THE FRACTIONATION OF β-HISTONE FROM CHICKEN ERYTHROCYTE NUCLEI

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Summary

Chicken erythrocyte β-histone, whose behaviour on moving boundary electrophoresis or on ultracentrifugation did not indicate any heterogeneity, has been resolved into seven components by starch-gel electrophoresis.

Substantial purification of these components has been achieved by exclusion chromatography on Sephadex G-200. The amino acid analyses and sedimentation behaviour of the fractions obtained confirm that β-histone from chicken erythrocyte nuclei is itself a family of closely related proteins. The implications of these findings are discussed.

I. Introduction

In 1954, Cruft, Mauritzen, and Stedman introduced a general classification of histones based on their electrophoretic mobilities. Of these, β-histones, the principal histone components of mammalian cell nuclei, represent an extreme example in amino acid composition containing, as they do, a higher content of arginine than any other known histones. β-histones also differ from other histones in their ability to undergo aggregation both with increasing ionic strength or pH, in contrast to 1·6 S γ-histones which do not aggregate with increasing ionic strength. The aggregation of β-histone is freely reversible and use was made of this property by Cruft, Mauritzen, and Stedman (1958*a*) to separate it from the α- and γ-components of the histone complex and to purify it to what was then regarded as a terminal stage.

The behaviour of the purified β-histone on moving boundary electrophoresis, on sedimentation (Cruft, Mauritzen, and Stedman 1958*a*, 1958*b*), and on chromatography on carboxymethylcellulose (Johns *et al.* 1960; Busch *et al.* 1962) was consistent with that of a homogeneous protein.

Satake, Rasmussen, and Luck (1960) separated two "arginine-rich" histones (designated III and IV) from calf thymus by chromatography on Amberlite IRC-50 with a guanidinium chloride gradient, but the amino acid composition of their fractions did not differ significantly. However, Hnilica and Busch (1962) isolated two arginine-rich histone fractions (designated 2a and 3c) from Walker 256 carcinoma, confirming the presence of more than one arginine-rich histone. Stedman (personal communication) has also reported the resolution of purified β-histone from calf thymus into several components on starch-gel electrophoresis.

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It had also been noted in this laboratory that "purified β-histones", prepared by a variety of methods, gave amino acid analyses which differed significantly, suggesting the presence of a number of β-histone components in varying proportions. The present report deals with the further resolution of purified β-histone from chicken erythrocyte nuclei on starch-gel electrophoresis and of the isolation and partial purification of these β-histones by exclusion chromatography on Sephadex G-200 in the presence of urea.

II. Material and Methods

(*a*) *Isolation and Purification of β-Histone*

Nuclei were isolated from chicken erythrocytes by a slight modification (Bellair and Mauritzen 1964*a*) of the acetic acid and saponin procedures of Stedman and Stedman (1951). The crude histone complex was obtained by repeated extraction of the defatted nuclei with ice-cold 0·1N sulphuric acid.

The histone complex was first separated into a β- plus 1·6 S γ-fraction and a crude α-fraction (containing the crude α-complex and 0·8 S γ-components) by precipitation of the former fraction with 17% ethanol at pH 6·5 as described by Cruft, Mauritzen, and Stedman (1958*b*). The β-histone was then separated from the 1·6 S γ-component by aggregation at pH 8·5 in 0·5M sodium chloride and sedimentation in the ultracentrifuge. It was then purified by repeated aggregation and disaggregation in the presence of 4M sodium chloride (Cruft, Mauritzen, and Stedman 1958*a*). As a further purification step, the procedure of Davidson and Shooter (1956), i.e. precipitation with 0·05N ammonia, was used. Finally, the β-histone was disaggregated by dissolving the protein in 0·05N hydrochloric acid. This protein solution was then dialysed against water and freeze-dried.

(*b*) *Starch-gel Electrophoresis*

Separation of histone fractions by starch-gel electrophoresis was performed with a vertical apparatus of the type described by Smithies (1959). The conditions used were as follows: starch concentration 14·8 g/100 ml; 4M urea (to minimize aggregation); sodium acetate–acetic acid buffer, prepared by titrating sodium acetate with acetic acid to pH 4·90 and diluting with 8M urea to a final concentration of 0·04M with respect to sodium acetate. Histone samples were dissolved in 6M urea prior to application to the gel. Separations were routinely performed with histone concentrations of 0·4 and 1·0%, the higher concentration being required to detect minor components and the lower to prevent overlap of the major electrophoretic components. Electrophoresis was continued for 3 hr at 18 V/cm.

