Qualitative Species Differences and Quantitative Tissue Differences in the Distribution of Lysine-rich Histones*

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SUMMARY

Species-specific differences in the distribution of lysine-rich histones were investigated in spleen tissue of three mammalian (calf, cat, and rat) and one avian (chicken) species. Tissue-specific differences in the distribution of the lysine-rich histones were studied in liver, kidney, spleen, and thymus tissues of the rat; liver, spleen, and thymus of the calf; and liver, spleen, and erythrocytes of the chicken. The lysine-rich histone fractions were extracted with aqueous trichloroacetic acid from 0.14 M NaCl-washed tissue homogenates and isolated nuclei. The extracts were fractionated by gradient elution chromatography on Amberlite IRC-50 with a linear gradient of guanidinium chloride.

Species-specific lysine-rich histone components were observed in the elution profiles for the spleen of calf, rat, cat, and chicken which showed three, five, four, and five distinct components, respectively. Studies on the electrophoretic mobility of the whole lysine-rich histone fractions in polyacrylamide gels showed that the rat spleen fraction contained a component not found in either calf spleen or chicken spleen. In addition, the chicken spleen fraction contained a component not found in the calf spleen fraction. Amino acid analysis of the individual chromatographic components of four different species showed that the over-all compositions were remarkably similar except for a single component found in four tissues of the rat. Treatment of this component with cyanogen bromide indicated that methionine was an integral part of the primary structure. This is the first report of the presence of methionine in a purified lysine-rich histone from any source.

Quantitative differences were found between a number of corresponding lysine-rich histones of different tissues of the same species, both mammalian and avian, but the complement of histones for a given species was identical in all tissues studied. Planimetric analysis of duplicate chromatographic profiles of three separate preparations of the rat thymus, spleen, liver, and kidney fractions showed that the thymus fraction contained a 4- to 7-fold greater amount of Component 1 compared to the liver, spleen, and kidney fractions. The kidney fraction contained about one-half the amount of both Components 2 and 5 compared to the thymus, liver, and spleen fractions. All four tissues contained similar relative proportions of Component 3, but the kidney fraction contained approximately twice as much of Component 4 compared to the other three fractions. Absorbance profiles of polyacrylamide gel electrophoresis patterns of the lysine-rich histone fractions of rat thymus, spleen, and liver provided similar results. However, precise quantitative determination of minor components was not possible because of the relatively small differences in mobility between certain components.

In recent years evidence has accumulated to support the hypothesis that histones are involved in some way in the process of gene regulation (2-5). The idea of a different histone for each gene (6, 7) implied that the histones contained the necessary biological specificity to combine with the appropriate genes and predicted a widespread species and tissue heterogeneity for these basic proteins. In the past, however, there has been a failure to find such heterogeneity (8, 9) and specificity (10, 11), and much emphasis has been placed on the over-all similarity of histone fractions derived from a variety of sources which are widely separated on the evolutionary scale (8, 11, 12). Although species-specific differences have been found for the histone complement of several nonmammalian species (13-15), the failure to show qualitative or quantitative (or both) species and tissue differences in the distribution of the histones has been considered a basic weakness of the hypothesis that histones function as gene regulators.

Previous studies on the chromatographic fractionation of the lysine-rich histones of calf thymus have shown that this histone fraction is comprised of a group of proteins which have similar molecular weights (16, 17) and closely related primary structures (18, 19). For this reason, it was believed that a similar chromatographic investigation might be useful for detecting differences in the complement of lysine-rich histones of different species and differences in the distribution of the lysine-rich histones of different tissues of the same species. The spleen appeared to be an eminently suitable tissue for the species studies because of the

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ease of isolation of this organ and its relatively high content of lysine-rich histones (20). The organs selected for a comparison of the tissue distribution of lysine-rich histones represent a relatively wide range. The results presented in this paper show that there are qualitative differences in the complement of the lysine-rich histones of different species and quantitative differences between certain of the corresponding components of different tissues of the same species.

**EXPERIMENTAL PROCEDURE**

**Sources and Preparation of Histone**—Calf thymus and spleen were obtained within 20 min of the animal's death, frozen in crushed, solid carbon dioxide, and extracted as soon as possible. Rat tissues were obtained from male, albino rats (Cheek-Jones Company, Houston, Texas, and Simonsen Laboratories, St. Paul, Minnesota) weighing 200 to 300 g which were fed ad libitum on Purina laboratory chow. Cat spleen was obtained from animals anesthetized with pentobarbital sodium. Chicken tissues were obtained from White Leghorn chickens. Chicken erythrocytes were isolated from fresh blood and washed three times with 0.14 M sodium chloride containing 0.01 M trisodium citrate. Isolated nuclei of fresh calf and rat liver were obtained by a modification of the method of Chauveau, Moule, and Rouiller (21) as described by Steele (22). All tissues were extracted initially with 0.14 M NaCl, pH 4, at 2-4°C by homogenization with a high speed blender within 30 min of their excision (23). Isolated nuclei were extracted with the same medium by homogenization with a Teflon-glass homogenizer. The lysine-rich histone fraction was prepared by extraction of the 0.14 M NaCl-washed homogenates and isolated nuclei with aqueous trichloroacetic acid as described previously (18). Some preparations of the lysine-rich histone fraction of nuclei of rat and calf liver were isolated in the presence of 0.05 M sodium bisulfite (24).

