manner, and extends over the entire reaction range investigated; it parallels the formation of form II (Myer, 1972a). The 3:1 NBS-modified preparation exhibits minimal reductase activity, whereas the succinate activity is lowered by about 30% only (Table I). The estimate of the amount of form II in the 3:1 NBS-modified preparation is of the order of 30–40%, which agrees rather well with the proportion of reduction of the succinate activity of the protein (Table III). Similar agreement exists between the estimated proportions of form II and the residual oxidase activity of the protein for the other two preparations as well (Tables I and III). Thus, consistent with earlier conclusions (Myer, 1972a), the NBS form (form I) with modified tryptophan-59 and methionine-65 and with unaltered, but definitely perturbed, central coordination configuration is the one with intact succinate oxidase activity and reduced NADH–cytochrome *c* reductase activity. The form with additional modification of methionine-80 and with structurally changed coordination configuration, form II, is the one which lacks both NADH–cytochrome *c* reductase activity and succinate oxidase activity. The latter is consistent with the well-accepted view that the replacement of methionyl sulfur from position 6 of heme iron results in elimination of the electron-transport property of the molecule. Detailed investigations of the purified preparations are being conducted to ascertain the two-function, two-structure relationships which emerge from these studies.

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Isolation and Purification of Histones from Avian Erythrocytes†

Lee A. Sanders*‡ and Kenneth S. McCarty

ABSTRACT: The six major histones present in avian erythrocytes were isolated and purified by a combination of techniques including selective extraction, oxidation, gel filtration on Sephadex G-100, and ion-exchange chromatography on Bio-Rex 70. The histones were identified by polyacrylamide gel electrophoresis and amino acid analysis. Amino acid compositions of the purified histone fractions are quite similar to corresponding fractions reported for calf thymus tissue. Histone F3, the only cysteine-containing histone, appears to be extracted exclusively in the monomer form; the dimerization of F3 under oxidative conditions facilitates its separation from the other histones by means of gel filtration. The purification procedure reported here has proven to be equally effective for the isolation of histones from avian reticulocytes as well as mature erythrocytes.

The histones isolated from a number of sources appear to be quite similar from species to species in terms of molecular size, charge, and even primary structure (Crampton *et al.*, 1957; DeLange *et al.*, 1969; Panyim *et al.*, 1971; DeLange and Smith, 1971). Many earlier procedures for histone fractionation and characterization have been recently reviewed by Hnilica (1967), Butler *et al.* (1968), Johns (1971), and

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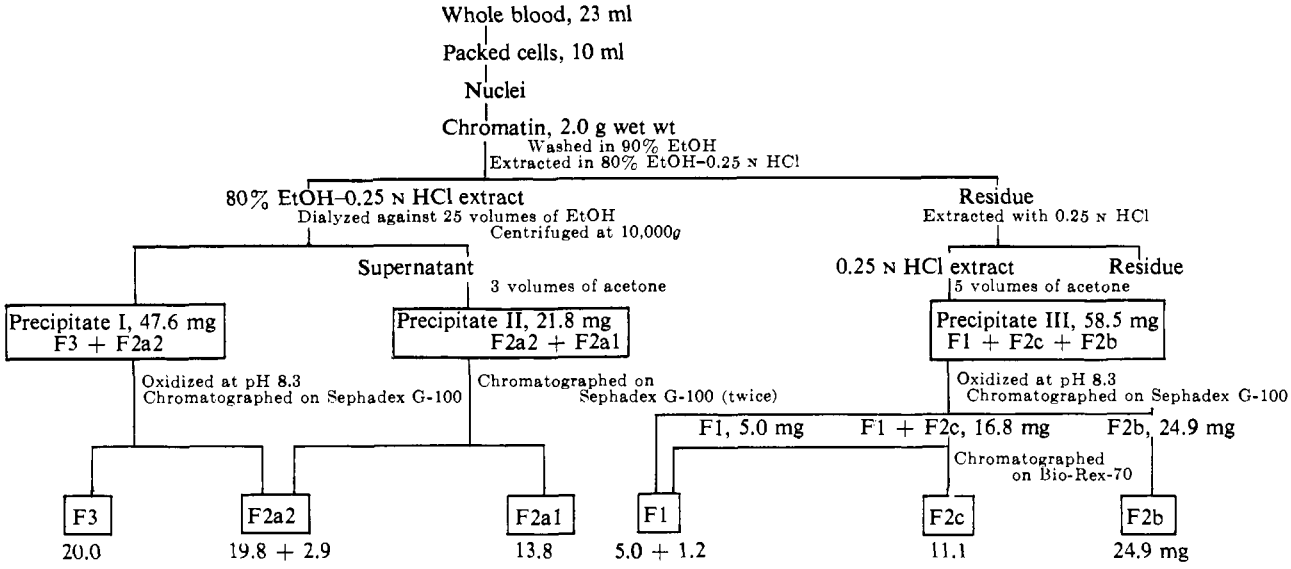
DIAGRAM I: Outline of the Procedure for the Isolation and Purification of Avian Erythrocyte Histones.[a]