(*c*) *Exclusion Chromatography*

Perspex columns, 180 by 6 cm, packed-bed volume 4·4 litres, were filled with Sephadex G-200 which was washed to remove fines, degassed under reduced pressure, and equilibrated with 0·03N hydrochloric acid in 4M urea, the same solution being used for elution. The columns were operated under a head of 20–25 cm of solvent. The β-histone was dissolved in 4M urea at a concentration of between 5 and 7% w/v. A

time-operated Locarte fraction collector was used to collect fractions of approximately 15 ml. Histone concentration was determined by measuring the absorption of the effluent fractions, diluted where necessary, at 230 and 280 mμ.

(*d*) *Recovery of Histone*

The contents of various tubes from the Sephadex fractionations were appropriately bulked to give from 10 to 12 fractions, dialysed against distilled water to remove acid and urea, and freeze-dried.

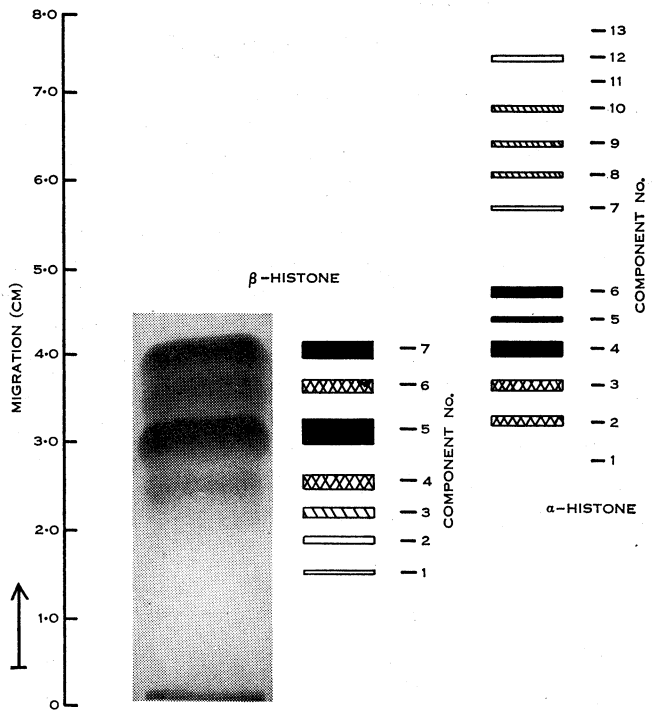

Fig. 1.—Starch-gel electrophoretic patterns of purified β-histone and crude α-histone.

(*e*) *Sedimentation*

Aggregation studies were made with a Spinco model E ultracentrifuge fitted with Schlerien optics, rotor temperature indicator, and control. Runs were performed at 20°C, a synthetic-boundary cell of the valve type being used.

(*f*) *Amino Acid Analysis*

Weighed samples (6–10 mg) of dried histone were suspended in 2 ml of constant-boiling HCl (β-histone does not dissolve readily in strong acid), frozen, and sealed *in vacuo* in glass tubes and hydrolysed by heating for 22·0 hr at 110±0·5°C. The hydrolysates were taken to dryness over NaOH *in vacuo* and dissolved in a suitable volume (usually 10 ml) of citrate buffer. Amino acids were estimated in 2- or 3-ml portions of the diluted hydrolysate on a Spinco model 120B amino acid analyser.

III. Results

(a) *Starch-gel Patterns*

When β-histone, purified as described by Mauritzen and Stedman (1959), is run on starch gel it separates into several components as depicted in Figure 1. The pattern obtained with crude α-histone is included in Figure 1 for comparison. The positions of bands 1, 2, and 3 are indicated but, as these constitute only a very small proportion of the purified β-histone (5%), they are not usually detectable in unfractionated samples. Maximum resolution was obtained at 0·04 ionic strength in presence of 4M urea. This is in contrast to the crude α-histones which resolve better on starch-gel electrophoresis at 0·08 ionic strength (Bellair and Mauritzen 1964*a*).

