# Nonhistone Chromosomal Proteins of Rat Tissues. A Comparative Study by Gel Electrophoresis†

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ABSTRACT: To investigate the extent of the limited heterogeneity and the possibility of tissue-specific nonhistone chromosomal proteins (NHC proteins) in greater detail than heretofore, we have compared the NHC proteins of seven rat tissues. The NHC proteins of rat liver, kidney, spleen, lung, thymus, thyroid, and brain were analyzed at a high level of resolution using two types of sodium dodecyl sulfate–polyacrylamide gels (molecular weight sieving). Comparison of the gel patterns leads to the following conclusions. There are 12–18 major NHC proteins (molecular weight bands) that make up the majority of the protein mass; in addition, there are many minor NHC proteins. Most of the NHC protein bands are present in most of the tissues examined; nonetheless, each tissue possesses a unique NHC protein pattern in qualitative (presence or absence of band) and quantitative (density of band) terms. A few NHC proteins appear to be specific to certain tissues. In particular, the brain NHC protein pattern is most different from all others and includes an exceptional number of high molecular weight bands.

Chromatin, the interphase form of the eukaryotic hereditary material, is a complex of DNA, RNA, histones, and nonhistone chromosomal proteins (NHC proteins).[1] Chromatin can be isolated by gentle techniques (Bonner *et al.*, 1968) and the biochemical properties of the complex studied *in vitro*. Isolated chromatin possesses the same DNA-dependent RNA polymerase template activity as the *in vivo* chromatin, to the extent that this has been tested. Transcription and hybridization competition experiments indicate that isolated chromatin maintains the limited stage- and tissue-specific patterns of transcription observed *in vivo* (Marushige and Bonner, 1966; Paul and Gilmour, 1966, 1968; Smith *et al.*, 1969; Chetsanga *et al.*, 1970). Accordingly, isolated chromatin is an appropriate starting material for the isolation and characterization of the macromolecules in association with the DNA, which are relevant to gene transcription. The histones, small, basic chromosomal proteins, have now been well characterized (for review see Elgin *et al.*, 1971; Busch *et al.*, 1972). There is considerable evidence showing that the binding of histones to DNA will prevent transcription by RNA polymerase (Shih and Bonner, 1970; Smart and Bonner, 1971). However, there are only a few different kinds of histones (5–12 depending on the organism), and there is little evidence that they can bind to DNA with much detailed sequence specificity. (See DeLange and Smith (1971) for a review of the data.) Tissue-specific histones have been found only in nucleated erythrocytes and in some sperm (reviewed in Elgin *et al.*, 1971). Thus, attention has recently been focused on the other macromolecular components of chromatin, the RNA and the NHC proteins.

The NHC proteins are a complex group of proteins containing 12–18 major components and at least 20–40 minor components as resolved by sodium dodecyl sulfate–disc gel electrophoresis (molecular weight sieving). Comparisons of the gel patterns of NHC protein (or nuclear acidic protein) from two–three tissues of a single organism indicate considerable similarity, although a few apparent tissue-specific protein bands are usually observed (Elgin and Bonner, 1970; Loeb and Creuzet, 1970; Shaw and Huang, 1970; Platz *et al.*, 1970; MacGillivray *et al.*, 1971, 1972; Teng *et al.*, 1971; Shelton and Neelin, 1971; Richter and Sekeris, 1972). To investigate the question of major, tissue-specific NHC proteins in greater detail we have compared the NHC protein patterns of seven rat tissues at a high level of resolution utilizing two types of sodium dodecyl sulfate–polyacrylamide gels (molecular weight sieving).

## Methods

*Preparation of Chromatin.* Chromatin was prepared from different rat tissues as previously described (Bonner *et al.*, 1968; Elgin and Bonner, 1970). Frozen tissue was ground in a Waring Blendor, the homogenate filtered, and a crude nuclear pellet collected by low speed centrifugation. After further washing, the nuclei were lysed in 0.01 M Tris (pH 8). The crude chromatin was washed two–four times in 0.01 M Tris (pH 8) and purified by centrifugation through 1.7 M sucrose–0.01 M Tris (pH 8). The chromatin was then sheared in a Virtis homogenizer at 30 V for 90 sec and centrifuged at 12,000*g* for 30 min; the supernatant, referred to as purified chromatin, was used immediately as the starting material for the preparation of chromosomal proteins. One alteration in this procedure was found necessary. In the case of tissues with a high lipid content (brain and lung), the crude nuclear pellet was washed by centrifugation through 1 M sucrose–0.01 M Tris (pH 8) at 27,000*g* for 15 min and then treated as above.