[a] Typical yields are presented for each of the histone fractions.

others. The presence of the erythrocyte-specific histone F2c (Neelin and Butler, 1961; Purkayastha and Neelin, 1966; Greenaway and Murray, 1971), however, presents certain problems in the development of a comprehensive fractionation procedure for avian erythrocyte histones.

In our laboratory a rapid and selective extraction procedure was desired that would give sufficient fractionation of avian erythrocyte histones to permit accurate determination of their specific activities after *in vitro* labeling studies. In particular, since it was of interest to do a comparative study of histone acetylation in immature avian reticulocytes *vs.* mature erythrocytes, a method was also needed which would give sufficient fractionation of the histones from each cell type. Previous fractionation procedures had proven to be less than satisfactory, both in the isolation of histones from cells at different stages of maturity and in the subsequent fractionation of the arginine-rich histones (Vidali and Neelin, 1968; Adams and Neelin, 1969).

The complete purification of the avian erythrocyte histones is described here. A slight modification of the procedure of Johns (1964) was utilized to obtain partial fractionation of the erythrocyte histones. Final purification of each of the six major histones was achieved after oxidation of F3 histone to the dimer form, followed by gel filtration on Sephadex G-100 [suggested by the earlier work of Cruft (1961) and Mauritzen *et al.* (1966)] and ion-exchange chromatography on Bio-Rex 70 resin.

A paper to follow (Sanders *et al.*, 1972)[1] utilizes the methods described here in a comparative study of histone acetylation and deacetylation in avian reticulocytes and erythrocytes.

Materials and Methods

Chemicals. Ultra Pure Gdn·HCl[2] was obtained from Heico, Inc. Selected white saponin was obtained from Coulter. Acrylamide and Temed were purchased from Eastman Chemicals. Ethylene diacrylate was obtained from Borden Chemical Co. Amido Black 10B was purchased from Hartman-Leddon Co.

Isolation of Erythrocyte Nuclei and Chromatin. Adult white Peking ducks weighing 2.5–3.0 kg were utilized for all of the studies described herein. Erythrocyte nuclei were isolated essentially as described by Murray *et al.* (1968) with the following modifications. Blood was collected from ether-anesthetized ducks either by slitting of the jugular veins or by cardiac puncture; clotting was prevented by the presence of citrate (10 ml of 10% trisodium citrate/100 ml of blood). Erythrocytes were sedimented by centrifugation at 600*g* for 10 min at 4° and both the plasma and buffy coat were removed by aspiration. Lysis of the erythrocytes was accomplished by vigorous stirring in 0.3% (w/v) saponin solution for 30 min. The final SSC-washed nuclear pellet (sedimented at 10,000*g*) was resuspended in six volumes of SSC and homogenized with a 50-ml Dounce homogenizer (Kontes glass, five strokes each with A and B pestles). The homogenate, which still contained many intact erythrocyte nuclei as observed by phase-contrast microscopy, was sonicated four times in 15-sec bursts (using a Model S 75 Branson sonifier at setting 8) to ensure complete disruption of the nuclei and to attain crude chromatin. The resultant crude chromatin was pelleted *via* centrifugation at 10,000*g* for 10 min at 4° before further use.

Isolation of Total Histones. Total histones were extracted from chromatin in 25–50 volumes of 0.25 N HCl (Phillips and Johns, 1959; Marzluff *et al.*, 1972).

Extraction and Purification of Histones F2a1, F2a2, and F3.[3] The method used in isolating histone proteins from chromatin is outlined in Diagram I. Histones F2a1, F2a2, and F3 were selectively extracted from the erythrocyte chromatin by a slight modification of the procedure of Johns (1964).

[1] Submitted for publication.

