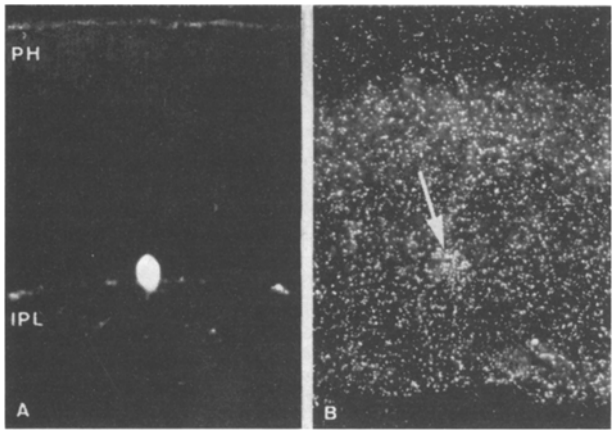
25 μCi ^{3}H-octopamine was injected intravitreally into albino rabbit eyes, which were then excized after 4 h and freeze-dried, fixed in formaldehyde vapor according to the FALCK and HILLARP method, embedded directly in epoxy resin (Durcupan, Fluka) in vacuo, sectioned, photographed in the fluorescence microscope and covered with autoradiographic stripping film (Kodak AR 10). Exposure times were 1 to 3 months.



Rabbit retina, 4 h after the injection of 25 μCi ^{3}H-octopamine intravitreally. A) fluorescence micrograph showing one dopamine-containing, strongly fluorescent cell body and several fluorescent terminals throughout the inner plexiform layer (IPL). The photoreceptors (PH) at the top are faintly autofluorescent, and the pigment cells above them are somewhat more autofluorescent. B) is a dark field micrograph of the autoradiogram of the same area. There is a diffuse distribution of silver grains all over the retina, with a slight increase in radioactivity in the dopamine-containing cell (arrow). × 340.

Fluorescence microscopy demonstrated the by now well-known dopaminergic junctional cells and the 3 sublayers of dopaminergic terminals in the inner plexiform layer (Figure A). The autoradiography showed mainly diffuse distribution of the radioactivity, but on close inspection it was observed that there was a slight increase in dopaminergic neurons (Figure B). The radioactivity of these cells was far less than that seen after the injection of tritiated catecholamines under comparable circumstances.

The experiments show that octopamine or a metabolite is only weakly accumulated by dopaminergic neurons and not at all in the recently detected indoleamine-accumulating retinal neurons[11]. They further show the absence in the rabbit retina of a selective, efficient neuronal accumulation of octopamine, but they do not show whether this is so because there are no neurons operating with octopamine, or because such hypothetical neurons have no efficient uptake and storage mechanism for their transmitter.

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Age-Dependent Increase of Thermal Stability of in situ Chromatin of Rat Liver and its Reversal after Hepatectomy

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Summary. An age-dependent increase of thermal stability of DNA in situ has been demonstrated in rat liver by means of microfluorimetry, which was reversed to a great extent in old regenerated liver.

Cellular ageing involves a progressive loss of the ability of the cell to maintain the homeostasis[2], which may be due to an alteration in the DNA-protein association determining the structure and physiological activity of chromatin. Numerous biochemical studies[3–7] showed an age-dependent increase of thermal stability of the extracted chromatin. It has also been revealed that the method of extraction, as well as the ionic strength of the medium used, have a strong influence on the results obtained. In order to avoid the problems of chromatin extraction[8], thermal denaturation experiments were performed on chromatin in situ of young and old rat hepatocytes. On the other hand, in order to reveal whether the age-dependent changes of thermal stability of chromatin are reversible, similar experiments were carried out also in regenerated liver of old animals. Some preliminary data of these experiments have been presented elsewhere[9].

Female Wistar rats of our own breed were used: A) 3 rats of 2 months of age (young group); B) 3 rats of 28 months of age (old group); C) 3 rats of 25 months of age

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Basic data and calculated parameters obtained in liver cell nuclei