*Preparation of Chromosomal Proteins.* Histones and NHC proteins were prepared from the chromatin essentially by the method of Elgin and Bonner (1970). In outline, the chromatin was extracted with 0.4 N $H_2SO_4$ at 4° for 30 min to remove histone; the pellet, collected by centrifugation, was washed once with 0.4 N $H_2SO_4$ and rinsed briefly with 0.01 M

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[1] Abbreviation used is: NHC proteins, nonhistone chromosomal proteins.

Tris (pH 8). The pellet was then solubilized in 0.1% sodium dodecyl sulfate–0.01 M Tris (pH 8) and the DNA was removed by centrifugation at 50,000 rpm for 24 hr at 25° in a Spinco SW 50 rotor (200,000*g*). The DNA forms a gelatinous pellet at the bottom of the tube; the entire supernatant is taken as the NHC protein preparation. The protein solutions were then dialyzed to the appropriate starting buffers for gel electrophoresis.

*Disc Gel Electrophoresis.* The acid-extracted histones were dialyzed against 8 M urea–0.01 M Tris (pH 8) and analyzed on 15% acrylamide gels, pH 4.3, in the presence of urea (Bonner *et al.*, 1968). The NHC proteins were analyzed using two sodium dodecyl sulfate gel systems, the phosphate-buffered system of Shapiro *et al.* (1967) and the Tris-glycine-buffered system of Laemmli (1970). Both systems separate proteins on the basis of molecular weight, but are effective over slightly different ranges. Only the latter gels are presented here for most of the comparisons, as there is considerable overlap of information in the two sets (see Elgin and Bonner (1970) for details on the use of the sodium dodecyl sulfate–phosphate gels). For the sodium dodecyl sulfate–Tris–glycine gels the NHC protein samples were dialyzed to 2% sodium dodecyl sulfate–5% β-mercaptoethanol–10% glycerol–0.065 M Tris, pH 6.8. The samples were run on 5 mm × 10 cm gels at 50 V for *ca.* 7.5 hr using the running buffer of 0.025 M Tris–0.192 M glycine–0.1% sodium dodecyl sulfate, pH 8.3. [The final gel composition is 10% acrylamide, 0.27% *N,N'*-bis(methyleneacrylamide), 0.2% sodium dodecyl sulfate, 0.75 M Tris (pH 8.8), 0.037% *N,N,N',N'*-tetramethylenediamine, and 0.03% ammonium persulfate (Laemmli, 1970).] After electrophoresis the proteins were fixed in the gels with 50% $Cl_3CCOOH$ and stained with Coomassie Brilliant Blue (King and Laemmli, 1972). All gels were photographed using an orange filter and scanned at 600 μm on a spectrophotometer with a 0.05-mm slit. Protein loads were arbitrarily adjusted for maximum band resolution for each sample; typically 100 μg of protein was used for sodium dodecyl sulfate–Tris-glycine gels. In scanning, the optical density scale was set to approximately equalize major peak heights of the gels being compared.

## Materials

Tissues were dissected from adult male Sprague-Dawley rats immediately after the rats were killed by exsanguination. Tissues were washed in saline, frozen in liquid nitrogen, and stored at −80° until used for making chromatin. Sodium dodecyl sulfate (Sipon WD) was obtained from Alcolac Chemical Corp. (Baltimore, Md.). Acrylamide, *N,N'*-bismethyleneacrylamide, *N,N,N',N'*-tetramethylenediamine, and ammonium persulfate were Electrophoresis Purity Reagents from Bio-Rad, Richmond, Calif. All urea used was purified by passage of a 10 M solution through a mixed bed ion exchange column (Barnstead DO 803). Other chemicals used were reagent grade.