[2] Abbreviations used are: Gdn·HCl, guanidine hydrochloride; Temed, *N,N,N′,N′*-tetramethylethylenediamine; SSC, 0.14 M NaCl–0.01 M trisodium citrate.

[3] The histone nomenclature used is that of Johns and Butler (1962) as modified by Hnilica (1964). The corresponding designations of Rasmussen *et al.* (1962) as modified by Neelin *et al.* (1964) and Fambrough *et al.* (1968) are given in parentheses as follows: F1 (I), F2a1 (IV), F2a2 (IIb1), F2b (IIb2), F2c (V), and F3 (III).

All operations were performed at 4° except where indicated. Isolated erythrocyte chromatin was washed twice by suspension in 15 volumes of 90% ethanol followed by sedimentation at 10,000*g*. The washed chromatin was extracted by vigorous stirring in eight volumes of 80% ethanol–0.25 N HCl for 24 hr followed by sedimentation at 10,000*g* for 10 min and reextraction in four volumes of fresh 80% ethanol–0.25 N HCl for 4 hr. The extracts were pooled and dialyzed against 25–30 volumes of absolute ethanol for 18 hr. It was determined by quantitative gel electrophoresis as described by Marzluff and McCarty (1970) that all of the F3 histone and approximately 80% of the F2a2 histone coprecipitated during the dialysis step. The precipitated F3 + F2a2 fraction was sedimented by centrifugation at 10,000*g* for 5 min, washed in ethanol, acetone, and ether, dried *in vacuo* at room temperature, and lyophilized from 0.1 N acetic acid. After oxidation of F3 histone to the dimer form as described below, the F3 was readily separated from F2a2 histone by gel filtration on Sephadex G-100.

Histone F2a1 plus the remaining F2a2 in the ethanol dialysis supernatant were precipitated by addition of three volumes of cold acetone, allowed to stand for 48 hr at −20°, washed in cold acetone, dried *in vacuo* at room temperature, and lyophilized from 0.1 N acetic acid. Histone F2a1 was resolved from F2a2 by gel filtration on Sephadex G-100 as described below.

Extraction and Purification of Histones F1, F2b, and F2c. The chromatin residue remaining after the above 80% ethanol–0.25 N HCl extractions contained predominantly histones F1, F2b, and F2c. The chromatin residue was extracted as above with four volumes of 80% ethanol–0.25 N HCl for 4 hr, and the extract was discarded. Histones F1, F2b, and F2c were then selectively extracted from the chromatin residue in eight volumes of 0.25 N HCl for 18 hr. After reextraction of the chromatin in four volumes of fresh 0.25 N HCl for 3 hr, the 0.25 N HCl extracts were combined, and the contained histones were precipitated by addition of five volumes of cold acetone (Johns, 1964). The histones were sedimented, washed, dried, and lyophilized as described earlier.

After oxidation at pH 8.3 as described below (in order to convert the small amounts of contaminating F3 histone to the dimer form), histones F1, F2b, and F2c were resolved by gel filtration on Sephadex G-100. Final purification of histone F2c, however, required ion exchange chromatography on Bio-Rex 70 resin as described below in order to remove small amounts of contaminating F1 and F3 (dimer) histones.

Oxidation of Histones. F3 + F2a2 histones were oxidized by incubation in 6 M Gdn·HCl–0.3 M Tris-HCl buffer at pH 8.3 (37°, 18 hr) at a protein concentration of 4 mg/ml. The F1 + F2b + F2c histone fraction was oxidized as above except that a higher protein concentration of 10 mg/ml was utilized to facilitate F3 dimer formation (Marzluff *et al.*, 1972), since only a relatively small amount of contaminating F3 histone was present in this fraction as indicated by polyacrylamide gel electrophoresis. After oxidation, the solutions were dialyzed exhaustively against distilled water and the histones were quantitatively recovered by addition of 0.67 volume of cold 50% trichloroacetic acid (w/v) and centrifugation at 10,000*g* for 10 min. The supernatants were discarded, and the oxidized histones were washed with acidified acetone (0.5 ml of 12 N HCl/l. of acetone), acetone, and ether. After drying *in vacuo* at room temperature, the oxidized histones were dissolved in 0.1 N acetic acid and lyophilized prior to gel filtration.