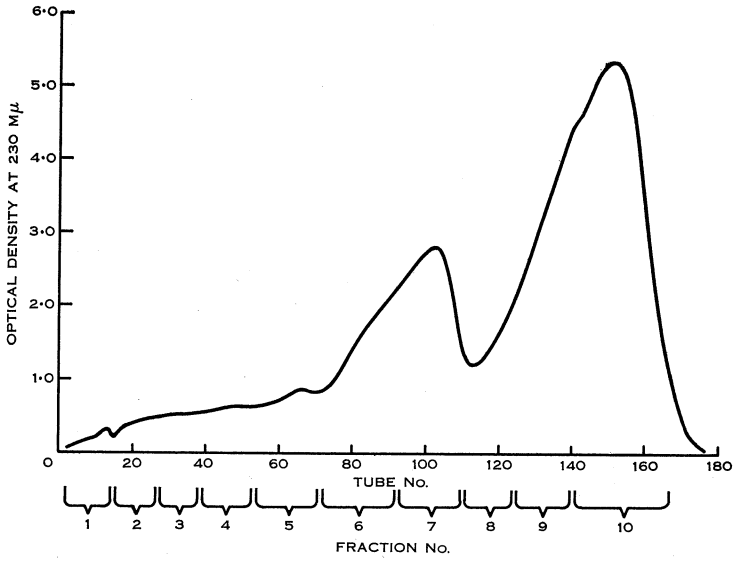

Fig. 2.—Elution pattern of purified β-histone on Sephadex G-200.

If the urea was omitted from the buffer then much of the histone stayed at the origin, being unable to penetrate the gel; the histone which did enter the gel tended to trail, obscuring the separation pattern. The demonstrable polydispersity of β-histone did not, of itself, mean that the β-histone contained several components, for starch-gel electrophoresis might merely have been resolving various aggregates of β-histone.

It was decided to use Sephadex G-200 to attempt a separation of β-histone as Cruft (1962) had obtained promising results with unfractionated histone on Sephadex G-75, while we had found exclusion chromatography useful for the resolution of α- and γ-histones (Bellair and Mauritzen 1964*b*). The separation was made in 0·03N hydrochloric acid in 4M urea as it was important to minimize the possible formation of aggregates.

A typical elution pattern is shown in Figure 2 which also indicates the fractions which were bulked for recovery. The elution pattern, like the starch-gel pattern, indicates heterogeneity of β-histone. The elution pattern is negatively skewed, i.e. the slope of the ascending side of the peak is less than that of the descending side. Pedersen

(1962) has noted similar behaviour with bovine serum albumin but this phenomenon was not observed on exclusion chromatography of the α-histones (Bellair and Mauritzen 1964*b*). The histone recovered from the fractions obtained from the Sephadex fractionation were then examined by starch-gel electrophoresis.

Initially, the histone samples for starch-gel electrophoresis were dissolved, following our usual practice, in water, and 50 μl of the solutions inserted into preformed slots in the gel. When this was done it was found that only part of the histone in Sephadex fractions 1–7 migrated into the gel while the bulk of the histone remained at the origin. The histones from Sephadex fractions 8–11, on the other hand, migrated cleanly into the gel without tailing. This suggested that the histone from earlier Sephadex fractions was so highly aggregated that it could not penetrate the gel. The histone samples were therefore disaggregated prior to electrophoresis by dissolving the protein in 6M urea. When the samples were prepared in this way, all the histone fractions migrated cleanly into gel without tailing and the electrophoretic bands were clearly separated.