## Results

*Reproducibility of NHC Protein Preparations.* It has previously been documented that chromatin prepared by these techniques will have a given mass ratio of DNA : histone : NHC protein with a standard deviation of *ca.* 10% (Elgin and Bonner, 1970). Thus, one might anticipate quantitative variations of a similar magnitude in the amount of a given NHC protein present. The preparation of NHC protein from chromatin and the running and scanning of gels are highly reproducible procedures (Elgin, 1971). The reproducibility of the total procedure, including the preparation of chromatin, is indicated in Figure 1. In this instance NHC proteins were prepared from two separate rat liver chromatin preparations made on different days; the proteins have been analyzed on sodium dodecyl sulfate–Tris-glycine gels. The scans show that one reproducibly obtains the same gel pattern of NHC proteins in terms of the position of the protein bands (relative mobility) and in terms of the relative quantitative values for the major protein bands. Differences in total protein load, as in the two scans shown in Figure 1, have little effect on these parameters. However, it should be noted that the dye binding response with Coomassie Brilliant Blue is typically linear only in the range of 1–20 μg of protein/band. Beyond this range the response curve flattens out to give an overall S shape, resulting in an overestimate of protein in fine bands and an underestimate of protein in heavy bands. Thus, changes in the apparent relative amounts of protein in given bands will be observed with changes in total protein load if those bands contain less than 1 or more than 20 μg of protein. The amount of dye bound per milligram of protein has been found to vary by a factor of two or less in a survey of six standard proteins (Elgin, 1971). In the following analysis the gel patterns will be compared for the most part in terms of the presence or absence of protein bands in a given position (relative mobility, reflecting molecular weights).

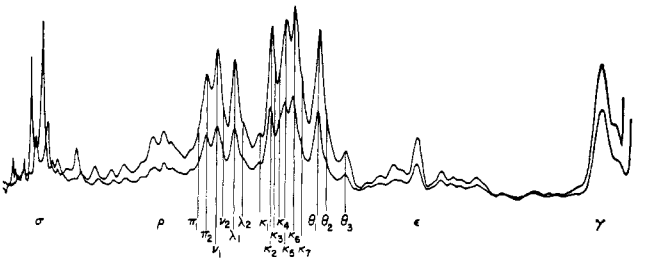

FIGURE 1: Reproducibility of NHC protein isolation procedure. Analysis on sodium dodecyl sulfate–Tris-glycine gels of NHC proteins from two independent preparations of rat liver chromatin. Gel origin at left.

*Comparison of Histones from Different Rat Tissues.* As anticipated from numerous earlier reports (*e.g.*, Fambrough *et al.*, 1968; Panyim and Chalkley, 1969), there are no significant qualitative differences in the histones of different rat tissues as analyzed by disc gel electrophoresis (Figure 2). Differences in the amount of histone III dimer (formed by cysteine oxidation) are observed; these are probably not biologically significant, as careful studies of rapidly isolated histones indicate that histone III exists in the reduced form in interphase chromatin *in vivo* (Sadgopal and Bonner, 1970; Dixon, 1972). No basic proteins other than histones are observed on the gels, indicating that our chromatin is free of contamination by ribonucleoprotein particles, etc. The histone gels constitute an important control on all the chromatin preparations used.

*Comparison of NHC Proteins.* SODIUM DODECYL SULFATE–PHOSPHATE GELS. Initial analyses of the NHC protein fraction utilized the sodium dodecyl sulfate–phosphate gel system. This gel system allows resolution of 14 major and seven additional minor components in the NHC protein fraction of rat liver; separation is accomplished over the mol wt range of 12,000 to several hundred thousand. Note that the protein has been reduced with β-mercaptoethanol and dissociated with sodium dodecyl sulfate; thus, the components will fractionate as individual polypeptide chains. The bands have previously