**Chromatography**—The crude lysine-rich histone preparation was fractionated by gradient elution chromatography on Amberlite IRC-50 (Bio-Rex 70, 10 meq per g dry weight, 200 to 400 mesh, Bio-Rad) according to the method of Kinkade and Cole (18) at 25-27°C with a linear gradient of guanidinium chloride (7% to 140%, 850 ml each) containing 0.1 M sodium phosphate buffer, pH 6.8. Column effluents were monitored at 218 rnp. The guanidinium chloride (Eastman grade) was purified by passage through a Norite-Celite column as described earlier (18) to remove ultraviolet absorbing impurities. The guanidinium chloride solution (7% to 140%, 850 ml each) containing 0.1 M sodium phosphate buffer, pH 6.8, was prepared by measuring ultraviolet absorption at 218 rnp with a Gilford model 2410 spectrophotometer. The concentration of guanidinium chloride solutions was determined with the aid of an Abbe-3L refractometer (Bausch and Lomb) as described previously (18). Column effluents and salt-free purified components were analyzed by discontinuous electrophoresis in 15% polyacrylamide gels (29); 20% gels were also prepared from the same stock solutions as follows: 1 part Solution A, 2 parts Solution C, and 3 parts ammonium persulfate (0.28%, w/v). The gels were stained for 16 hours with Amido schwarz (1%) dissolved in 7% acetic acid and electrolytically destained. The gels were monitored for absorbance at 580 µm with a gel scanner (Gilford model 2410) at a speed of 1 cm per min with a slit width of 0.05 mm.

**Amino acid analysis** was performed on protein hydrolysates (6 N HCl at 110° for 22 hours) with an automatic amino acid analyzer (model 120C Beckman-Spinco) according to the method of Moore and Stein (29). The samples were prepared for hydrolysis as recommended by Crestfield, Moore, and Stein (30).

**Cyanogen Bromide Cleavage**—The protein at a concentration of 1% was treated with cyanogen bromide (Eastman grade) in 70% (v/v) formic acid at 25°C (31). A molar ratio of cyanogen bromide to methionine of 160:1 was used; the calculations were based on the composition of the protein and the assumption that lysine-rich histones of rat and calf thymus have similar molecular weights (16, 17). The reaction was allowed to proceed for 24 hours, after which time the mixture was either diluted with water and lyophilized or chromatographed on Sephadex G-25 (medium grade) to remove formic acid and cyanogen bromide decomposition products.

**RESULTS**

**Fractionation of Species-specific Lysine-rich Histones**—Previous studies (18) have shown that the whole lysine-rich histone fraction of calf thymus tissue may be resolved into three distinct components by chromatography on Amberlite IRC-50. Fig. 1 presents representative chromatographic profiles of the whole lysine-rich histone fraction of spleen tissue of four different species.
obtained by chromatography on Amberlite IRC-50. The profiles for the spleen of the calf (Fig. 1a), cat (Fig. 1b), rat (Fig. 1c), and chicken (Fig. 1d) show that the preparations were resolved into at least three, four, five, and five components, respectively. These results indicate that there are species-specific differences among the lysine-rich histone fractions of the spleen of these four species. The most striking difference was seen for Component 5 of rat spleen (Fig. 1c), since this component was eluted at a higher guanidinium chloride concentration than any of the other spleen components of the rat or other species. Other differences were observed in the sequence of elution of components of the other species. For example, Component 4 of chicken spleen (Fig. 1d) was eluted prior to Component 4 of rat spleen (Fig. 1c) and Component 5 of chicken spleen was eluted between Components 4 and 5 of rat spleen. Furthermore, Component 5 of chicken spleen was eluted after Component 3 of calf spleen (Fig. 1a) and Component 4 of cat spleen (Fig. 1b), and thus did not appear to be represented by a similar component in the calf and the cat. In addition, Component 2 of the cat spleen fraction (Fig. 1b) was eluted between Components 1 and 2 of the calf spleen (Fig. 1a).

Electrophoretic Resolution of Species-specific Lysine-rich Histones—Because previous studies (18) had suggested that the various lysine-rich histone components of calf thymus were poorly resolved by polyacrylamide gel electrophoresis, it was of interest to investigate this problem with several of the whole fractions from other species. Fig. 2 presents the representative patterns obtained by electrophoresis in 15% as well as 20% polyacrylamide gels. The lysine-rich histone fractions of chicken spleen (Gel 1) and calf spleen (Gel 4) were resolved into two major bands (Gel 1, a and b, and Gel 4, b and c, respectively) while the rat spleen preparation (Gel 3) was separated into three major bands (b, d, and f). It should be pointed out that the whole lysine-rich histone fraction obtained by trichloroacetic acid extraction of the crude deoxyribonucleoprotein residue of certain tissues may contain traces of other histone fractions and degradation products that also produce visible bands. For example, the chicken spleen preparation (Gels 1 and 6) contained a rapidly migrating band (a) which was not found in any of the chromatographic components of the lysine-rich histone fraction. It is possible that this band may represent the serine-rich histone which is characteristic of the chicken erythrocyte fraction, since the spleen probably contained a relatively large amount of erythrocytes. Fig. 2 also shows that the rat spleen (Gel 3) and the calf spleen (Gel 4) contained a band (a) which migrated slightly ahead of the fastest migrating major band in each of these fractions. These fast moving bands probably represent degradation products, since they were absent from the chromatographically purified components of these two species and the whole fractions obtained under optimal conditions of isolation (18).

