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

Our previous research[1] concerning the possible correlations between thymus and senescence considered the effect of the administration of an aqueous extract of calf thymus on a few hematic parameters and on the nucleic acids content of the liver of old rats. We have observed that in the liver the thymus extract modifies the extractability of DNA in the polymerized state. This result leads to the hypothesis of a different chemical-physical 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 treated as compared to untreated old rats.

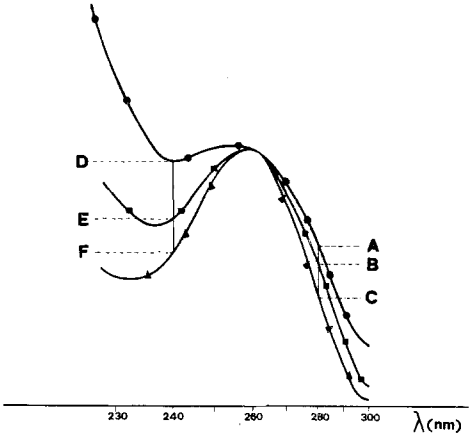

Fig. 1. Absorption spectra between 220 and 300 nm of the chromatin (●—●), deoxyribonucleoproteins (■—■) and DNA (▲—▲) extracted from rat liver. Segments $\overline{AB}$, $\overline{DE}$ are demonstrative of spectrum modifications of the chromatin when subjected to deproteinization in low ionic strength medium ; $\overline{BC}$, $\overline{EF}$ are demonstrative of spectrum modifications of the deoryribonucleoproteins when subjected to deproteinization in the presence fo 1 *M* $NaClO_4$ (see Table).

Materials and methods. The preparation of the aqueous thymus extracts and the method of administration were effected according to methods described previously[1]. The experiments were carried out on groups of 6 old rats (26 months). The extraction of the chromatin was carried out using the method of MARUSHIGE and BONNER[2]; the extraction of the deoxyribonucleoproteins was effected according to MARMUR[3] and KAY et al.[4], or according to BRAM and RIS[5]. The nucleoproteins were deproteinized by chloroform-isoamylic alcohol (24:1 v/v) according to SEVAG et al.[6], in a different condition of ionic strength. The RNA was removed from nucleoproteins by ribonuclease digestion[3].

Results and discussion. A) The hyperchromaticity of DNA-protein complexes extracted by 0.5 *M* NaCl[7,8] was studied, after heating at 100 °C for 45 min and sub-

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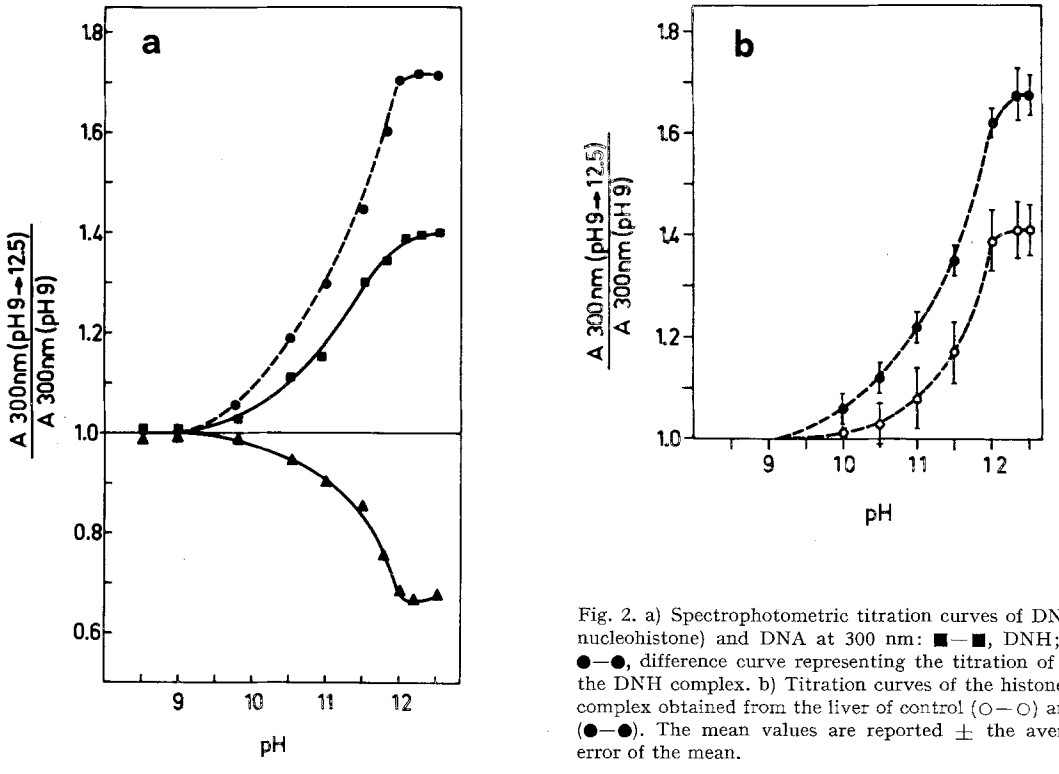

Fig. 2. a) Spectrophotometric titration curves of DNH (deoxyribonucleohistone) and DNA at 300 nm: ■—■, DNH; ▲—▲, DNA; ●—●, difference curve representing the titration of the histone in the DNH complex. b) Titration curves of the histones in the DNH complex obtained from the liver of control (○—○) and treated rats (●—●). The mean values are reported ± the average quadratic error of the mean.