Gel Filtration. Samples of oxidized F3 + F2a2 histones (24 mg) and F2a2 + F2a1 histones (43 mg) were dissolved in 0.5 and 0.8 ml of 0.01 N HCl, respectively, and chromatographed separately on Sephadex G-100 equilibrated with 0.01 N HCl (Mauritzen *et al.*, 1966). The column was 2.4 × 165 cm and 3.2-ml (12 min) fractions were collected at ambient temperature. Protein was monitored by reading eluate absorbance at 235 nm with a Beckman DU spectrophotometer (Model 2400). A larger sample of oxidized F1 + F2c + F2b histones (125 mg) was dissolved in 3.0 ml of 0.01 N HCl and chromatographed on a 4.5 × 165 cm column of Sephadex G-100 equilibrated with 0.01 N HCl; 7.3 ml (12 min) fractions were collected. Protein was monitored as above.

Ion-exchange chromatography was performed on Bio-Rex 70 resin (200–400 mesh, Bio-Rad Laboratories, Richmond, Calif.) as described by Marushige and Dixon (1971) and modified by Cieplinski and Huang.[4] The resin was cycled by the method of Bonner *et al.* (1968) and a 4.5 × 8 cm column was prepared. Histone samples were layered in 5.0 ml of 8% (w/v) Gdn·HCl in 0.1 M sodium phosphate buffer at pH 6.8, and eluted at ambient temperature with a stepwise gradient of 8% Gdn·HCl (125 ml), 10% Gdn·HCl (125 ml), 13% Gdn·HCl (125 ml), 13% Gdn·HCl (125 ml), and 40% Gdn·HCl (100 ml) in the presence of 0.1 M sodium phosphate buffer at pH 6.8. Flow rate was maintained at 60 ml/hr, and 5-ml fractions were collected. The eluate protein was monitored at 235 nm, and the Gdn·HCl concentration was monitored *via* index of refraction. After each usage the column was regenerated with 100 ml of 8% Gdn·HCl at pH 6.8. Gdn·HCl was removed from the pooled column fractions by exhaustive dialysis against 0.1 N acetic acid, and the purified histones were recovered by lyophilization.

Electrophoresis. Disc gel electrophoresis was performed at pH 2.8 by a slight modification of the methods of Panyim and Chalkley (1969a,b). Gels (10 × 0.4 cm) contained 15% acrylamide, 2.5 M urea, 0.5% Temed (w/v), and 0.125% $(NH_4)_2S_2O_8$ (w/v) in 0.9 N acetic acid. Ethylene diacrylate [0.1% (v/v)] was utilized as the cross-linker to allow the gels to be solubilized (Choules and Zimm, 1965). After preelectrophoresis of the gels for 8 hr at 0.89 mA/tube, samples (10–40 μg) were layered in up to 40 μl of 0.5 M sucrose–0.001 N HCl and electrophoresis was performed for 6.5 hr at 0.89 mA/tube. Gels were stained for 5 hr in 1% Amido Black 10B in 7% acetic acid and destained in 7% acetic acid. (During the process of staining and destaining the gels reproducibly swelled to a length of 13.5 cm.) The gels were scanned at 615 nm with a Gilford linear transport scanner (Model 2410).

Cysteine Content Determination. The cysteine content of histone F3 was determined as cysteic acid after performic acid oxidation (Hirs, 1967).

Amino Acid Analysis. Amino acid analyses were performed on a Beckman automatic amino acid analyzer (Model 121). Duplicate samples of each purified histone were analyzed after hydrolysis *in vacuo* (in 6 N HCl containing 0.1% phenol) for 24 and 72 hr at 110°. Values for serine and threonine were corrected for losses (<10%) occurring during hydrolysis by assuming first order decay. Values for valine, leucine, and isoleucine were obtained from the 72-hr hydrolysates.

Results and Discussion

Erythrocyte Total Histone. Figure 1 shows the gel electrophoresis pattern of total histone isolated from the avian

[4] W. Cieplinski and R. C. Huang, personal communication.