In an attempt to determine whether species-specific differences could be detected by electrophoresis, mixtures of whole lysine-rich histone fractions of spleen tissue of several species were studied by the co-electrophoretic method (18). The mobilities of the various components were reproducible for a given set of conditions, and the gels were aligned with respect to the origins. The gel patterns in Fig. 2 show that Band b of rat spleen (Gel 3) was not present in the chicken spleen (Gel 1), and Band b of the chicken spleen (Gel 6) was absent in the calf spleen fraction (Gel 4). In general, it was found that more components were resolved by chromatography than by electrophoresis. For example, chromatographic Component 1 of calf spleen and Component 5 of chicken spleen were eluted at markedly different guanidinium chloride concentrations (Fig. 1), but had identical electrophoretic mobilities on polyacrylamide gel. An exception to this finding was observed in the case of chromatographic Component 9 of rat spleen and Component 2 of calf spleen which were eluted at approximately the same guanidinium chloride concentration but had widely different mobilities on polyacrylamide gel electrophoresis.

Tissue Distribution of Lysine-rich Histones of Rat—Fig. 3 presents the chromatographic profiles for the whole lysine-rich histone fractions of the thymus (Fig. 3a), kidney (Fig. 3b), liver (Fig. 3c), and spleen (Fig. 3d) tissues of the rat. A comparison of these profiles shows that each of the four tissues contained the same complement of lysine-rich histones, but that...
FIG. 2. Polyacrylamide gel electrophoresis of the whole lysine-rich histone fraction of the spleen of: 1, chicken spleen, 10 μg; 2, co-electrophoresis of chicken spleen, 10 μg and rat spleen, 20 μg; 3, rat spleen, 20 μg; 4, calf spleen, 5 μg; 6, co-electrophoresis of calf spleen, 5 μg and chicken spleen, 10 μg. Gels 1 to 3 were 20% polyacrylamide and were run at 5 mA per gel for 2 hours. Gels 4 to 6 were 15% polyacrylamide and were run for 55 min at 10 mA per gel.

FIG. 3. Chromatographic profiles (Amberlite IRC-50) for the lysine-rich histone fraction of thymus, kidney, liver, and spleen tissue of the rat. The conditions were the same as described in the legend to Fig. 1. a, thymus, 23 mg; b, kidney, 26 mg; c, liver, 28 mg; d, spleen, 27 mg. The elution profiles for the thymus, liver, and spleen were normalized at the peak absorbance value of Component 5 of the thymus profile.

some of the components were present in different amounts in the different tissues. Electrophoretic studies showed that the mobilities in polyacrylamide gel for the corresponding components of each tissue were identical. Additional evidence for correspondence between the lysine-rich histones of each tissue was provided by cochromatography studies on the whole fractions of several tissues of the rat. Fig. 4 presents the profiles for the individual fractions of rat thymus and spleen and the profile obtained for an approximately equal mixture of the two fractions. These results show that both of the tissues contained the same complement of lysine-rich histones.

To investigate possible differences in the relative amounts of individual components of different tissues of the rat, the lysine-rich histone fraction was prepared on three separate occasions from pooled samples of the four tissues studied above. Each preparation was chromatographed twice and the resulting elution profiles were analyzed by planimetry. The data in Table I show that significant quantitative differences were found...
between some of the corresponding lysine-rich histones of the different tissues. The thymus fraction contained a 4- to 7-fold greater amount of Component 1 compared to the liver, spleen, and kidney fractions. In addition, the thymus of the rat contained a higher proportion of Component 2 than the kidney and spleen, and the kidney contained about one-half of the amount of Component 2 found in the other tissues. The proportion of Component 3 was relatively similar for all four of the tissues studied; however, the spleen fraction appeared to contain slightly more of Component 3 than either the kidney or thymus fractions. On the other hand, the kidney fraction contained approximately twice the amount of Component 4 compared to the other three tissues, and the thymus fraction contained the smallest amount of Component 4. The kidney fraction contained about one-half of the amount of Component 5 found in the other three tissue fractions. It is interesting to note that the distribution of the lysine-rich histones was remarkably similar for the liver and spleen fractions of the rat. The same qualitative differences were observed when the lysine-rich histone fractions were prepared from isolated nuclei of rat liver and 0.14 M NaCl-washed homogenates of rat thymus in the presence of sodium bisulfite (24).