Data	Control	50 °C	60 °C	70 °C	80 °C	90°C
	Young					
$\alpha \pm$ SD	0.188 ± 0.016	0.210 ± 0.022	0.213 ± 0.011	0.239 ± 0.033	0.307 ± 0.044	0.349 ± 0.040
(n)	(30)	(30)	(29)	(30)	(30)	(25)
Relative DNA content (%)	100	89.62	86.47	82.39	83.87	77.57
(n)	(60)	(60)	(60)	(60)	(60)	(60)
S% ± SD (not corrected for DNA loss)	—	16.61 ± 11.87	18.21 ± 6.32	31.64 ± 11.87	52.39 ± 8.16	60.16 ± 5.56
S% ± SD (corrected for DNA loss)	—	25.27 ± 10.64	29.28 ± 5.46	43.68 ± 9.78	60.07 ± 6.84	69.09 ± 4.31
	Old					
$\alpha \pm$ SD	0.170 ± 0.013	0.179 ± 0.006	0.194 ± 0.019	0.220 ± 0.025	0.262 ± 0.070	0.327 ± 0.114
(n)	(40)	(40)	(40)	(40)	(40)	(40)
Relative DNA content (%)	100	100[a]	86.79	93.40	100[a]	86.46
(n)	(40)	(40)	(40)	(40)	(40)	(40)
S% ± SD (not corrected for DNA loss)	—	8.60 ± 4.47	19.23 ± 11.07	35.58 ± 7.80	48.64 ± 14.37	62.28 ± 12.45
S% ± SD (corrected for DNA loss)	—	8.60 ± 4.47	22.16 ± 9.61	38.09 ± 7.29	48.64 ± 14.37	72.03 ± 10.77
	Old regenerated					
$\alpha \pm$ SD	0.168 ± 0.013	0.175 ± 0.013	0.196 ± 0.013	0.237 ± 0.038	0.251 ± 0.025	0.322 ± 0.044
(n)	(40)	(40)	(40)	(40)	(40)	(40)
Relative DNA content (%)	100	83.10	88.90	100[a]	86.80	77.60
(n)	(80)	(40)	(60)	(40)	(40)	(30)
S% ± SD (not corrected DNA loss)	—	6.63 ± 10.27	22.26 ± 7.30	41.60 ± 11.21	46.14 ± 6.91	61.98 ± 6.12
S% ± SD (corrected for DNA loss)	—	22.33 ± 8.53	30.89 ± 6.49	41.60 ± 11.21	53.25 ± 6.00	70.50 ± 4.75

n, stands for total number of nuclei measured in 3 animals. [a] Values being not significantly different from the control. Every other value showed a significance level $p < 0.1\%$ as compared to the control of the same group.

(hepatectomized group). Two-thirds hepatectomy was carried out according to HIGGINS and ANDERSON[10], and the regenerated livers were studied 20 days thereafter, since by that time the volume and weight of the new livers reached those of the control old group[11]. The rats were killed by decapitation. Smears were prepared from small pieces of liver on slides. Fixation and further treatment of preparation was carried out according to the method of RIGLER et al.[12] described for tissue cultures, modified by Zs.-NAGY and Zs.-NAGY[13], and Zs.-NAGY[14,15]. The main points of the method are:

1. Thermal denaturation of the smears at different temperatures (50–90 °C) in SSC-formalin[12], the control smears were kept at 20 °C. 2. Staining with acridine-orange[12]. 3. Calculation of $\alpha = \frac{F_{590}}{F_{530}}$ where F represents UV-excited fluorescence intensities of the stained nuclei at the wavelengths[12] indicated measurable also by microfluorimetry[15]. 4. Calculation of the percentual value of single stranded DNA (S %) unsing the formula of RIGLER et al.[12]. The controls are considered as containing 100% double stranded DNA. 5. Measurement of the relative DNA content of the nuclei on parallel smears by Feulgen microfluorimetry. Since it has been shown that only single stranded DNA can be lost during thermal denaturation, the values for S % obtained before were corrected for the DNA-loss[13]. 6. Statistical analysis was carried out using the Student's t-test. Since the hepatocyte populations of the different animals within the same group showed a fairly similar behaviour under identical conditions, the data obtained were pooled together considering all the hepatocytes as one population and were not treated per single animal.

The results are shown in the Table. As expected, the thermal denaturation causes an increase in the value of α, involving an increased strand separation of DNA. The values of S % corrected for DNA-loss are plotted also in the Figure showing significant differences between young and old animals at all the temperatures except 90 °C, whereas the thermal denaturation curve of the regenerated old liver proved to be identical with that of young animals, except 80 °C.