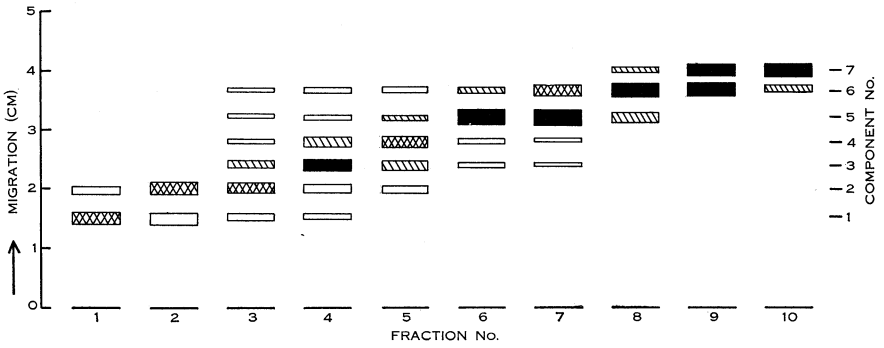

Fig. 3.—Starch-gel electrophoretic patterns of Sephadex fractions 1–10. Increasing intensity of shading indicates increasing order of concentration.

The starch-gel patterns obtained from the various Sephadex fractions are depicted in Figure 3. Fraction 11, which is not shown but which amounted to approximately 0·5% of the β-histone, was a mixture of α-histones, as suggested by the starch-gel pattern and the amino acid composition of this fraction. Fractions 1–5 contain mainly the slower-moving components while fractions 6, 8, and 10 represent considerable purification of components 5, 6, and 7, which together comprise 85–90% of the original β-histone. It is provisionally proposed to designate these histones $\beta_1, \ldots, \beta_7$ in increasing order of electrophoretic mobility under the conditions used.

(*b*) *Amino Acid Analyses*

Amino acid analyses were performed on histones from the various fractions and the results for these are shown in Table 1. Included in this table for comparison are the average values for a series of "purified" β-histone preparations from chicken erythrocytes. The percentage represented by each of the fractions of the total β-histone is also given together with the starch-gel components found in each fraction.

It can be seen from the amino acid composition of these fractions that:

(1) All the fractions, and therefore presumably all the components separable on starch-gel, are true β-histones in as much as they possess the composition characteristic of this type of protein (Mauritzen and Stedman 1959, 1960), i.e. they contain large amounts of both arginine and lysine while the lysine : arginine ratio varies from 0·75 in fraction 6 to 1·06 in fraction 2. Moreover, the sum of the contents of serine and proline is low (4–12%) while the sum of tyrosine and phenylalanine contents (4·9–5·8%) and aspartic and glutamic acids (13–19%) are relatively high in contrast to similar values for the various α-histone components (Bellair and Mauritzen 1964*b*).

(2) However, considerable variation occurs in composition with respect to other amino acids. For example, the glycine content of fraction 10 is double that of fractions 2–8, while conversely, the alanine content of fraction 10 is less than two-thirds of the alanine content of fractions 2–8. Similarly the amounts of serine and proline, tyrosine and phenylalanine, aspartic and glutamic acids, methionine, and the two leucines also vary significantly. While the amounts of these individual amino acids vary, the sum of the contents of the pairs glycine and alanine, the two leucines, and phenylalanine and tyrosine are relatively constant. Thus differences in composition of the fractions are most striking when the ratios of these pairs of amino acids are compared as has been done for alanine and glycine and for tyrosine and phenylalanine (Table 1).

All the β-histones examined contained a small amount of ninhydrin-positive material which was eluted immediately after lysine and which was presumably ε-*N*-methyl lysine (Murray 1963). Another interesting feature is the presence of cystine in fractions 3, 4, 6, and 7 for, hitherto, it had been believed that all histones were devoid of either cystine or cysteine. Because of hydrolytic conversion to cystine it was not possible to say whether this histone originally contained cystine or cysteine.

As mentioned earlier, components 5, 6, 7 constitute 85–90% of the pure β-histone, and these were found in purest form in fractions 6, 8, and 10 respectively. Fractions 6 and 10 were therefore re-run on smaller Sephadex columns and the elution pattern obtained along with the corresponding starch-gel patterns of the subfractions are shown in Figure 4, while the amino acid compositions of fractions 6–4 and 10–2 (which represent almost pure β_5 and β_7 respectively) are recorded in Table 1.

(*c*) *Aggregation Studies*

Purified β-histones have been shown to undergo aggregation and disaggregation reactions in a characteristic manner (Cruft, Mauritzen, and Stedman 1958*a*). Moreover, as indicated above by the initial experiments on starch-gel electrophoresis, Sephadex fractions 1–7 had given evidence in contrast to fractions 8–10 of an increased propensity to aggregate. It was therefore decided to compare the aggregation behaviour of some of the β-histone fractions with that of the original "pure" sample of histone. Sephadex fractions 2, 3, 5, 6, 8, and 10 were used. Fractions 6, 8, and 10 represented principally components 5, 6, and 7 respectively, while fractions 2, 3, and 5 were mixtures of components 1, 2, 3, and 4.