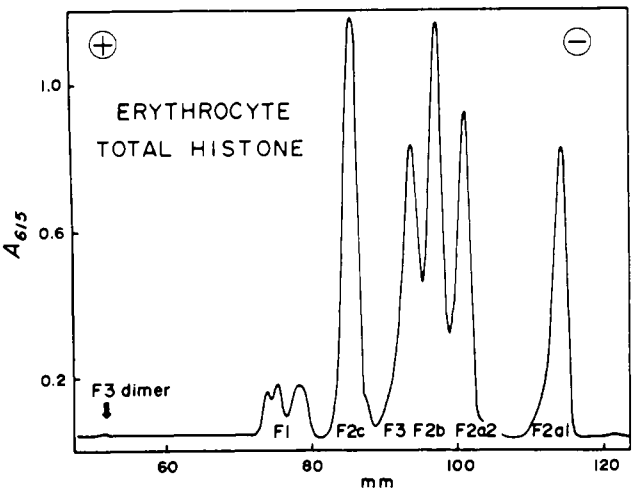


FIGURE 1: Electrophoresis of erythrocyte total histone. Disc gel electrophoresis was performed at pH 2.8 as described under Materials and Methods. Migration distances, given in millimeters, actually refer to position in the gel after staining and destaining. Note that negligible F3 dimer is present in the total histone as isolated here.

erythrocyte by extraction in 0.25 N HCl. Identification of the various bands is in agreement with that of Panyim *et al.* (1971). It is important to note that negligible F3 dimer (F3-S-S-F3) is present in the total histone as isolated. This finding is in agreement with that of Sadgopal and Bonner (1970) for the interphase chromatin of HeLa cells, but is in disagreement with that reported by Adams and Neelin (1969) who observed F3 dimer in their partially purified avian histone fractions. However, these avian histones had been exposed to Gdn·HCl at close to neutral pH—a condition which we have found facilitates F3 dimer formation (Figures 2 and 3).

The finding of a relatively low proportion of histone F1 in avian erythrocytes (3.5% of the total histone as determined by quantitative gel electrophoresis) is in agreement with the earlier studies of Dick and Johns (1969), and appears to be due to the unique relative proportions of the avian erythrocyte histones, rather than to inadequacies of the extraction procedures.

The erythrocyte-specific F2c histone migrates between histones F1 and F3 in this electrophoretic system. Slight asymmetry of the F2a1 band is apparently due to partial acetylation of this histone as discussed by Sanders *et al.* (1972).

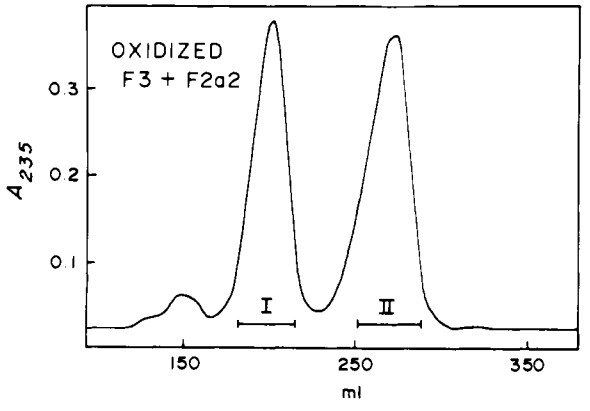


FIGURE 2: Gel filtration of oxidized F3 + F2a2 histones. F3 + F2a2 histones were isolated, oxidized, and chromatographed on Sephadex G-100 as described under Materials and Methods. Column fractions were combined as indicated.

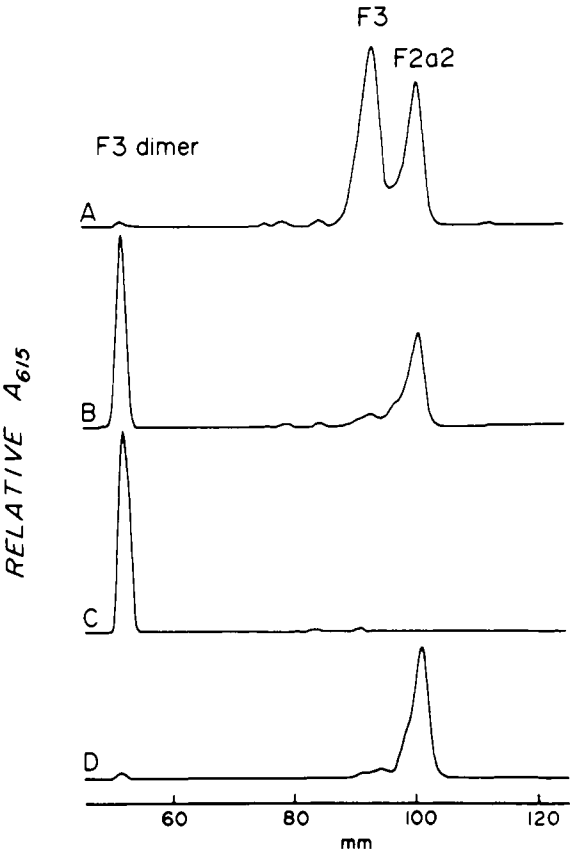


FIGURE 3: Electrophoresis of various F3 and F2a2 histone samples. (A) Starting F3 + F2a2. (B) F3 + F2a2 after oxidation at pH 8.3. (C) Peak I of Figure 2. (D) Peak II of Figure 2.