Electrophoresis of Lysine-rich Histones of Different Tissues of Rat—The relative mobilities of the five chromatographic components of the lysine-rich histone fraction of the rat (cf. Fig. 3) were found by co-electrophoresis of mixtures of individual chromatographic components (18) to be 3 > 5 > 4 > 1 > 2. Fig. 5 shows representative electrophoretic patterns for the lysine-rich histone fraction of liver, spleen, and thymus tissues of the rat. Electrophoresis in 20% polyacrylamide gels resolved all five of the chromatographic components. However, only the three major bands were detected by spectrophotometric scanning of the gels, because of the small amount of separation between some of the components. Although this method could not be used to determine the relative amounts of each of the individual lysine-rich histones of the rat, the patterns in Fig. 5 show that the liver, spleen, and thymus tissues contain different relative amounts of the three major components. The shoulder on the leading edge of the fastest migrating lysine-rich histone of rat spleen (Fig. 5c) corresponds to chromatographic Component 5. The electrophoretic mobility of this component was found to be concentration-dependent, a finding which agrees with earlier studies (32) on the electrophoretic mobility of certain histones in starch gels.

**Table I**

Distribution of chromatographic components of lysine-rich histone fraction of liver, kidney, spleen, and thymus tissues of rat

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Chromatographic component*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Thymus</td>
<td>%</td>
</tr>
<tr>
<td>Liver</td>
<td>15 ± 0.9</td>
</tr>
<tr>
<td>Spleen</td>
<td>4 ± 0.5</td>
</tr>
<tr>
<td>Kidney</td>
<td>4 ± 0.7</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Tissues compared</th>
<th>Level of significance of differences between components b</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thymus/liver</td>
<td>p &lt; 0.001</td>
</tr>
<tr>
<td>Thymus/spleen</td>
<td>p &lt; 0.001</td>
</tr>
<tr>
<td>Thymus/kidney</td>
<td>p &lt; 0.05</td>
</tr>
<tr>
<td>Liver/spleen</td>
<td>p &lt; 0.01</td>
</tr>
<tr>
<td>Liver/kidney</td>
<td>p &lt; 0.001</td>
</tr>
<tr>
<td>Spleen/kidney</td>
<td>p &lt; 0.05</td>
</tr>
</tbody>
</table>

* Values reported as percentage of total lysine-rich histone fraction. Each value is the mean ± standard error of three different preparations (N = 3) each of which was chromatographed two times (cf. Fig. 3).

b Blank space indicates no significant difference at p < 0.05.

![Fig. 4. Chromatographic profiles (Amberlite IRC-50) for the lysine-rich histone fraction of rat thymus and spleen and a mixture of the two fractions. The conditions were the same as described in the legend to Fig. 1. The elution profile for the mixture (C—C) was found to be equal to the sum of the individual profiles for the rat thymus (18 mg), A—A, and the rat spleen (16 mg), B—B.](image-url)
and thymus contain the same three components, but that these components were present in different relative amounts in each tissue. For example, the liver contained a smaller amount of Component 1 than the thymus, and the spleen contained a relatively larger amount of Components 1 and 3 compared to the thymus. The relative proportions of the three components were nearly identical (±3%) in all of the preparations chromatographed. Co-electrophoresis of the corresponding individual components of each of these tissues showed that the components of the liver, spleen, and thymus had the same mobilities. Furthermore, it was found that, like the thymus (18), chromatographic Component 3 of liver and spleen actually contained two components, designated 3a and 3b (Fig. 6c). Cochromatography of the whole lysine-rich histone fraction of thymus with that of liver and spleen also showed that each tissue contained the same complement of lysine-rich histones.

The whole lysine-rich histone fractions of three tissues of the chicken were also fractionated by chromatography on Amberlite IRC-50 to investigate the possibility of tissue-specific differences in another class of animals. The elution profiles for the liver (Fig. 7a), spleen (Fig. 7b), and erythrocyte (Fig. 7c) tissues of the chicken show that all three tissues contained the same major components, but that some of the components were present in different amounts. The erythrocyte fraction appeared to contain more of Components 1 and 3 than either the liver or the spleen. The spleen, on the other hand, appeared to contain greater amounts of Component 4 than the liver or erythrocytes. The relative proportions of Components 1, 2 through 4, and 5 were nearly identical (±5%) in all of the preparations chromatographed. Cochromatography studies on the whole lysine-rich histone fraction of chicken erythrocytes with those of liver and spleen provided additional evidence to support the idea that each tissue contained the same components. Furthermore, electrophoretic studies showed that corresponding components from each of the three tissues had identical mobilities in polyacrylamide gel.

