# CHANGES IN LIVER NUCLEAR HISTONES DURING BOVINE ONTOGENY

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## 1. Introduction

The function of histones in chromatin is not yet well enough understood. Histones are thought to be involved in the control of genetic activity. Studies have been carried out to find age-related changes in the electrophoretic patterns and in the relative quantities of different histone fractions which could be explained by changes in the template activity of chromatin during development [1–5]. According to model structures chromatin is composed of repeating nucleosome units, each unit consisting of about 200 base pairs of DNA associated with an octamer of pairs of histones H2A, H2B, H3 and H4 [6,7] while the H1 histones are probably bound to DNA on the outside of the nucleosome [8]. H1 histones show species and tissue specificity [9], and the H1 histones of mammalian tissues undergo changes during the prenatal and postnatal period [9,10]. The different location of the H1 histones in chromatin compared with the nucleosome core histones may reflect their specific role in the regulation of the template activity. Therefore the relationship between development and the chemical nature of histones is important.

In this work we compared the relative amounts of the main histone types from liver during ontogeny. Our results showed a striking increase in the relative amounts of the H1 histones during the whole ontogeny, and the appearance of the H1° histone during the bovine postnatal period.

## 2. Experimental

The age of fetal calves was estimated according to the insemination date. Adult cows were used as controls.

The bovine livers were removed immediately after decapitation and stored at −80°C until used. The liver nuclei were isolated principally as in [11] with the exception that phenylmethylsulfonylfluoride (100 μmol) was used as a protease inhibitor [12] and the sucrose homogenate was centrifuged at 81 000 × *g* for 90 min in a SW 27 rotor. The nuclei were checked for purity by phase contrast microscopy. Histones were extracted from the purified nuclei with 0.2 M $H_2SO_4$ solution containing 100 μmol phenylmethylsulfonylfluoride, were precipitated with 94% ethanol at −20°C and dried with ether. Histone fractions were isolated from adult bovine liver according to [13]. H1 histones were extracted from isolated total histones with 5% perchloric acid [14]. Purified calf thymus histone fractions, obtained from Sigma (Sigma, St Louis, MO), were used as standards. Protein concentrations were determined with Folin-Ciocalteau phenol reagent [15].

Total histones and H1 histones (perchloric acid extracts of total histones) were analyzed by electrophoresis in 2.5 M urea as in [16]. The pre-electrophoresed gels were loaded with 40 μg total histones of each age in 40 μl 0.9 M acetic acid, with 100 μl perchloric acid extract of the total histones of each age or with 20 μg each histone fraction in 40 μl 0.9 M acetic acid. The electrophoresis was continued for 7 h at 1.5 mA/gel. The gels containing total histones were stained with Procion Navy (ICI Ltd.) and were destained by diffusion according to [17]. The gels containing H1 histones (perchloric acid extracts of the total histones) were stained with Coomassie Brilliant Blue R 250 (Merck, Darmstadt) and were

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destained by diffusion according to [18]. The amounts of different histone fractions relative to that of total histones were estimated from the densitometric records at 610 and 550 nm, respectively, by using a Quick Scan densitometer with peak area integrator (Helena Labs.).

## 3. Results

Electrophoresis of adult bovine liver histones was carried out both in short (12 cm) and in long (24 cm) gels. As repeated separations on short gels of the same histone extract showed less variance in histone proportions than separations in long gels, the former were used for the estimates of the relative amounts of liver histones [17].

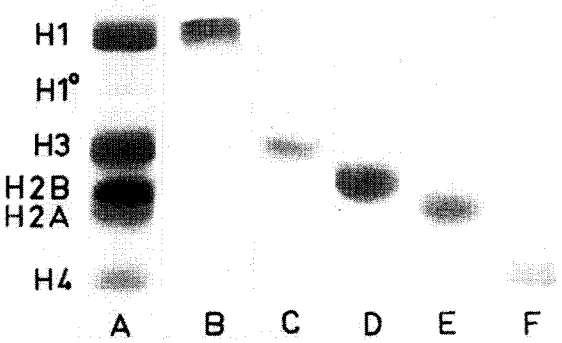


Fig.1. Acetic acid–urea–polyacrylamide gel electrophoresis [16] of total bovine liver histones, and the identification of the main histone fractions. (A) Total liver histones; (B) thymus H1 histones; (C) thymus H3 histones; (D) thymus H2B histones, (E) liver H2A histones; (F) liver H4 histones. Purified calf thymus histone fractions were obtained from Sigma. Electrophoresis was run at 1.5 mA/gel for 7 h; gels were stained with Procion Navy. The positions of the histone fractions are indicated in sample (A).

