesis receives support from the results of DENKHAUS et al.[5] who demonstrated, using microfluorimetric determinations, that the number of electrostatic bonds between nucleohistones and DNA decreases with age while more stable bonds are formed.

[1] D. AMICI, G. L. GIANFRANCESCHI, G. MARSILI and L. MICHETTI, preceding article.
[2] D. AMICI and G. L. GIANFRANCESCHI, Ital. J. Biochem. in press.
[3] R. I. GOLUBITSKAJA, V. N. NIKITIN and N. I. TSINOVEI, Molec. Biol. Stareniya, 9 (1969).
[4] I. O. WALKER, J. Molec. Biol. *14*, 381 (1965).
[5] W. DENKHAUS, D. HEIMER and I. THRUN, Gerontologia *16*, 1 (1970).

Activity Models of 1- and 2-Year-Old Rats by Electronic Registration

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The activity of male rats was measured by a two channel Type DS Animex activity meter. Total movements and also 'energy rich' (large and quick) movements were registered and in 30 min periods the groups were compared. The differences of large numbers between 1- and 2-year-old animals were compared and statistically evalueted by *t*-test.

First spontaneous activity of 8 h, between 19.00 h and 03.00 h, was registered. After a first increased activity, which we do not count as part of the spontaneous period, with both groups appear rhythmic increases of activity. Both age groups did not show differences.

Then social activity was tested by keeping 2 animals together. 1-year-old animals activity is increased up to 2 h and then decreases. 2-year-old animals showed a quicker decrease of activity which increased later, and finally no differences were present between the two groups. Large movements always appear more often with the young animals.

[1] J. ASCHOFF, in *Handbuch der Zoologie* (Springer, Berlin 1962), vol. 8, Teil 2.
[2] D. A. OLEWINE, C. H. BARROWS and N. W. SHOCK, J. Geront. *19*, 230 (1964).
[3] M. E. SIME and H. KAY, J. Geront. *17*, 81 (1962).

Age, Ether Narcosis and 'Weck'-Amines

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It was tested whether, with the same concentration of ether, the time from the start of narcosis to the surgical stage (stage III of G. THOMAS) differed in young and old rats. Furthermore it was determined whether the time of narcosis death was different, with the same concentration of ether, for young and old rats, and finally whether a certain dose of 'Weck'-amine counteracted the ether narcosis in the same time in young and old rats.

For these tests 84 male albino Wistar rats were used in 2 groups: 42 rats were 4 months old (average weight 152 g) and 42 rats were 24 months old (average weight 380 g). Narcosis was made in round glass cages of 4 l capacity into which 20 ml of ethyl ether was sprayed at 28 °C. The rat was placed in the cage which was sealed. The time taken to reach narcosis stage III, was registered. The test was repeated in a weeks time with the same animal, and after one more week, it was left in the narcosis cage until death occured with stopping of breathing and heart-beat.

After the first narcosis test 10 young and 10 old rats were injected s.c. with 1 mg/kg with 'Weck'-amine Benzedrine and the time taken for the animal to awaken was registered. Criteria for being awake were blink reflex and getting on its feet and attempting to run.

The differences between young and old rats were statistically significant, and showed that 1. old rats reached a state of narcosis almost twice as rapidly as young rats; 2. that narcosis death occured twice as rapidly in old rats as in young; whereby the individual differences between the old rats were greater than between the young rats. 3. the awaking from narcosis in response to the 'Weck'-amine injection was significantly more rapid in young rats than in the old ones.

Influence of Age Distribution of Penicillin V in Rats

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Diffusion constants of penicillin *V* through biological surfaces were studied. Sprague-Dawley rats of 3, 12–16, 19–21 and 24 months age were used.

The velocity of intestinal absorption and excretion velocity is highest in 12-month-old animals. They are lower in 24-month-old ones than in 3-month-old ones. Also the uptake velocity into liver and lung decreases with aging. The differences were significant by *t*-test.

The highest penicillin concentrations were found in liver and lung in the 3-month-old and in the kidney of 19–21-month-old rats. The maximal concentration in the blood are highest in old animals. The blood concentration is thus not always relevant to the therapeutic concentration, and in young animals such concentrations are reached much more quickly.

