The halophilic malic enzyme was strongly inhibited by acetyl-CoA and NADH (Figure A) and rather weakly inhibited by oxaloacetate and glyoxylate (Figure B). The former inhibitions presented sigmoidal kinetics, suggesting that they are probably allosteric. The effects of oxaloacetate and glyoxylate, on the other hand, apparently followed hyperbolic kinetics. All the inhibitors, which were competitive towards the substrate L-malate (ref.[7] and unpublished results), were more effective in the presence of 3 *M* KCl than in the presence of 1 *M* NH_4Cl; this was particularly significant for NADH, which was very little effective in the presence of the latter monovalent cation activator (Figure A). Since the apparent K_m values for both substrates were the same in the presence of either salt, it seems likely that the inhibitory effects themselves required a high salt concentration, similar to that reported as present in living halophile cells[8]. A similar finding was reported by KUSHNER et al.[13,14] for the inhibition of the aspartate transcarbamylase from *H. cutirubrum* by CTP. The results shown in the Figure were obtained with Mg^{2+} as divalent cation activator; similar results were obtained in the presence of Mn^{2+}.

The experimental data presented here suggest that the malic enzyme from *H. cutirubrum*, in spite of its truly halophilic character, shown by its absolute requirement of high salt concentrations for both activity and stability[6], is similar to the malic enzymes from other microorganisms in both its kinetic and regulatory properties. The low concentrations of acetyl-CoA and NADH (about 10 μM) effective for 50% inhibition in the presence of 3 *M* KCl (Figure A) suggest that these metabolites may play an important role in the regulation of the enzyme activity in vivo.

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Food-Induced Changes in UV-Absorption Spectra of Isolated Chromatin from Liver of Rats under Controlled Feeding Schedules

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Summary. We have measured the UV-spectra of liver chromatin extracted at 2 different times of day, corresponding to low or high rate of RNA synthesis from rats maintained under controlled feeding schedules. Results show that food intake does modify the UV-spectra of liver chromatin.

The control of the feeding schedules on which the laboratory rat is maintained has been proved to be a very useful experimental method to study the physiological mechanisms which regulate nuclear RNA synthesis.

Liver cells respond to food intake with an increased rate of RNA synthesis[1] in rats maintained under the controlled feeding schedules developed by POTTER et al.[2]. Such increased rates of RNA synthesis can be ascribed both to an activation of nuclear genome by increasing the availability of chromatin DNA template[3,4] and to an increased activity of pre-existing RNA polymerase molecules[4].

Recently it has been shown that isolated nuclei and chromatin can be studied by optical methods[5-7] and that the changes of the UV-spectrum correspond to different functional states of chromatin and rates of RNA synthesis[8].

In the present communication, we report food-induced changes in UV-absorption spectra of purified chromatin obtained from the liver of starved or fed rats in relation

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UV-absorption of liver chromatin at 2 different times of day and in different feeding conditions from rats under controlled feeding schedules

Time of day	Feeding condition	Chromatin absorbance at 258 nm (O.D. units/mg DNA)	Nucleoplasmic RNA synthesis in purified nuclei[1] (pmoles AMP/mg DNA)	Actinomycin-D binding to chromatin[3] (μg/mg DNA)	Chromatin apparent K_M for form-B RNA polymerase[4] (μM deoxynucleotides)
09.00 h	Starved since 17.00 h of previous day	8.672 ± 0.09 (9)	740 ± 59	30.6 ± 2.3 (6)	53.4
15.00 h	Fed from 09.00 h	9.690 ± 0.12 (10)	1289 ± 83	56.9 ± 2.9 (6)	32.2
15.00 h	Starved on the day of experiment (since 17.00 h of previous day)	8.763 ± 0.08 (6)	720 ± 62	31.2 ± 2.7 (6)	51.8

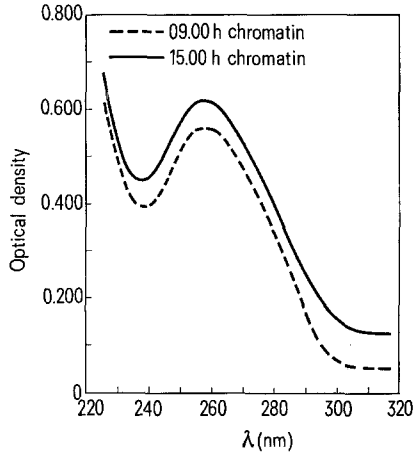
Measurements were performed in 1 cm path length cuvettes on chromatin solutions containing 50–100 μg DNA/ml. Data are expressed as O.D. units at 258 nm/mg DNA ± SEM; in parentheses is the number of experiments performed in different occasions.

to corresponding functional states of genome previously reported[1,3,4].