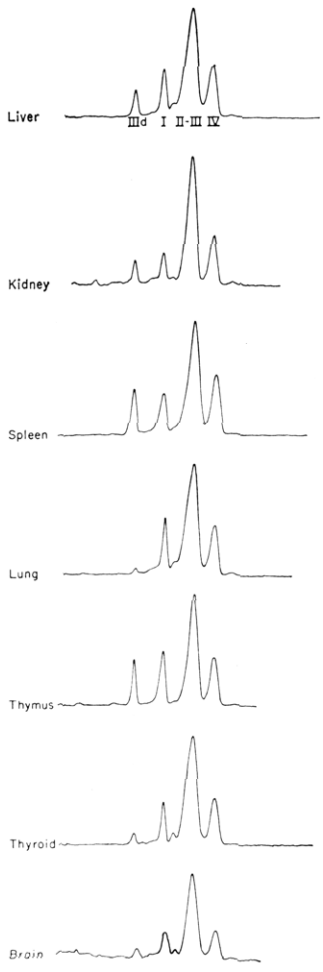


FIGURE 2: Histones of different rat tissues, urea gels. Gel origin at left.

been assigned arbitrary Greek letter names for purposes of discussion. These names and molecular weight markers are given in Figure 3 for orientation. Arbitrary vertical guide lines have been put on the figures to assist in comparisons. In our own analysis the scans were compared by superimposing

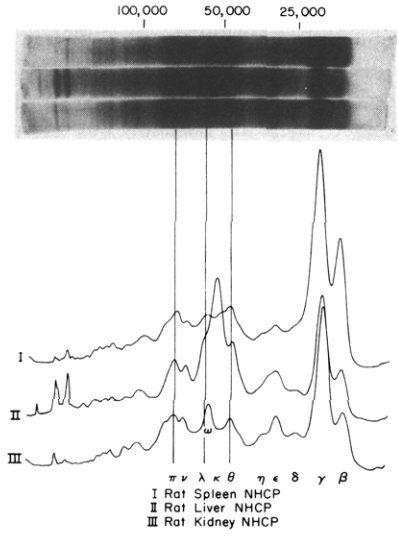


FIGURE 3: Comparison of the NHC proteins of rat spleen, liver, and kidney using sodium dodecyl sulfate–phosphate gels for analysis.

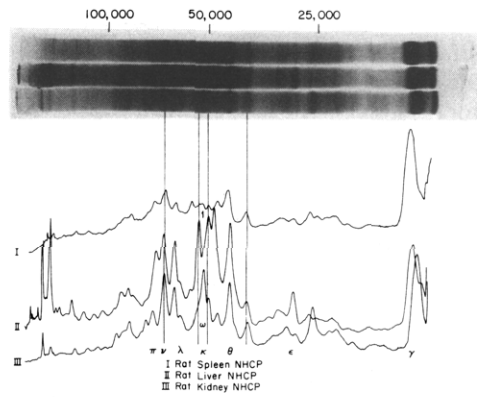


FIGURE 4: Comparison of the NHC proteins of rat spleen, liver, and kidney.

them directly onto each other using a light box. The liver NHC protein pattern is used as the standard for comparison throughout this study.

Comparative analysis utilizing these gels shows a high degree of homology among the NHC protein patterns of different tissues. The homology is particularly strong in the low molecular weight region (below 40,000) (see Figure 3). All tissues possess the bands $\beta$, $\gamma$, and $\epsilon$. Again in the high molecular weight region (top one-third of the gel, mol wt above 100,000) the homology in comparison with rat liver NHC protein bands is very striking for spleen and kidney (Figure 3) and lung (not shown) NHC protein. The most significant variations in the NHC protein band patterns occur in the middle molecular weight regions (40,000–100,000). For proteins of greater than 25,000 the homologous results were obtained in both sodium dodecyl sulfate gel systems. These will be discussed in detail only for the sodium dodecyl sulfate–Tris-glycine gels, which show better resolution.

Some comparisons of a quantitative nature can be made. Assuming equal dye binding coefficients, one can estimate that the sum of the protein in the major rat liver NHC protein bands ($\beta$, $\gamma$, $\epsilon$, $\theta$, $\kappa$, $\lambda$, $\nu$, $\pi$) accounts for at least 75% of the protein. Preliminary chemical work suggests that these bands together contain 10–15 different proteins (Elgin and Bonner, 1972), indicating that there are major NHC proteins of low and middle molecular weight. The general features of the NHC protein distribution over the molecular weight axis are the same for six of the tissues studied. There is a significant relative increase in high molecular weight proteins in brain. The low molecular weight protein bands are a prominent feature of the pattern in all cases, particularly from thymus (not shown). Observations on rat liver chromatin and NHC proteins have indicated that little or no proteolysis of the NHC proteins occurs under the conditions used (Elgin, unpublished observations). However, the question has not yet been examined in other tissues, and therefore the significance of the relative prominence of the low molecular weight bands in the thymus pattern is not known.