The absence of distinct chromatographic resolution of Component 4 of chicken erythrocytes (Fig. 7c) compared to that found for the liver (Fig. 7a) and spleen (Fig. 7b) fractions suggested the presence of another component between Components 3 and 4. This component appeared to occur in greater amounts in the erythrocyte fraction than in the liver and spleen fractions. The spleen fraction was chosen to investigate this question because of a more favorable ratio between the amounts of Components 3 and 4 compared to that found in the erythrocyte fraction. To verify the reproducibility of elution volumes for the various components, selected regions of the initial elution profile for the spleen (Fig. 8a) designated A, B, and D were pooled and rechromatographed on the same column (Fig. 8b). This profile (Fig. 8b) shows the presence of a new component (3a) eluting between Components 3 and 4 of the spleen fraction. To establish that Component 3a actually precedes Component 4, the region of the profile (Fig. 8b) designated E was rechromatographed on the same column together with the components present under area C of Fig. 8a. The elution profile for this mixture of components (Fig. 8c) also shows that Component 3a was eluted between Components 3 and 4. Similar experiments showed that Component 3a was also present in the liver and erythrocyte fractions. Component 3a was not separated from Components 3 and 4 by electrophoresis in polyacrylamide gels under the conditions used in this study. The electrophoretic patterns (Fig. 9) for Components 3, 3a, and 4 of the spleen (Gels 1 to 3) and erythrocyte tissue (Gels 4 to 6) show only two main bands.

Amino Acid Analysis—Amino acid analysis was performed on chromatographic components of the lysine-rich histones of cat...
spleen, chicken erythrocyte, calf thymus, and rat thymus, each of which was found to migrate as a single band in polyacrylamide gel electrophoresis. Table II presents the data on the amino acid composition of the components of the cat, chicken, and calf tissues and the one significantly different component of rat thymus. The over-all amino acid compositions of the various lysine-rich histone components were remarkably similar with the exception that Component 3 of rat thymus contained methionine in an amount equivalent to tyrosine and phenylalanine. This component was also found in three other tissues of the rat: spleen, liver, and kidney. Methionine has been reported previously as occurring in the lysine-rich histone fraction obtained by other workers (13, 33); however, its presence was generally considered to result from contamination by other histone fractions and their degradation products (34, 35). Other studies have shown that methionine is completely absent from the purified components of calf thymus lysine-rich histone (18). In a later section evidence is presented to support the conclusion that methionine is an integral component of the primary structure of rat lysine-rich histone.

Previous studies suggested that the presence of histidine in the lysine-rich histone fraction may be attributed to contamination by degradation products of other histone fractions (34, 35). In addition, histidine has been shown to be completely absent from purified calf thymus lysine-rich histones (18). The data in Table II indicate that histidine was present in Components 1 and 4 of the cat spleen fraction and Components 1, 2 + 3, and 4 of the chicken erythrocyte fraction, but absent from the thymus fractions. These results confirm previous studies in which histidine has been found in chromatographically purified lysine-rich histones of chicken erythrocyte (13) and chicken liver.1

A comparison of the data (Table II) for the valine content of the various chromatographic components of an individual species shows that the initial components have a higher content of valine than those eluted with higher concentrations of guanidinium chloride. If one assumes that the various com-

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1 Dr. R. D. Cole, personal communication.
I. Fractionation and Analysis

Effluent Volume (ml)

Fig. 8. Chromatographic profiles (Amberlite IRC-50) for the lysine-rich histone fractions of chicken spleen. The conditions were the same as described in the legend to Fig. 1: a, whole fraction, 30 mg; b, rechromatography of protein recovered from areas A, B, and D of a; c, rechromatography of protein recovered from area C of a and area E of b.

II. Amino Acid Analysis

Components have the same molecular weight as the calf thymus lysine-rich histones (21,000) (16, 17), then the largest decrease would correspond to Components 1 and 5 of the chicken erythrocyte fraction which were calculated to contain 13 and 8 residues of valine, respectively.

Amino acid analysis was also performed on a series of chromatographic components from several tissues of the same species to investigate possible differences in the composition of corresponding components of different tissues. Each component was purified by rechromatography of pooled fractions representing the central portion of each peak. The protein for amino acid analysis was collected only from the central portion of the peaks of the rechromatographed fractions. The data presented in Table III indicate that corresponding components from different tissues of the same species have essentially the same over-all amino acid compositions, and the differences presented are probably within the error of the experimental methods. No corrections were made for the slow release or destruction of amino acids during hydrolysis.

Treatment with Cyanogen Bromide—The whole lysine-rich histone fraction of rat thymus and its methionine-containing component (Component 3, Fig. 3a) were treated with cyanogen bromide in an attempt to obtain evidence for a suspected methionyl peptide linkage. The gel electrophoretic patterns in Fig. 10 show the whole lysine-rich histone fraction of rat thymus before (Gel 10a) and after (Gel 10b) treatment with cyanogen bromide. The results show that cyanogen bromide treatment caused a marked decrease in the intensity of the band which represents the methionine-containing Component 3 (fastest migrating band of the three major rat thymus bands). In addition, a new electrophoretic component was produced which migrated faster than any of the bands corresponding to the chromatographic components of the whole, untreated rat thymus lysine-rich histone fraction. This new component