[1] G. HOFECKER, Vet. med. Diss. Wien (1969).
[2] H. NIEDERMÜLLER, Wiss. techn. Diss. Wien (1972).
[3] S. ULLBERG, Acta radiol., Stockholm Suppl. 118 (1954).

Intestinal Water Absorption in Young and Old, Germfree and Conventional Rats

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In germfree animals more liquid contents and distension of the lower bowel are widespread characteristics. These anomalies have been described primarily in rodents, but have been observed also in germfree dogs, pigs and human infants. With progress of age these anomalies become more pronounced. In a colony of aging Swiss-Webster mice, at natural death, the weight of the full cecum averaged in germfree 20.0 $\pm$ g; 5.5 in conventional controls 1.4 $\pm$ 0.6 g. At 3 months of age, mice of this colony averaged in their cecal weights approximately for the germfree 7% and for the conventional 1%. The prevalent lesion at natural death of germfree mice was associated with the enlarged cecum. Most frequently, the muscle tone became reduced to the point that the intestinal propulsive movements were severely impaired or came to a complete standstill. The lower bowel was greatly distended and filled ad maximum by semiliquid contents. In conventional controls, at natural death, similar changes were never observed. In these animals, inflammatory changes prevalied.

In the development of the germfree anomalies, inhibition of water absorption from the lower bowel appears to play a major role. This is brought about by the accumulation of mucus and related substances and by the lack of mucus degrading enzymes in the intestinal contents. Such conditions inhibit water absorption from the bowel at least by the accumulated mucus, i.e. a non-absorbable colloid, which attracts water into the intestinal lumen. Thus in germfree rat cecal contents, colloid osmotic pressure values of approximately 100 mm Hg have been reported resulting in a pressure gradient of 60–70 mm Hg between lumen and blood plasma; in conventional controls this gradient appeared negligible. Mucus, consisting primarily of acid mucopolysaccharides, sequesters a large proportion of diffusible cations, most sodium, in the intestinal lumen. Under these circumstances of the germfree gut, the diffusible anions, mainly chloride, assume very low values. When in an in vivo experiment, the natural contents of the germfree animal's cecum are replaced with saline (i.e. when diffusible ions for water transport are plentiful) water absorption becomes normal or is even greater than in conventional controls. This leads to chronic diarrhea in these animals; the abnormal composition of the intestinal contents and not an impairment of mucosal function is the essential factor.

The mechanism of loss of intestinal muscle tone is not clear. In germfree animals it occurs conjointly with the water absorption inhibition, and they become worse with age and appears implicated in the death. Seeding if the conventional flora via a fecal inoculum into the intestinal tract even at adult age, makes the mentioned anomalies disappear within 2–3 weeks. It seems that the intestinal flora carries out indispensable regulatory function in the maintenance of GI normality by enzymes needed for luminal degradation of the host's bioactive metabolites. The identity of the flora is unknown. Chronic diarrheas which occur in conventional life on neutralization of the flora by oral antibiotic treatment or on shifts of the flora, conditioned by old age, may develop on the disappearance of such synergistic elements from the intestinal tract.

[1] H. A. Gordon, E. Bruckner-Kardoss and B. S. Wostmann, J. Geront. *21*, 380 (1966).
[2] H. A. Gordon and B. S. Wostmann, *Germfree research: Biological effect of gnotobiotic environments* (Ed. J.B. Heneghan: Academic Press, New York 1973), p. 593.
[3] K. Loeschke and H. A. Gordon, Proc. Soc. exp. Biol. Med. *133*, 1217 (1970).

Changes of Fibre Pattern in the Diaphragmatic Muscle of Aging Rats

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The proportional distribution of red, intermediate and white fibres of the diaphragmatic muscle (D) has been examined histochemically in rats of 15 days up to 28 months. Distinction of the 3 fibre types was based on the succinate dehydrogenase reaction; fat and glycogen content have been examined as well[1]. The red, intermediate and white fibres of the costal and hiatal region of the D were counted in an area of 1 mm^2 and their relative distribution calculated. Fresh weights and total areas of the D were also determined.