In order to improve resolution of the band patterns in the middle molecular weight region, sodium dodecyl sulfate–Tris-glycine gel analysis has been used. This gel system offers increased resolution in this molecular weight range, but does not separate proteins below 18,000 in mol wt.

SODIUM DODECYL SULFATE–TRIS-GLYCINE GELS. The increased resolving power in this gel system and the use of longer gels allow resolution of 45 NHC protein bands from rat liver (Figure 4). The general conclusions derived from the

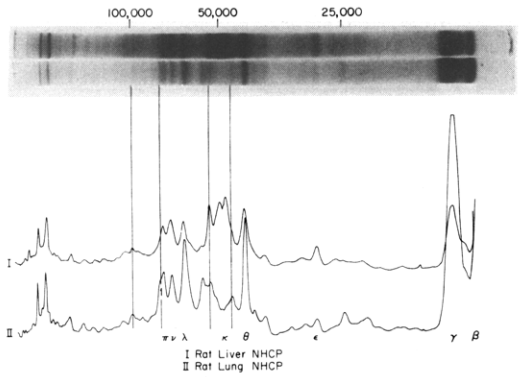


FIGURE 5: Comparison of the NHC proteins of rat liver and lung.

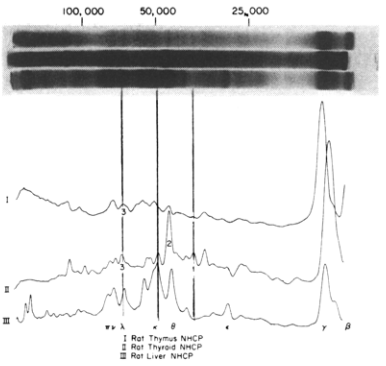


FIGURE 6: Comparison of the NHC proteins of rat thymus, thyroid, and liver.

above studies are substantiated by the experiments with the sodium dodecyl sulfate–Tris-glycine gels. The NHC protein patterns of different tissues show considerable homology. There is a spectrum of differences among the patterns; they range from those that are very similar (*e.g.*, kidney and spleen, Figure 4) to those that are very different (*e.g.*, liver and brain, Figure 7). The protein bands observed on the sodium dodecyl sulfate–Tris-glycine gels have been correlated with those on the sodium dodecyl sulfate–phosphate gels on the basis of molecular weight assignments (Table I). [Note that this represents a revision of molecular weights previously obtained using a 5% acrylamide–sodium dodecyl sulfate gel system and confirmation of results obtained using a 10% acrylamide–sodium dodecyl sulfate gel system (Elgin and Bonner, 1972).] The increased resolution allows one frequently to discern several distinct bands in a molecular weight region occupied by one apparent band in the sodium dodecyl sulfate–phosphate gel system. These bands have been labeled by using subscripts with the previously assigned Greek letter. The detailed sodium dodecyl sulfate–Tris-glycine gel nomenclature is given in Figure 1.

Comparative analysis of the NHC protein patterns of the different rat tissues on sodium dodecyl sulfate–Tris-glycine gels reveals similar homology to that noted before (Figures 4–7). Data on the presence or absence of a band at a given relative mobility in the middle molecular weight range (40,000–100,000) are tabulated in Table II. Generally the NHC protein patterns fall into three groups on the basis of homologies: liver, kidney, spleen, and lung; thymus and thyroid; and brain. Within each group there is considerable homology in the high molecular weight region (above 100,000) and low molecular weight region (below 40,000) of the protein spectrum. In the middle molecular weight region protein bands not observed in NHC protein preparations from the first group of tissues are found in preparations from brain and from thymus or thyroid. These bands are numbered in order of increasing molecular weight in a given NHC protein pattern and listed in Table II as "new 3" etc. Under the greater resolving power of this system, "new" bands in the sodium dodecyl sulfate–phosphate system can turn out to be the consequence of quantitative shifts among the band subcomponents. For example, band $\omega$ in rat kidney NHC proteins (Figure 3) represents a significant increase in $\kappa_4$, discernible as a shoulder on $\kappa_5$ in the rat liver NHC proteins (Figure 4). Nonetheless, there do seem to be certain NHC protein bands that are present only in a given tissue (*e.g.*, brain), or group of tissues (*e.g.*, thymus and thyroid). In addition to the numbered middle molecular weight protein bands, several unique high molecular weight protein bands are observed in the brain NHC proteins.