Fig. 9. Polyacrylamide gel (15%) electrophoretic patterns of chromatographic fractions of chicken spleen and erythrocyte lysine-rich histones. Gels 1 to 3 are from areas 3, 3a, and 4, respectively, of the spleen profile (Fig. 7b) and gels 4 to 6 are from areas 3, 3a, and 4, respectively, of the erythrocyte profile (Fig. 7c). Electrophoresis was performed at 10 ma per gel for 55 min and gels were treated as described in Fig. 5.
### Table II

**Amino acid composition of lysine-rich histone components of different species**

<table>
<thead>
<tr>
<th>Amino Acid</th>
<th>Cat spleen component&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Chicken erythrocyte component</th>
<th>Calf thymus component</th>
<th>Rat thymus component&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mole %&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1 2 3</td>
<td>4 5</td>
<td>1 2 3</td>
</tr>
<tr>
<td>Glutamic acid</td>
<td>3.1 3.4 3.1 3.3 3.2 3.2</td>
<td>2.3 1.5 1.6 2.1</td>
<td>1.9 1.8 1.9 2.2</td>
<td></td>
</tr>
<tr>
<td>Alanine</td>
<td>26.8 26.0 24.4 25.2 25.9 26.0</td>
<td>26.1 25.6 26.6</td>
<td>25.7 26.0 26.2</td>
<td></td>
</tr>
<tr>
<td>Proline</td>
<td>9.3 8.6 9.7 8.6 9.5 9.6</td>
<td>9.0 9.4 9.7 9.0</td>
<td>9.3 9.7 9.5 8.8</td>
<td></td>
</tr>
<tr>
<td>Threonine</td>
<td>5.2 5.7 5.5 5.8 5.1 5.1</td>
<td>4.4 3.4 3.5 4.6</td>
<td>5.2 5.5 5.1 4.6</td>
<td></td>
</tr>
<tr>
<td>Tyrosine</td>
<td>0.5 0.3 0.4 0.4 0.4 0.4</td>
<td>0.4 0.4 0.4 0.4</td>
<td>0.5 0.5 0.4 0.4</td>
<td></td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>0.5 0.6 0.6 0.6 0.5 0.5</td>
<td>0.7 0.8 0.9 0.5</td>
<td>0.5 0.5 0.4 0.5</td>
<td></td>
</tr>
<tr>
<td>Lysine/arginine</td>
<td>1.8 2.0 1.6 1.7 2.0 2.1</td>
<td>0.1 0 0.1 0.2</td>
<td>0.1 0 0 0</td>
<td></td>
</tr>
<tr>
<td>Lysine/arginine</td>
<td>13.0 16.4 19.5 16.2</td>
<td>13.4 14.6 13.8 11.7</td>
<td>16.2 21.9 17.9 9.4</td>
<td></td>
</tr>
<tr>
<td>Basic amino acids/acidic amino acids</td>
<td>4.5 4.0 4.6 5.0</td>
<td>4.5 5.8 5.8 5.8</td>
<td>6.2 6.6 6.3 4.2</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> The components in the table refer to the protein recovered from the fractions designated by the numbered components shown in Fig. 1.

<sup>b</sup> Values reported as moles of amino acid per 100 moles of total amino acids recovered after 22-hour hydrolysis in 6 N HCl at 110°.