TABLE 1

AMINO ACID COMPOSITION OF β-HISTONE FRACTIONS OBTAINED BY EXCLUSION CHROMATOGRAPHY ON SEPHADEX G-200

Values as molar percentage of total amino acids. No correction has been made for the destruction of serine or threonine on hydrolysis

	Purified β-Histone†	Fraction No:											
		1	2	3	4	5	6	7	8	9	10	6–4§	10–2§
Fraction as a percentage of total recovered	—	0·8	1·5	2·0	3·0	4·5	10·6	15·0	7·2	18·4	36·4	—	—
Starch-gel components present in fraction*	**5, 7** (6, 3, 4)	**1** (2)	**2** (1)	**2, 3** (1, 4)	**3** (4, 2, 5)	**4** (3, 5, 6)	**5** (6)	**5** (6)	**6** (5,7)	**6** (7)	**7** (6)	**5**	**7**
Lysine } B	9·4	8·0	10·1	9·6	9·3	8·1	8·7	8·7	9·9	10·7	10·0	8·4	10·8
Histidine } B	1·7	1·9	2·3	2·5	2·2	2·3	1·5	1·5	1·8	2·0	1·9	1·5	1·9
Arginine } B	10·8	8·9	9·5	9·7	10·2	11·3	12·2	12·6	11·2	11·9	13·9	12·8	13·6
Amide NH_3	7·6	‡	‡	‡	‡	‡	‡	‡	‡	7·90	7·0	9·3	10·0
Aspartic acid } A	5·3	6·3	5·7	5·7	5·4	5·3	4·5	4·3	5·4	4·4	5·2	4·2	5·2
Glutamic acid } A	10·0	12·5	12·0	11·7	11·3	10·7	11·6	12·8	10·7	8·1	7·5	11·4	8·0
Threonine	6·4	6·4	6·4	6·5	6·5	6·8	6·9	6·8	6·1	6·7	6·4	6·9	6·1
Serine	3·4	7·2	5·3	5·6	4·7	4·5	4·2	4·1	4·7	3·7	2·5	3·7	2·3
Proline	3·6	4·5	5·0	4·3	4·2	4·2	4·2	4·1	4·0	2·9	1·7	4·3	1·9
Glycine	9·6	9·0	7·1	7·3	6·7	6·8	6·0	6·0	7·9	11·7	14·9	6·1	14·5
Alanine	12·3	11·8	12·2	12·3	13·4	12·8	13·2	14·4	12·7	11·6	8·4	14·0	8·5
Valine	6·3	5·5	4·4	5·3	5·2	5·5	4·8	4·8	5·6	6·3	7·4	5·1	7·4
½ Cystine	—	—	Trace	0·5	0·5	Trace	0·5	0·4	—	—	—	0·5	—
Methionine	0·9	0·2	1·3	0·8	1·1	1·1	1·5	1·2	1·0	0·9	0·9	1·5	0·9
Isoleucine	4·9	4·2	4·4	4·4	4·5	5·0	4·9	5·1	5·0	4·9	5·5	5·4	5·3
Leucine	10·0	8·2	8·6	8·6	9·6	9·6	9·8	9·8	9·4	9·4	8·5	9·7	8·4
Tyrosine	2·8	2·1	2·6	2·3	2·2	2·4	2·3	2·2	2·5	2·9	3·5	2·3	3·3
Phenylalanine	2·6	2·9	3·1	3·5	3·2	3·4	3·1	3·1	2·5	2·0	2·0	3·2	1·9

TABLE 1 (*Continued*)