On interpreting these results, one has to consider the derivative melting profile of chromatin. According to LI et al.[16], it displays 4 maxima, i.e. 4 different melting points: I) at 47 °C, free DNA; II) at 57 °C, DNA bound by non-histone proteins; III) and IV) at 72 and 82 °C, DNA regions bound by less or more basic parts of histones, respectively. This means that the single stranded DNA measured at 50 °C in our experiments may correspond to

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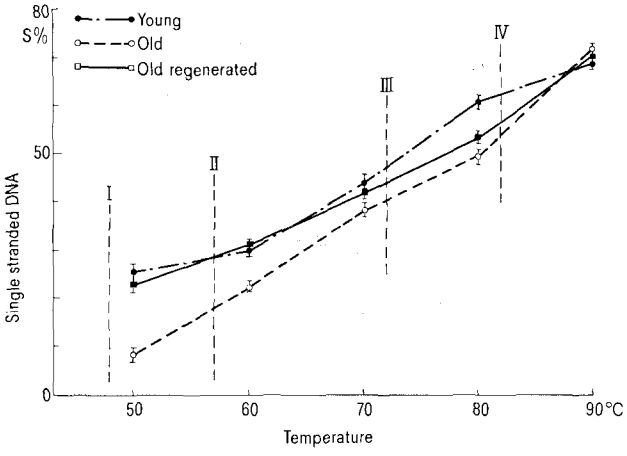
the protein-free DNA fraction of the nucleus, amounting to 25% in the young animals and only to 8% in the old ones. It is remarkable that the regenerated old liver reached almost the young value. The percentage of protein free DNA in young livers is of the same order as observed in other tissues using also quite different techniques by others (18–25%)[13,16–18]. One can assume, therefore, that, for the normal function of interphase cell nucleus, about this proportion of DNA should be in a protein-free state. In the old animals this value decreased to a high extent which must have serious functional consequences. The percent of single stranded DNA obtained at 60 °C is to be considered as the sum of free DNA and DNA stabilized by non-histone proteins, i.e. in the young animals only about 4% of DNA is stabilized by acidic proteins; in old rats this value increased to about 13%, whereas in the regenerated old liver it was between these values. Following a similar logic, one can see that the more basic part of histones (melting point at 82 °C)[16] is binding a higher portion of DNA in the old liver than in the young one, and again the regenerated old liver lies between these values.



Thermal denaturation curves of nuclear DNA in rat liver. S = single stranded DNA in % of DNA-phosphate. Dotted lines and roman numbers indicate the sites of peaks of derivative melting profile[15]. The plots are taken from the Table. (Means ± SEM). Significance values: A) Young-old comparison: $p < 0.1\%$ at each temperature, except 90 °C where N.S. B) Young-old regenerated comparison: N.S. except 80 °C where $p < 0.1\%$. C) Old-old regenerated comparison: $p < 0.1\%$ at 50 and 60 °C; $p < 2\%$ at 80 °C; N.S. at 70 and 90 °C.

We believe that our results allow us to conclude a certain reversibility of the age-dependent condensation of chromatin by mitoses, supporting the idea that true ageing phenomena are present first of all in the post-mitotic cells[19].

From a methodical point of view, our results show that the in situ thermal denaturation with parallel measurement of the DNA-loss, introduced in our laboratory[13], is a valid method for studying the physico-chemical properties of chromatin.

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Exocrine Pancreatic Enzymes in Cycloheximide Treated Rats

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Summary. Cycloheximide, even in a dose of 0.25 mg/kg administered s.c. to rats stimulated by pancreozymin and secretin, inhibited lipase activity in pancreatic juice. Lipase activity in serum of control animals was inhibited by cycloheximide. The secretion of trypsin and chymotrypsin was also decreased.

Cycloheximide, an antibiotic isolated from *Streptomyces griseus*[1], inhibits synthesis of proteins in yeast and mammalian systems by interfering with both the initiation and elongation of polypeptide chains on polyribosomes[2]. The drug has been used extensively to study the relationship between a number of biological processes and protein synthesis[3–9]. Moreover, cycloheximide has been used clinically[10] recently to produce defervescence in chronically febrile patients with Hodgkin's disease[11]. In our laboratories we extend the earlier observation[12] that cycloheximide affects gastric secretion. Especially gastric acidity was almost completely abolished by a single dose of the drug[13]. In this report evidence is presented that exocrine secretion of some enzymes in the pancreas and in the serum of rats are also inhibited following the administration of this drug.

Materials and methods. Experiments were performed on 100 male Wistar rats (240–250 g) kept on a standard diet. The first group of animals was treated s.c. with one dose, or with the same dose of cycloheximide for 3 consecutive days. The animals were killed on the 2nd or the 4th day,

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