### Table III

**Amino acid composition of corresponding lysine-rich histone components of different tissues of same species**

<table>
<thead>
<tr>
<th></th>
<th>Cat&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Component 2</th>
<th>Component 3</th>
<th>Component 3</th>
<th>Component 4</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Thymus</td>
<td>Liver</td>
<td>Thymus</td>
<td>Liver</td>
<td>Thymus</td>
</tr>
<tr>
<td>Aspartic acid</td>
<td>1.9 1.9</td>
<td>1.8 2.0</td>
<td>1.9 1.9</td>
<td>2.2 2.4</td>
<td>2.2 2.1</td>
</tr>
<tr>
<td>Threonine</td>
<td>5.2 5.7</td>
<td>5.5 5.8</td>
<td>5.1 5.1</td>
<td>4.6 5.0</td>
<td>5.0 5.3</td>
</tr>
<tr>
<td>Serine</td>
<td>6.0 5.8</td>
<td>6.2 6.4</td>
<td>6.2 5.8</td>
<td>7.5 7.5</td>
<td>7.3 7.9</td>
</tr>
<tr>
<td>Glutamic acid</td>
<td>3.1 3.4</td>
<td>3.1 3.5</td>
<td>3.2 3.2</td>
<td>4.4 4.4</td>
<td>4.0 4.3</td>
</tr>
<tr>
<td>Proline</td>
<td>9.3 8.6</td>
<td>9.7 9.6</td>
<td>9.5 9.6</td>
<td>8.8 8.2</td>
<td>7.9 9.3</td>
</tr>
<tr>
<td>Glycine</td>
<td>0.2 0.4</td>
<td>0.0 0.0</td>
<td>0.0 0.7</td>
<td>8.2 7.1</td>
<td>7.1 7.9</td>
</tr>
<tr>
<td>Alanine</td>
<td>26.8 26.0</td>
<td>24.4 25.2</td>
<td>25.9 26.0</td>
<td>24.6 24.5</td>
<td>25.2 23.1</td>
</tr>
<tr>
<td>Valine</td>
<td>5.0 5.1</td>
<td>4.8 5.2</td>
<td>3.8 4.1</td>
<td>5.2 5.3</td>
<td>5.2 5.8</td>
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<tr>
<td>Methionine</td>
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<td>0 0</td>
<td>0 0</td>
<td>0.4 0.4</td>
<td>0.4 0.4</td>
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<tr>
<td>Isoleucine</td>
<td>0.9 1.0</td>
<td>0.8 0.9</td>
<td>0.8 0.9</td>
<td>1.7 1.6</td>
<td>1.6 2.2</td>
</tr>
<tr>
<td>Leucine</td>
<td>4.0 3.9</td>
<td>3.9 4.0</td>
<td>3.0 3.0</td>
<td>4.7 5.0</td>
<td>4.6 5.1</td>
</tr>
<tr>
<td>Tyrosine</td>
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<td>0.5 0.5</td>
<td>0.4 0.4</td>
<td>0.5 0.5</td>
<td>0.4 0.4</td>
</tr>
<tr>
<td>Phenylalanine</td>
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<td>0.5 0.6</td>
<td>0.4 0.4</td>
<td>0.5 0.5</td>
<td>0.6 0.6</td>
</tr>
<tr>
<td>Lysine</td>
<td>29.1 29.1</td>
<td>30.7 27.8</td>
<td>30.4 30.0</td>
<td>26.3 24.7</td>
<td>26.7 23.4</td>
</tr>
<tr>
<td>Histidine</td>
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<td>0 0</td>
<td>0 0</td>
<td>0 0</td>
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<tr>
<td>Arginine</td>
<td>1.8 1.9</td>
<td>1.4 1.5</td>
<td>1.7 1.8</td>
<td>2.7 2.8</td>
<td>2.5 3.2</td>
</tr>
<tr>
<td>Lysine/arginine</td>
<td>16.2 15.3</td>
<td>21.9 18.5</td>
<td>17.9 16.7</td>
<td>9.4 8.8</td>
<td>10.7 7.3</td>
</tr>
<tr>
<td>Basic amino acids/acidic amino acids</td>
<td>6.2 5.9</td>
<td>6.6 5.5</td>
<td>6.3 6.2</td>
<td>4.2 4.0</td>
<td>4.7 4.1</td>
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</table>

<sup>a</sup> Values reported as moles of amino acid per 100 moles of total amino acids recovered after 22-hour hydrolysis in 6 N HCl at 110°.

<sup>b</sup> The components in the table refer to the protein recovered from the fractions designated by the numbered components shown in Fig. 6 (calf) and Fig. 3 (rat).
of this lysine-rich histone with cyanogen bromide which presence of a single methionine residue was obtained by treat-

the thymus lysine-rich histones (16, 17). Further evidence for the.

ular weight (21,000) identical with that found for the calf.

analysis of this methionine-containing component indicated that.

this is the first demonstration of a methionine-containing lysine-rich histones of rat contained methionine. Aside from the fact that.

the extraction procedure.

between the lysine-rich histone complements of different species.

were the same or nearly identical for a wide range of trichlor-

histones from mammary gland tissue showed that the absolute.

components. Amino acid analysis of Component 3 of rat.

thymus after treatment with cyanogen bromide showed that.

the cleavage reaction was about 60% complete.

of particular interest was the finding that one of the lysine-rich.

histone fractions from calf, chicken, and cat.

spleen with cyanogen bromide, as well as treatment with 70%.

formic acid, produced no new, faster migrating electrophoresic.

components. Amino acid analysis of Component 3 of rat.

thymus after treatment with cyanogen bromide showed that.

DISCUSSION

The present work shows that the method of deNooij and.

Westenbrink provides whole lysine-rich histone preparations.

of spleen, liver, kidney, and erythrocyte tissues which are.

comparable to those obtained previously from thymus (18) and.

mammary gland (25). These studies also support the previous.

conclusion (18, 25) that the relative proportions of the different.

lysine-rich histones were almost identical in all preparations.

derived from the same tissue, since the variation in the relative.

proportions of the components of four different tissues of the.

rat was less than ±2%, of three different tissues of the chicken was.

less than ±5%, and of three different tissues of the calf was less.

±3%. Similar studies (25) on the extraction of lysine-rich.

histones from mammary gland tissue showed that the absolute.

amounts and the relative proportions of the extracted histones.

were the same or nearly identical for a wide range of trichlor-

acetic acid concentrations. Therefore, the differences observed.

between the lysine-rich histone complements of different species.

cannot reasonably be explained by differences in the efficiency of.

the extraction procedure.

Of particular interest was the finding that one of the lysine-rich.

histones of rat contained methionine. Aside from the fact that.

this is the first demonstration of a methionine-containing lysine-

rich histone, it represents a clear example of species specificity.

at the level of the primary structure of a histone. Amino acid.

analysis of this methionine-containing component indicated that.

it contained a single methionine residue, if one assumes a mole-

cular weight (21,000) identical with that found for the calf.

thymus lysine-rich histones (16, 17). Further evidence for the.

presence of a single methionine residue was obtained by treat-

ment of this lysine-rich histone with cyanogen bromide which.

produced a new, fast migrating zone containing two closely.

migrating bands.