	Purified β-Histone†	Fraction No:											
		1	2	3	4	5	6	7	8	9	10	6–4§	10–2§
Fraction as a percentage of total recovered	—	0·8	1·5	2·0	3·0	4·5	10·6	15·0	7·2	18·4	36·4	—	—
Starch-gel components present in fraction*	**5, 7** (6, 3, 4)	**1** (2)	**2** (1)	**2, 3** (1, 4)	**3** (4, 2, 5)	**4** (3, 5, 6)	**5** (6)	**5** (6)	**6** (5,7)	**6** (7)	**7** (6)	**5**	**7**
B (Lys+His+Arg)	21·9	18·8	21·8	21·8	21·7	21·7	22·4	22·8	22·9	24·6	25·8	22·7	26·3
A (Asp+Glu)	15·3	18·8	17·7	17·4	16·7	16·0	16·1	17·1	16·1	12·5	12·7	15·6	13·2
Leu+Ileu	14·9	12·4	13·0	13·0	14·1	14·6	14·7	14·9	14·4	14·3	14·0	15·1	13·7
Ala+Gly	21·9	20·8	19·3	19·6	20·1	19·6	19·2	20·4	20·6	23·3	23·3	20·1	23·0
Ser+Pro	7·0	11·7	10·3	9·9	8·8	8·7	8·4	8·2	8·7	6·6	4·2	8·0	4·2
Tyr+Phe	5·4	5·0	5·7	5·8	5·4	5·8	5·4	5·3	5·0	4·9	5·5	5·5	5·2
Ala : Gly	1·3	1·3	1·7	1·7	2·0	1·9	2·2	2·4	1·6	1·0	0·6	2·3	0·6
Tyr : Phe	1·1	0·7	0·8	0·7	0·7	0·7	0·7	0·7	1·0	1·5	1·8	0·7	1·7

* Principal component(s) in bold face type, minor components in parenthesis.

† Average values for four different preparations.

‡ Ammonia values are not recorded for these fractions as they were spuriously high due to contamination with urea.

§ Values for $(B+NH_3)-A$ for fractions 6–4 and 10–2 were 16·4 and 23·1, respectively.

In order to determine whether the isolated β-histone fractions aggregated to a greater or lesser extent than the parent material, the most sensitive region of the aggregation process (i.e. primary aggregation or the conversion of 1·6 S to 10 S material) was chosen, and it was decided, for comparative purposes, to choose conditions whereby approximately 40% of the original "pure" β-histone would have undergone primary aggregation (conversion to 10 S form). The straightforward procedure would have been to dissolve the freeze-dried samples directly in buffer of

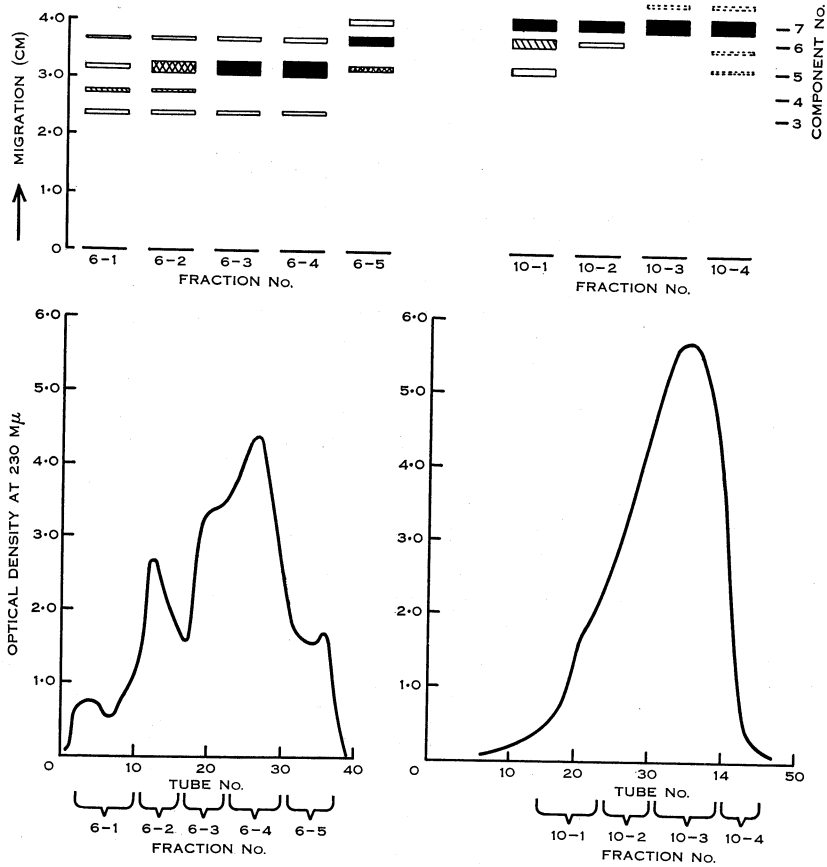

Fig. 4.—Elution curves and starch-gel electrophoretic patterns of Sephadex fractions 6 and 10, re-run on Sephadex G-200.