Methods. 7-week-old male albino rats of Wistar strain, obtained from the departmental animal house, weighing 200–220 g have been used in these experiments. The rats were housed since weaning in an air-conditioned windowless room with an inverted and displaced lighting schedule in which lights were on from 21.00 h to 09.00 h in a 24 h cycle. The food, a purina chow lab diet, was supplied just before the lights were switched off, and was removed 8 h later according to the '8 + 16' feeding schedule developed by POTTER et al.[2]. Water was supplied ad libitum.

Rat liver chromatin was extracted from the purified nuclei[9] as indicated by BUTTERWORTH et al.[10]. Chromatin resuspended in *Tris*-HCl 1 m*M*, pH 8, was sheared and diluted to 50–100 µg of DNA/ml with the same buffer. Optical measurements were done with a Cary-15 spectrophotometer. DNA was determined by the diphenylamine reaction of BURTON[11], and proteins by the method of LOWRY et al.[12].

Results and discussion. The Figure reports the absorption spectra of liver chromatin extracted at 09.00 h or at 15.00 h from rats under the controlled feeding schedules of POTTER et al.[2]. These 2 times of day have been chosen as representative of low (09.00) or high (15.00) rate of RNA synthesis respectively in our experimental conditions[1,3,4]. There is a marked difference between the UV-absorption spectra of liver chromatin extracted at 09.00 h from that extracted at 15.00 h. This difference is represented by an increase in magnitude of the band at 258 nm in chromatin extracted at 15.00 h. No red- or blue-shifts of the main band are observed.



Absorption spectra of liver chromatin extracted at 2 different times of day from rats maintained under controlled feeding schedules. Measurement conditions were as described in the text. The above experiment was performed with a chromatin solution containing 64 µg DNA/ml.

A control measurement performed on chromatin extracted at 15.00 h from rats starved on the day of the experiment showed that the UV-absorption spectrum was overlapping with the one obtained from rats killed at 09.00 h.

Data reported in the Table show that the food intake is able significantly to increase the band at 258 nm by 12% over the corresponding value obtained from chromatin extracted at 09.00 h or at 15.00 h from rats denied food on the day of the experiment. In the Table data are also reported from previous papers[1,3,4], showing the behaviour of functional parameters obtained in the same experimental conditions. It appears that, at a time of day when there is an increased rate of RNA biosynthesis[1] involving also a modification of the template capacity of chromatin[3,4], measured as capacity of binding actinomycin-D or its homologous enzyme, there is also a corresponding increase of the main band of the UV-spectrum.

It is believed that the difference in UV-absorption spectra is due to different states of chromatin condensation[5,6]. The more flattened spectrum of different chromatins is interpreted as presumably due to local areas of condensed chromatin within the nucleus ('granular chromatin')[6].

Therefore the perturbation of the chromophoric portion of liver chromatin following food intake appears to reflect perturbations in nucleoprotein structure induced by mechanisms related to food intake, which result in an increased rate of RNA synthesis[1,4].

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5-Hydroxytryptamine: Autoradiographic Evidence for Uptake Into Fibroblast Cell Nuclei

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Summary. After incubation with tritiated 5-HT, activity is autoradiographically localized over fibroblast nuclei. This may indicate an effect of 5-HT directly on the nuclei of these cells.

Among the many roles of 5-hydroxytryptamine (serotonin; 5-HT) in the organism, an effect on the proliferation of fibroblasts was suggested by the occurrence of fibrosis in the heart and other organs of patients with carcinoid syndrome (see FIORE-DONATI[1] for references). In addition, experiments with injection of 5-HT into subcutis[2] and joint spaces[3] apparently lead to local proliferation of connective tissue. BOUCEK and ALVAREZ[4] have elegantly demonstrated a specific effect of micromolar concentrations of 5-HT on growth and division of cultured fibroblasts, and this effect has been further corroborated recently (BOUCEK and NOBLE[5]).

The present study originated during an attempt to define transmitter substances at certain neuromuscular junctions in the Atlantic hagfish[6]. Tritiated 5-HT is

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