In considering the quantitative aspects of the data it is apparent that there are major NHC proteins. Over 50% of the protein above mol wt 18,000 in the rat liver pattern is contained in the eight major bands, $\pi_2$, $\nu_1$, $\lambda_1$, $\kappa_2$, $\kappa_5$, $\kappa_6$, $\theta_1$, and $\epsilon$; this estimate is made from the relative peak areas from the gel scan (see Figure 5). Again the brain NHC protein pattern is unique in the relatively large amount of high molecular weight protein observed (Figure 7).

TABLE I: Comparative Molecular Weights of NHC Proteins.

| NHC Protein | Mol Wt: Sodium Dodecyl Sulfate–Phosphate Gels | Mol Wt: Sodium Dodecyl Sulfate–Tris-Glycine Gels |
|---|---|---|
| $\pi_2$ | 81,000 | 80,000 |
| $\nu_1$ | 73,000 | 74,000 |
| $\lambda_1$ | 66,000 | 66,000 |
| $\kappa_2$ | 55,000 | 56,000 |
| $\kappa_5$ | 55,000 | 55,000 |
| $\kappa_6$ | 55,000 | 48,000 |
| $\theta_1$ | 44,000 | 43,000 |
| $\epsilon$ | 30,000 | 28,000 |

## Discussion

The results of this study substantiate the concept of extensive molecular weight homology among the patterns of the NHC proteins of different tissues of a given organism. This discussion is, of course, subject to the limitations of the analytical technique used (molecular weight sieving) and the

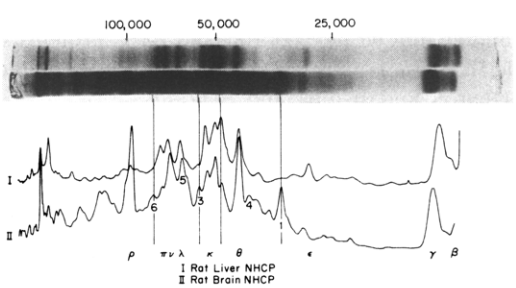


FIGURE 7: Comparison of the NHC proteins of rat liver and brain.

TABLE II: Presence (+) or Absence (0) of NHC Proteins in Rat Tissues.[a]

| Protein | Liver | Kidney | Spleen | Lung | Brain | Thymus | Thyroid |
|---|---|---|---|---|---|---|---|
| | | | | | New 6 | | |
| $\pi_1$ | + | + | + | ++ | 0 | + | + |
| $\pi_2$ | ++ | 0 | + | ++ | + | 0 | ++ |
| $\nu_1$ | ++ | ++ | ++ | ++ | ++ | ++ | ++ |
| $\nu_2$ | 0 | + | + | 0 | ++ | 0 | 0 |
| | | | | | | New 3 | New 3 |
| $\lambda_1$ | ++ | ++ | + | ++ | 0 | ++ | 0 |
| | | | | | New 5 | | |
| $\lambda_2$ | + | + | + | 0 | + | + | + |
| $\kappa_1$ | + | 0 | + | ++ | 0 | + | + |
| | | | | | New 3 | | |
| $\kappa_2$ | ++ | + | 0 | ++ | ++ | ++ | 0 |
| $\kappa_3$ | 0 | 0 | + | 0 | 0 | + | ++ |
| $\kappa_4$ | + | ++ | + | + | + | + | ++ |
| $\kappa_5$ | ++ | ++ | + | + | ++ | ++ | 0 |
| $\kappa_6$ | ++ | 0 | 0 | + | + | 0 | ++ |
| $\kappa_7$ | + | + | + | + | + | + | 0 |
| | | | | | | New 2 | New 2 |
| $\theta_1$ | ++ | ++ | ++ | ++ | ++ | 0 | 0 |
| $\theta_2$ | + | + | 0 | + | 0 | + | + |
| | | | | | New 4 | | |
| $\theta_3$ | + | + | + | + | + | 0 | + |
| | | | | | | New 1 | New 1 |