the desired pH and ionic strength. This experimental course might have been defeated if the various fractions had been fortuitously brought to different degrees of aggregation during isolation, for we have established (Bellair and Mauritzen, unpublished data) that while the aggregation of β-histone is freely reversible it proceeds much more readily in the forward than reverse direction. It was therefore decided to ensure complete disaggregation by dissolving the histone fraction in 6M urea and to remove the urea and substitute the desired buffer by dialysis at low temperature as earlier studies (Cruft, Mauritzen, and Stedman 1958*a*) had shown that aggregation is

extremely slow at 2°C. The dialysed solutions would then be allowed to aggregate for 48 hr at 20°C, after which time alterations in the state of aggregation take place very slowly (Cruft, Mauritzen, and Stedman 1958*a*). The conditions finally chosen were as follows: 15 mg histones were dissolved in 0·75 ml 6M urea to ensure complete initial disaggregation; the urea was then removed by dialysis against four 200-ml changes of 0·1M sodium acetate–acetic acid buffer, pH 5·8, over a period of 72 hr at 0°C. The dialysed solutions were then allowed to aggregate by standing for 48 hr at 20°C.

The uncorrected sedimentation coefficients of fast and slow components and the percentage aggregation (i.e. the percentage of the aggregated component) are shown in Table 2. All of the fractions have undergone aggregation to a greater extent than the parent material, while fractions 6 and 10 were almost entirely in the aggregated form. Considerable differences can also be seen in the sedimentation coefficients of the different fractions: thus the sedimentation coefficient of the aggregated

TABLE 2

AGGREGATION OF β-HISTONE FRACTIONS

Fraction No.	Percentage Aggregation	Sedimentation Coefficients (uncorrected)	
		Fast Component	Slow Component
Purified β-histone	41	7·2	1·6
2	60	7·4	3·8
3	65	7·3	2·5
5	80	6·0	2·2
6	95	5·5	2·1
8	52	12·5	1·5
10	95	10·9	0·7

component of fraction 6 is 5·5 S while that of fraction 8 is 12·5 S. Similarly the unaggregated form of fraction 10 has a value of 0·7 S while that of fraction 2 has a value of 3·8 S. The apparently low value (0·7) for unaggregated fraction 10 (β_7) is possibly spuriously low for this fraction did contain a small amount of α-histone (see Fig. 4) which has a value of 0·8 S and which would not have aggregated under the conditions used. The extent to which the various fractions underwent aggregation also varied and was not simply related to the purity of the fraction.

IV. DISCUSSION

The resolution of "pure" β-histone on starch-gel electrophoresis into several components has been confirmed. The separation of these components on a preparative scale has been achieved by exclusion chromatography on Sephadex G-200 in the presence of 4M urea.

The amino acid composition of these partially purified components and a study of their aggregation properties leaves no doubt that these are true β-histones, i.e. their chemical composition and properties sets them apart from all other known types of

histone. In this sense, the earlier purification of β-histones is upheld, for the present study has shown that β-histone contains only minute traces of α- or γ-histones.

The principal components of "purified" β-histone are β_5, β_6, and β_7, and of these β_5 and β_7 have been isolated in almost pure form as judged by starch-gel electrophoresis. The amino acid composition of these two β-histones is strikingly different with respect to their contents of glycine, alanine, tyrosine, and phenylalanine, and while the amounts of these amino acids vary, the sums of glycine plus alanine and tyrosine plus phenylalanine are nearly constant. The net charge of β_5 and β_7 also varies.

Hnilica and Busch (1962) has reported the amino acid composition of two arginine-rich histones (3c and 2a) from Walker 256 carcinoma. These histones correspond to β_5 and β_7 of the present study in that fraction 3c contains more alanine than glycine and more phenylalanine than tyrosine while the converse is true of fraction 2a.