The present work also provides evidence that the quantitative.

differences between the lysine-rich histones of different tissues.

of the same species are not the result of proteolytic degradation,

since the same quantitative differences were observed when the.

lysine-rich histone fraction was isolated in the presence of sodium.

bisulfite, a strong inhibitor of histone proteolysis (24). In.

addition, polyacrylamide gel electrophoresis patterns of the.
different preparations were devoid of the faster moving bands.

characteristic of degraded preparations (34, 36). Because.

previous work has shown that nonhistone contaminants do not.

elute from Amberlite IRC-50 in the region of the lysine-rich.

histones (8, 18, 25), it is concluded that the observed differences.

in the distribution of the lysine-rich histone components of differ-

tent tissues of the same species represent real, endogenous.

differences.

Recent studies by other workers (37) have suggested that the.

complement of lysine-rich histone is different in different organs.

of the same animal. Although this conclusion is different from.

that presented in this paper, there may be a reasonable explana-

tion for this discrepancy. The chromatographic data presented.

by Bustin and Cole (37) show that the major peaks of rabbit.

thymus lysine-rich histone do not have the same elution volumes.

as the major peaks of rabbit mammary gland lysine-rich histone.

The present work shows that the resolution of chromatographic.

peaks may be altered by the presence of components normally.

undetected by a single chromatographic fractionation with the.
system used in this work and by Bustin and Cole (37). For.

example, the elution pattern for chicken erythrocyte lysine-rich.

histone (Fig. 7c) appears to differ from those obtained for chicken.

liver (Fig. 7a) and spleen (Fig. 7b) in that the latter two show a.
definite peak for Component 4. However, this is not a qualita-

tive difference, since there are differences in the amount of Com-

ponent 3a present in these tissues (Fig. 8). Moreover, a peak of.

Component 3a is not visible in the liver, spleen, or erythrocyte.

profiles, and Component 3a is not resolved by polyacrylamide gel.

electrophoresis (Fig. 9) under the conditions used in the present.

study. In addition, the range of the differences in amino acid.

compositions of corresponding lysine-rich histone components of.

different tissues of the same species presented in this paper is.
similar to that reported by Bustin and Cole (37) for a correspond-

ing component of rabbit thymus and mammary gland.

The original hypothesis that histones functioned as genetic.

repressors (6, 7) gave rise to a model whereby a single, specific.

gene interacted with a single, specific histone. Of relevance in.

this regard are the findings presented in this paper, as well as.

those of Bustin and Cole (37), that there are distinct species-

specific differences among the lysine-rich histone complements of.

different mammalian and avian species. If histones are involved.

in the control of genetic information, a certain amount of spec-

ificity must be inherent in their primary structures. The finding.

that the lysine-rich histones of a given species show clear differ-

eces in their content of valine (19), despite remarkably similar.

ever-all amino acid compositions, may have some significance in.

this regard. While such observations are consistent with the one.
gene-one histone model of histone function, they do not rule out.

the possibility that these differences may be a reflection of evolu-

tionary variation, such as observed for hemoglobin (38) and.
cytochrome c (39), rather than an expression of some functional.

differences.
On the other hand, there is an accumulation of data which tend to weaken the one gene-one histone model. Thus, numerous studies (9, 20, 40) have shown that there are only three major classes and six recognizable molecular types of histones, and these have been subfractionated into a total of approximately 10 components (8, 10). In addition, there is a rather large body of evidence which emphasizes the striking similarities of the histones from many different sources (8–11). Such considerations have led a number of investigators to consider other molecular species such as RNA and hormones (41–43) or processes such as histone acetylation (44), methylation (45), and phosphorylation (46, 47) as mechanisms whereby histones may acquire the degree of specificity required of the one gene-one histone model.

The failure of the one gene-one histone model to account for all of the experimental data concerning the histones has led to the formulation of alternative models. One such model is that involving the interaction of a group of genes with a single specific histone. Such an approach has been taken by Goodwin (48, 49) and maintains that different relative concentrations of histones might serve to modulate the number of distinct cell states and thus decrease the requirement for a high degree of histone specificity. Additional evidence for a decreased requirement of histone specificity has come from work which shows that there are quantitative differences among the major histone classes of both plants (27) and animals (51, 52).

Within the context of a functionally significant relationship between histone and genetic expression, one is tempted to speculate on the possible kinds of regulative roles that might be associated with the different classes of histones. Although the lysine-rich histones represent the smallest proportion of the total histones, the observed qualitative species specificity and quantitative tissue-specific distribution of this group suggest that it might be concerned with a different type (or types) of biochemical control mechanism than the other major classes of histones. It is of interest in this regard that Fambrough, Fujimura, and Bonner (27) have shown an increase in the relative proportion of the lysine-rich histone class of pea cotyledons during germination.

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Qualitative Species Differences and Quantitative Tissue Differences in the Distribution of Lysine-rich Histones

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