	A) Chromatin		B) Deoxyribonucleoproteins	
	Δ (A 280 nm/A 260 nm); [a] ($\overline{AB}$)	Δ (A 240 nm/A 260 nm); [a] ($\overline{DE}$)	Δ (A 280 nm/A 260 nm); [a] ($\overline{BC}$)	Δ (A 240 nm/A 260 nm); [a] ($\overline{EF}$)
Control	0.075 ± 0.015	0.126 ± 0.012	0.033 ± 0.013	0.161 ± 0.024
+ Thymus	0.015 ± 0.005	0.071 ± 0.022	0.068 ± 0.004	0.208 ± 0.011

[a] The above differential values are function of $\overline{AB}$, $\overline{DE}$, $\overline{BC}$, $\overline{EF}$ segments reported in Figure 1. The mean values are reported $\pm$ the average quadratic error of the mean.

sequent rapid cooling. A higher hyperchromaticity Δ A 260 nm/A 260 nm can be seen in the deoxyribonucleoproteins of the control (0.0694 $\pm$ 0.0075) as compared to the treated animals (0.0306 $\pm$ 0.0098). B) Spectrophotometric analysis was made of the chromatin. The ratios A 280 nm/A 260 nm and A 240 nm/A 260 nm of the chromatin obtained from the liver of the control or of the treated animals did not show significant differences: this indicates an apparent homogeneity of the chromatin[9]. On the same chromatin preparations, the differential variations Δ (A 280 nm/A 260 nm) and Δ (A 240 nm/A 260 nm) were subsequently calculated after deproteinization in low ionic strength medium (μ 0.018) Table A: the values for the control animals were significantly higher than for the treated animals. These results suggest the hypothesis of the presence in the chromatin of the control animals of a higher amount of non-histone proteins or of proteins whose interactions with DNA can be overcomed by deproteinization by chloroform at low ionic strenght. This is in agreement with the results of GOLUBITSKAJA[10] which demonstrate that senescence is accompained by an increase (21%) of the non-histone proteins of rat liver nuclei.

C) Spectrophotometrical analysis were made on deoxyribonucleoproteins extracted by sodium lauryl sulfate[3,4] from the liver of old animals untreated or treated with thymus extract (Table B). The differential variations Δ (A 280 nm/A 260 nm) and Δ (A 240 nm/A 260 nm) between the deoxyribonucleoproteins preparations obtained following deproteinization by chloroform in low ionic strength medium (μ 0.018), and the preparations derived from subsequent deproteinization by chloroform in presence of 1 *M* $NaClO_4$ (μ 1.018), were calculated: the presence of 1 *M* $NaClO_4$ helps the dissociation of all the histone components bound to the nucleic acid by electrostatic interactions[11]. These differential variations were significantly higher in the animals treated with thymus extract as compared to the controls. This indicates a greater concentration of histone proteins in the deoxyribonucleoproteins of the liver of treated rats which can be dissociated from DNA in the presence of 1 *M* $NaClO_4$.

D) A subsequent series of experiments was carried out on preparations of deoxyribonucleohistones (DNH) according to the method of BRAM and RIS[5], obtained from the liver of old rats untreated or treated with thymus extracts. A spectrophotometrical titration of the DNA and DNH, according to WALKER's method[12], was carried out on these preparations; this method is based on the variation of absorbance at 300 nm shown by DNA, DNH and histones when the pH is increased from 10 to 12.5. The above variations are due to the different ionization, correlated with the variation of the pH, of the cytidine and guanine groups as concerns the DNA, and of the phenolic groups of the tyrosine as concerns the histone proteins. Figure 2a shows the pattern of the DNH and DNA extinction variations when the pH goes from 7 to 12.5: the curve representing the contribution of the histones to the spectrophotometrical variations observed for the DNH is obtained by substracting the DNA spectrum from the DNH spectrum. Figure 2b shows the titration curves of the histones in the DNH complex obtained from the liver of control and treated rats. The data demonstrate that in the DNH complex of the treated rats there is a significant increase of the concentration of the histone proteins bound to DNA by electrostatic interactions which can be overcomed in the presence of 1 *M* $NaClO_4$.

If interpreted in the light of results obtained by other authors[13,14], according to which the stabilization of the interactions between DNA and proteins increases with senescence, these data could appear to contradict the results described in our preceding work[1] 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.[15] who demonstrated that the number of electrostatic bonds between nucleohistones and DNA decreases with age as more stable bonds are formed.

Riassunto. Un estratto acquoso di timo modifica le interazioni tra DNA e proteine nel fegato di ratti vecchi aumentando il numero dei legami tra le proteine istoniche ed il DNA.

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