[a] ++ indicates a dominant protein band; see Figures 4–7.

sensitivity of protein detection. The major conclusions may be summarized as follows.

(1) There are 12–18 major proteins (defined here by molecular weight bands) that make up the majority of the NHC protein mass. In addition there are many minor NHC proteins, the total number probably being limited at this time only by the resolving power of the analytical system employed.

(2) Most of the NHC protein bands are present in most of the tissues examined. Nonetheless, each tissue possesses a unique pattern of NHC proteins, both in terms of the presence or absence of the protein bands and in terms of which protein bands are quantitatively dominant.

(3) A few NHC protein bands were found in all tissues examined, *i.e.*, $\gamma$, $\epsilon$, $\kappa_4$, and $\nu_1$.

(4) A few NHC protein bands appear to be specific to certain tissues (brain) or a subgroup of tissues (thymus and thyroid).

(5) Comparisons of the NHC protein gel patterns show a continuum of differences, ranging from those that are very much alike (*e.g.*, kidney and spleen) to those with marked differences (*e.g.*, liver and brain). It is also possible to place the tissues examined in three groups according to the analysis of the middle mol wt (40,000–100,000) NHC proteins shown in Table II. These groups are (1) liver, lung, spleen, and kidney; (2) thyroid and thymus; and (3) brain.

It should be stressed that this analysis indicates the presence of a limited number of major NHC proteins. Thus, it will be possible to isolate these proteins and to characterize them chemically. Preliminary chemical characterization of isolated rat liver NHC protein fractions also suggests that there is limited heterogeneity in that about eight N-terminal end groups are found for five molecular weight bands (one for $\epsilon$, one for $\theta_1$, and four–six for a fraction containing $\theta_2$, $\kappa$, $\lambda$, and $\pi$) (Elgin and Bonner, 1972). The results on the limited tissue specificity of the major NHC proteins reported here are in agreement with those of MacGillivray *et al.* (1972). These authors have recently reported finding little tissue specificity in the NHC protein patterns of mouse liver, spleen, and kidney, but a striking increase in the high molecular weight NHC proteins of mouse brain. The observations reported here using rat tissues further emphasize the uniqueness of the brain NHC proteins.

*A priori* it seems reasonable to assume that the NHC protein fraction will include structural proteins (such as those at the chromocenter), enzymes of chromosomal metabolism (polymerases, nucleases, etc.), and repressor or activator molecules related to the extent and tissue specificity of chromatin transcription. Proteins of the last category involved in the specific transcription of one or a few genes would be present in amounts too small to be detected by present analytical techniques. However, those proteins involved in large, coordinated changes in template activity (for example, in hormone response or in control of developmental programs) might be observed using disc gel electrophoresis. One might also observe shifts in the relative amounts of NHC proteins in tissue comparisons reflecting differences in cell division rates, differences in heterochromatin/euchromatin ratios, etc. Several of these possibilities could be invoked to explain the NHC protein pattern relationships observed here. In particular, it is interesting to note that studies on the transcription *in vivo* of unique DNA sequences in different mouse tissues have found that while *ca.* 4% of the DNA is transcribed in most tissues (liver, kidney, spleen), up to 11% is transcribed in brain (Grouse *et al.*, 1972). The pattern of rat brain NHC proteins is unusual in the amount of high molecular weight protein and differs more from that of other rat tissues than

those patterns differ from one another (Figures 4–7). Further investigation to determine whether or not there is any functional relationship between these observations is planned. It should be emphasized that all observations of alterations in the NHC protein pattern indicate small changes within a relatively stable pattern. This fact, coupled with the observations of considerable homology and little tissue specificity in studies of the NHC protein patterns from different tissues such as that reported here, suggest that the bulk of the proteins function in universal, nonspecific structural and enzymic roles. Further investigation to determine the general and the specific roles of these proteins in chromosomal architecture and metabolism is certainly warranted.