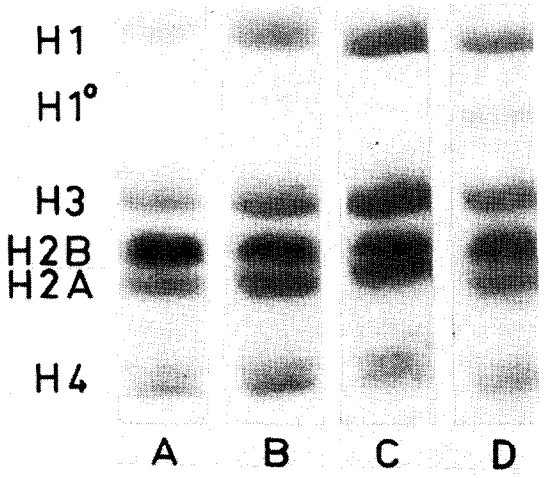


Fig.2. Acetic acid urea gel electrophoresis of total bovine liver histones. (A) Embryo (1 month); (B) embryo (3 months); (C) embryo (9 months); (D) adult cow. Electrophoresis was conducted as in fig.1. Gels were stained with Procion Navy.

Total histones, analyzed by electrophoresis, separated into 5 main fractions. The histone fractions were identified by the criteria in [19]. Confirmation of these identifications was obtained by fractionating bovine liver histones [13], and also with purified calf thymus histone fractions obtained from Sigma. Figure 1 shows the electrophoretic pattern of total bovine liver histones (A) and the identification of the main histone fractions (B–F). H1° histone was identified according to its electrophoretic mobility [14,20], perchloric acid solubility [14] and according to its presence in calf liver [20], but not in calf thymus histones [14].

Table 1
Quantitative analysis of total liver histones of calf embryos and adult cows estimated from densitometry and expressed as percentage of total histones

| | Age | H1 | H1° | H3 | H2B | H2A | H4 | *n* |
|---|---|---|---|---|---|---|---|---|
| Prenatal | 1 month | 8.8 | | 18.5 | 30.4 | 15.8 | 26.5 | 4 |
| | 3 months | 13.6 | | 17.5 | 30.2 | 15.3 | 23.3 | 12 |
| | 9 months | 17.1 | (traces) | 16.1 | 30.5 | 15.1 | 21.3 | 4 |
| Postnatal | Adult | 17.8 | 4.9 | 16.3 | 27.1 | 14.7 | 19.3 | 12 |

The standard deviations of the experiments with bovine liver histones ranged between ± 6–9%
Values expressed in the table are mean values of duplicate experiments of *n* different animals

The electrophoretic patterns of the total liver histones during bovine ontogeny are shown in fig.2, and the electrophoretic patterns of their respective perchloric acid extracts in fig.3. According to these results the amount of the H1 histone increased during ontogeny. The H1 histone of the calf embryos of 1, 3 and 9 months of age appeared as one component while the H1 histone of the adult cow separated into two components (fig.2,3).

Quantitative changes during ontogeny were found in the relative amounts of the main histone fractions (fig.4, table 1). The relative amount of the H1 histones increased according to the densitometric scans of the total histones (fig.4; 1A–4A) as also according to the densitometric scans of their respective perchloric acid extracts (fig.4; 1B–4B) during ontogeny. The relative amount of the H1 histone in the embryo of 1 month of age was 8.8%, of 3 months of age 13.6%, of 9 months of age 17.1% and in the adult cow 22.7% according to the areas of the densitometric records and expressed as percentage of total histones (table 1). The relative amount of the H1 histones increased ~2-fold during the prenatal period and almost 3-fold during the whole ontogeny. The mean value for the H1° histone in the adult cow was 4.9% (table 1).