The aggregation properties of the various β-histone fractions are strikingly different; thus fractions 2, 3, 5, and 6, which represent components 2, 3, 4, and 5, respectively, all have higher sedimentation coefficients and therefore presumably higher molecular weight than the original β-histone or β_6 or β_7. The order of elution from Sephadex is in agreement with the unaggregated values and the fact (Bellair and Mauritzen 1964*b*) that histones of higher net charge are eluted more slowly from Sephadex.

The demonstration of β-histones which contain cystine, albeit in small amount, is of interest, for previously all purified histones were believed devoid of this amino acid and, in recent years, the presence of cystine has frequently been taken as an indication of contamination with non-histone protein. None of our unfractionated histone samples contained any detectable cystine as the amount of cystine containing β-histone in the total unfractionated material is low.

It might be argued, as it has been in the past, that the components isolated in the present study are artefacts. If artefacts, these could have arisen in two ways: (1) by proteolytic degradation by nuclear cathepsins; or (2) by partial chemical degradation during isolation and fractionation. The first possibility can be largely discounted for, while the present study was made on β-histone from nuclei isolated at neutral pH with saponin in physiological saline, the β-histone from nuclei isolated by the acetic acid procedure, which precludes the possibility of proteolytic activity, gave exactly the same pattern on starch-gel electrophoresis as did the β-histone derived by the saponin procedure. The second possibility, that of chemical modification, can also be discounted for the present preparation had never been subjected to greater acidity than 0·1N H_2SO_4 for a few hours at 2°C or 0·03N HCl acid at 20°C during fractionation.

The present work therefore suggests that the β-histone from chicken erythrocyte nuclei is itself a family of very closely related proteins. Mauritzen and Stedman (1959) demonstrated that the purified β-histones obtained from nuclei of chicken erythrocytes, of liver cells, and of spleen cells possessed different amino acid composition. As these β-histone preparations were believed at that time to be homogeneous proteins, the results were held to constitute evidence of cell specificity of this group of proteins.

Clearly, the differences observed earlier could easily be accounted for by the β-histones from these three sources containing varying amounts of the components now shown to be present in one of them. The clear-cut demonstration of cell specificity of β-histones therefore still awaits unequivocal proof.

Moreover, the present work, coupled with that of Neelin and Neelin (1960), Irvin *et al.* (1963), and Bellair and Mauritzen (1964*b*) suggest that there may be as many as 25–30 different histones found in a single cell type.

At present, exclusion chromatography offers most promise for the fractionation of the histone complex. However, as the elution volumes of α-, β-, and γ-histones extensively overlap, clear-cut separations cannot be obtained by direct chromatography of the crude histone complex as has been suggested by Cruft (1962). This is illustrated in Table 3, where it can be seen that, in the case of both α- and β-histones, the bulk of the protein is eluted at fractional elution volumes of between 0·26 and 0·73.

TABLE 3

FRACTIONAL ELUTION VOLUMES* OF VARIOUS STARCH-GEL COMPONENTS OF α- AND β-HISTONES ON SEPHADEX G-200

β-histone									
Starch-gel component	1	2	3	4	5	6	7		
Fractional elution volume	0·26	0·28	0·34	0·39	0·53	0·67	0·73		
α-histone									
Starch-gel component	2	3A	4	5	6	8	9	10	11
Fractional elution volume†	0·26‡	0·49‡	0·32‡	0·36‡	0·42‡	0·69	0·73	0·77	0·82

* Elution volume as fraction of total bed volume.

† Values from Bellair and Mauritzen (1964*b*).

‡ Sephadex G-100.

Satisfactory separations are only obtained if the histone complex is first resolved by selective aggregation or precipitation into β-, 1·6 S γ-, and α-fractions (Bellair and Mauritzen 1964*b*; Hnilica 1964).

Recent studies by Huang and Bonner (1962) and Allfrey, Littau, and Mirsky (1962) with crude histone preparations have suggested that arginine-rich β-histones are potent inhibitors of RNA synthesis in isolated cell nuclei, and the separation of the components described above will enable the biological function of this group of histones to be tested more adequately.

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