References


Bonner, J., Chalkley, G. R., Dahmus, M., Fujimura, F., Huang, R. C., Huberman, J., Jensen, R., Marushige, K., Ohlenbusch, H., Olivera, B., and Widholm, J. (1968), *Methods Enzymol. 12B*, 3.

Busch, H., Choi, Y. C., Daskal, I., Inagaki, A., Olson, M. O. J., Reddy, R., Ro-Choi, T. S., Shibata, H., and Yeoman, L. C. (1972), *Acta Endocrinol.* (Copenhagen), *Suppl. No. 168*, 33.

Chetsanga, C. J., Poccia, D. L., Hill, R. J., and Doty, P. (1970), *Cold Spring Harbor Symp. Quant. Biol. 35*, 629.

DeLange, R. J., and Smith, E. L. (1971), *Annu. Rev. Biochem. 40*, 279.

Dixon, G. H. (1972), *Acta Endocrinol.* (Copenhagen), *Suppl. No. 168*, 110.

Elgin, S. C. R. (1971), Ph.D. Thesis, California Institute of Technology, Pasadena, Calif.

Elgin, S. C. R., and Bonner, J. (1970), *Biochemistry 9*, 4440.

Elgin, S. C. R., and Bonner, J. (1972), *Biochemistry 11*, 772.

Elgin, S. C. R., Froehner, S. C., Smart, J. E., and Bonner, J. (1971), *Advan. Cell Mol. Biol. 1*, 1.

Fambrough, D. M., Fujimura, F., and Bonner, J. (1968), *Biochemistry 7*, 575.

Grouse, L., Chilton, M. D., and McCarthy, B. J. (1972), *Biochemistry 11*, 798.

King, J., and Laemmli, U. K. (1972), *J. Mol. Biol. 62*, 465.

Laemmli, U. F. (1970), *Nature (London) 227*, 680.

Loeb, J. E., and Creuzet, C. (1970), *Bull. Soc. Chim. Biol. 52*, 1007.

MacGillivray, A. J., Cameron, A., Krauze, R. J., and Rickwood, D. (1972), *Biochim. Biophys. Acta 277*, 384.

MacGillivray, A. J., Carroll, D., and Paul, J. (1971), *FEBS (Fed. Eur. Biochem. Soc.) Lett. 13*, 204.

Marushige, K., and Bonner, J. (1966), *J. Mol. Biol. 15*, 160.

Panyim, S., and Chalkley, R. (1969), *Biochemistry 8*, 3972.

Paul, J., and Gilmour, R. S. (1966), *J. Mol. Biol. 16*, 242.

Paul, J., and Gilmour, R. S. (1968), *J. Mol. Biol. 34*, 305.

Platz, R. D., Kish, V. M., and Kleinsmith, L. J. (1970), *FEBS (Fed. Eur. Biochem. Soc.) Lett. 12*, 38.

Richter, K. H., and Sekeris, C. E. (1972), *Arch. Biochem. Biophys. 148*, 44.

Sadgopal, A., and Bonner, J. (1970), *Biochim. Biophys. Acta 207*, 227.

Shapiro, A. L., Vinuela, E., and Maizel, J. V. (1967), *Biochem. Biophys. Res. Commun. 28*, 815.

Shaw, L. M. J., and Huang, R. C. (1970), *Biochemistry 9*, 4530.

Shelton, K. R., and Neelin, J. M. (1971), *Biochemistry 10*, 2342.

Shih, T. Y., and Bonner, J. (1970), *J. Mol. Biol. 48*, 469.

Smart, J. E., and Bonner, J. (1971), *J. Mol. Biol. 58*, 675.

Smith, K. D., Church, R. B., and McCarthy, B. J. (1969), *Biochemistry 8*, 4271.

Teng, C. S., Teng, C. T., and Allfrey, V. G. (1971), *J. Biol. Chem. 246*, 3597.