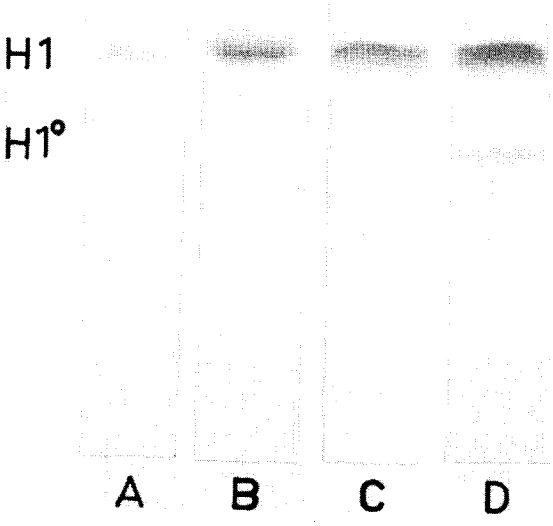


Fig.3. Acetic acid urea gel electrophoresis of the H1 histones extracted with perchloric acid from total bovine liver histones. (A) Embryo (1 month); (B) embryo (3 months); (C) embryo (9 months); (D) adult cow. Electrophoresis was performed as in fig.1. Gels were stained with Coomassie Brilliant Blue R250.

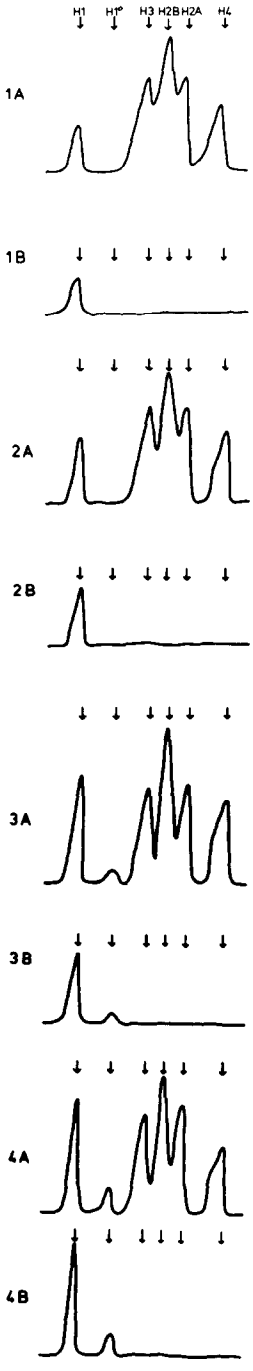


Fig.4. Densitometric scans of total bovine liver histones and their perchloric acid extracts. Total histones are presented by (A) and their perchloric acid extracts by (B). (1A,1B) Embryo (1 month); (2A,2B) embryo (3 months); (3A,3B) embryo (9 months); (4A,4B) adult cow.

Traces of the H1° histone were also found in the embryo of 9 months of age (fig.4), the amount being however, too small to be quantitated from the densitometric records.

Developmental changes in the relative amounts of the other main histone types were rather slight as compared with those found in the H1 histones during bovine ontogeny (fig.4, table 1).

## 4. Discussion

The role of histones in the regulation of the genetic activity has been under discussion during the last decade. H1 histones are thought to regulate genetic activity by interacting with chromosomal DNA [21]. The gene expression may involve modification of the histones, resulting in weakening of their association with DNA. Selective removal of H1 histones can be brought about by using different concentrations of NaCl solutions [21]. This enables transcription leading to synthesis of a gene-dependent protein.

The amount of H1 histones increases during ontogeny in calf brain [9], decreases in chicken embryo liver [22] and is higher in adult cow thymus than in calf thymus [3]. The present results show that the amount of the H1 histone increases ~2-fold during the bovine prenatal period and almost 3-fold during the whole ontogeny. These results confirm our preliminary findings one bone marrow [23], brain [24] and liver [25] histones.

The amount of the H1° histone increases in rat liver and spleen chromatin during the postnatal period [9] and is in rat hepatomas inversely related to their growth rate [26]. It has been suggested that the H1° histone is an inhibitor of DNA synthesis, and that the quantity of the H1° histone relative to total histone is inversely related to the number of dividing cells in the tissue [27]. This agrees with the present results as also with our preliminary findings on the amount of the H1° histone from calf and cow liver [25].

The increase in the amount of the H1 histones during the bovine prenatal period and the appearance of the H1° histone during the bovine postnatal period may reflect an age-related condensation of chromatin associated with an age-related reduction of RNA synthesis and template activity. Consequently, the template activity would be the greater, the earlier the developmental stage in question.

The amount of H2A, H2B, H3 and H4 histones has been reported to stay quite constant during rat and mouse postnatal periods [5]. Our results show a slight decrease in the relative amounts of these histones during bovine ontogeny which might be due either to the increase in the relative amount of the H1 histones or might reflect postsynthetic modifications of these histones related to a decrease in the template activity and protein synthesis.

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