During the developing period of the rat (15–20 days post partum) all the fibres appear to be red, with a small, rather uniform diameter of ca. 15–20 μm. At the age of 5–6 weeks there occurs a rapid differentiation into white and intermediate fibres, connected with an increase of the diameter, especially of the white and intermediate ones; therefore the number of fibres per mm^2 decreases rapidly. Since the total area of the D is increasing proportionally no real fibre loss is occurring.

From 3 to 28 months there is a slow but constant increase of intermediate and white fibres and a respective decrease of red fibres. The increase of the total area of the D continues only up to the age of ca. 15 months, then remains constant or even begins to decrease slowly, so a real overall decrease of fibres can be assumed (ca. 6% from 5 to 28 months). The comparison of fat in young and old rats D shows a respective decrease, and a similar pattern is seen for glycogen.

The slow but constant transformation of red into intermediate and white fibres is brought into connection with the decreasing respiratory activity of the organism, thus forcing the muscle fibres to adapt to the more anaerobic conditions[2].

[1] G. F. Gauthier and H. A. Padykula, J. Cell Biol. *28*, 333 (1966).
[2] M. Ermini, Acta geront. *3*, 141 (1973).

Myofibrillar and Mitochondrial ATPase Activity of Red, White, diaphragmatic and Cardiac Muscle of Young and Old Rats

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The specific activities of the myofibrillar Ca^{2+}, Mg^{2+}-stimulated ATPase and of the azide inhibited Mg^{2+}-stimulated mitochondrial ATPase have been measured in different striated muscles – white and red skeletal muscle of hind limbs, diaphragm and heart – of young (4–6 months) and old rats (over 20 months).

In young animals the myofibrillar ATPase of the white (W) muscle (2.29 ± 0.37 μmol *Pi*/mg prot./5 min, 25 °C; *n* for all values = 5) is more active than the red (R) (1.65 ± 0.13) and the diaphragmatic (D) muscle's one (1.57 ± 0.31), but similar to the heart (H) muscle (2.47 ± 0.56). This surprisingly high value must be caused by a strong presence of mitochondria within the washed myofibrils, if one considers that normally the myosin and purified actomyosin ATPase of the H are found to be similar to those of red skeletal muscles[1].

The mitochondrial ATPase of the muscle whole homogenate (in presence of EGTA and Mg^{2+}, inhibited by azide) is highest in the H (5.67 ± 0.98 μmol *Pi*/mg prot./5 min, 25 °C) and lowest in the W (1.28 ± 0.26); in R (1.65 ± 0.68) and D (1.95 ± 0.33) it is higher than in W, but still much lower than in H. Significant differences between R and W can be seen particularly considering the percent inhibition by azide[2] (R: 63 ± 12%; W: 24 ± 13%). The D (70 ± 6%) and H (73 ± 9%) show similar inhibition as R.

In old animals the values for the myofibrils of W (2.10 ± 0.66), R (1.75 ± 0.52), D (1.54 ± 0.44) and H (2.21 ± 0.32) are not significantly different from the young animal's ones. But the differences between W and R and W and D are not significant any more! Similarly the mitochondrial ATPase appears not to be reduced significantly, although all the mean values are lower (W: 1.12 ± 0.33; R: 1.31 ± 0.69; D: 1.78 ± 0.21; H: 4.12 ± 1.43). While the inhibition by azide is similar in R (63 ± 12%) and D (70 ± 6%) in young animals, the R's ATPase is less inhibited (50 ± 16%) than the D's one (69 ± 10%) in old animals.

We conclude that particularly the mitochondrial ATPase and it's inhibition by azide reveals a clear difference between the R and W type of striated muscle. The same is true for the myofibrillar ATPase except in the case of the H. Aging does not seem to influence these enzymes much, but the disappearing significance of difference between W and R myofibrillar ATPase as well as the less inhibited mitochondrial

[1] K. Büchi and E. Jenny, Experientia *27*, 1396 (1971).
[2] M. Ermini, Experientia *26*, 173 (1970).

ATPase of R could be interrpreted as a consequence of the organism's overall reduced respiratory activity[3], thus adapting the R's metabolic situation more to the W anaerobic type.

[3] M. ERMINI, Acta geront. *3*, 141 (1973).

Biochemical Aspects of the Aging of the Sea Mussel *Mytilus gallo-provincialis*

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The sea mussel *Mytilus galloprovincialis* has been examined as a possible model for gerontological research of biochemical direction. The sessility of these animals and the tendency to grow in colonies is allowing to collect within a very limited area mussels of different ages at the same time, thus guaranting relatively similar living conditions for every member of a chosen population.

Hoping that the length of the shells might be in relation with the animal's age[1], it was tried to 'calibrate' the shell's length with the biological age of it's proprietor determining certain biochemical parameters known to change in mammals during aging.

The content of water in percent of the animals body weight, and the aceton-ether extractible lipids were determined in whole animals with increasing length of the shells (6–7 groups between 35 and 80 mm), and of the adductor posterior and byssus retractor muscles the contents of total arginine and arginine phosphate (AP) were determined, as well as the myofibrillar ATPase activity. With increasing lengths of the shells the whole body water content seems to increase slowly from 82% (40–50 mm) to 85% (over 60 mm). The lipids are rather irregular but seem to decrease from 10% (dry body weight) to 5%.

The content of total arginine is higher in the adductor than in the byssus retractor and remains constant for all lengths (13–15 µmol P*i*/ fresh wt.), while in the retractor there can be observed a decline from 11.5 µmol (40–50 mm) to 7 µmol (70–75 mm). The AP is rather low in both muscles (1–2 µmol/g) and shows no age dependent changes, the variations being quite large, probably due to methodical inadequacies. The specific activity of the myofibrillar ATPase is higher in the adductor (0.318 µmol P/mg prot./5 min, 25 °C) than in the byssus retractor (0.2) as it is expected considering the former as 'phasic', the latter as 'tonic' muscle. With increasing length of the shell there is a continuous decline of the adductor's ATPase to 0.175 (63–65 mm) while the byssus retractor shows an activity of 0.105 at this length. An analogy to the mammals' quite expressed decrease of phosphocreatine during aging[2] has not been seen for the mussel's AP in relation to it's length.

The water and fat tests and the result of the determination of the myofibrillar ATPase activity, however, could signify that the mussel is still growing while already aging, thus permitting to conclude from it's lengths to it's age[3]. If this can be verified by further investigations the gerontological researcher can be provided easily with these abundant animals whose biological age is relatively simple to estimate.

[1] L. HARANGHY, Acta biol. hung. *16*, 57 (1965).
[2] M. ERMINI, Gerontologia *16*, 65 (1970).
[3] H. INAGAKI, C.r. Acad. Sci., Paris *274*, 1828 (1972).

Ultrastructural Maturation of Myocytes

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Ultrastructural maturation of myocytes is generally missing in culture: Fibrils and mitochondria do not dispose themselves in an ordered parallelity extended to several cells, and these cells are not jointed together by intercalated discs. These signs of maturation do not appear in cultures, even though the formerly trypsinized myocardial cells of chick embryo associate in clumps and pulsate as so-called mini hearts during weeks and even months. Therefore, the factor inducing maturation of myocytes in normal heart development is not present in the monolayers and cell agglomerations. The poor function of rhythmical contraction does not contain this 'agent', responsible for maturation, not taking into account that the conditions of culture, which maintain the pulsating activity for such a long time, do not provide it either (Film).

However a cell conglomeration, which, besides rhythmical contractions had some kind of functional task, was found in culture. From a purely mechanical point of view, this task is the same as the one accomplished by myocytes in the heart wall. This contraction in the heart acts against a tension provided by blood. In culture, the beating activity of some united cells acted against a traction, since the monolayer incorporated them to the cord which was formed by the rolling up of this monolayer. Through electron microscopical investigations of this portion of the pulsating cord, pulsation which is extended by linear stresses of fixed parts of the cord, we detected intercalated discs and a certain parallelity of mitochondria and myofibrils. This is characteristic during Hamburger-Hamilton stage 15 of maturation to 41.

Therefore, whereas the pulsating activity alone can only maintain the already existing development stage of heart cells, it may be concluded from our actual observations that the causal momentum of ultrastructural maturation is identical to the tonus against which the myocytes normally have to contract.

PRAEMIA

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