Isolation and Purification of Histones F2a1, F2a2, and F3. When chromatin was extracted with 80% ethanol-0.25 N HCl, histones F2a1, F2a2, and F3 were selectively removed. During dialysis of this extract against absolute ethanol, approximately 80% of the F2a2 histone coprecipitated with histone F3 as determined by quantitative gel electrophoresis. Figures 2 and 3 show the subsequent separation and purification of histones F2a2 and F3. As shown, after oxidation of F3 to the dimer form (F3-S-S-F3), this histone could be

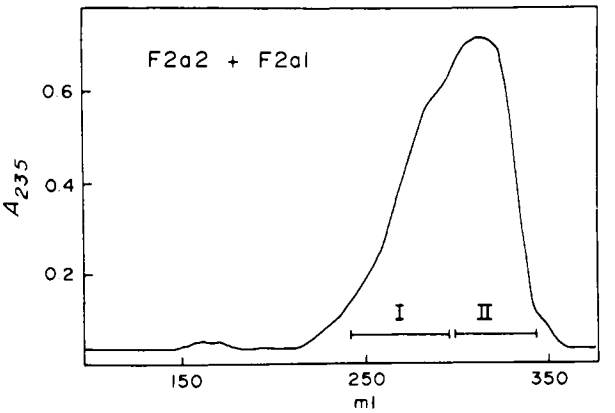


FIGURE 4: Gel filtration of F2a2 and F2a1 histones isolated as described under Materials and Methods.

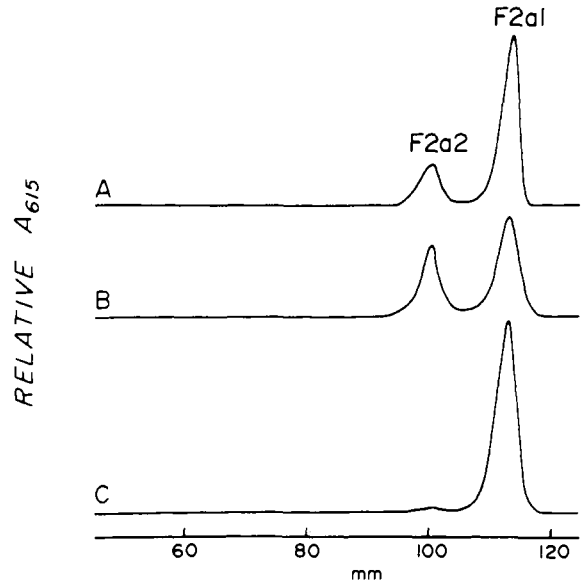


FIGURE 5: Electrophoresis of various F2a2 and F2a1 histone samples. (A) Starting F2a2 + F2a1. (B) Pool I of Figure 4. (C) Pool II of Figure 4.

resolved from F2a2 by gel filtration on Sephadex G-100 (Figure 2). The small peak eluting at 150 ml, the column void volume, was apparently nucleic acid free of protein as indicated by an $A_{260/280}$ ratio of 1.72 (in SSC–0.25 M sucrose, pH 7.5) and an inability to manifest any Amido Black stainable bands on gel electrophoresis.

The supernatant remaining after dialysis of the 80% ethanol–0.25 N HCl extract against absolute ethanol contained the remaining F2a2 and all of the F2a1 histone. As shown in Figures 4 and 5, these histones could be partially resolved by gel filtration on Sephadex G-100. Complete resolution could be achieved by refiltration of pool I (Figure 4) on the same column of Sephadex G-100.

Isolation and Purification of Histones F1, F2b, and F2c. The chromatin residue remaining after the 80% ethanol–0.25 N HCl extraction contained predominantly histones F1, F2b, and F2c with only very small amounts of contaminating histones F2a1, F2a2, and F3 (as determined by gel electrophoresis). After extraction from the chromatin residue in

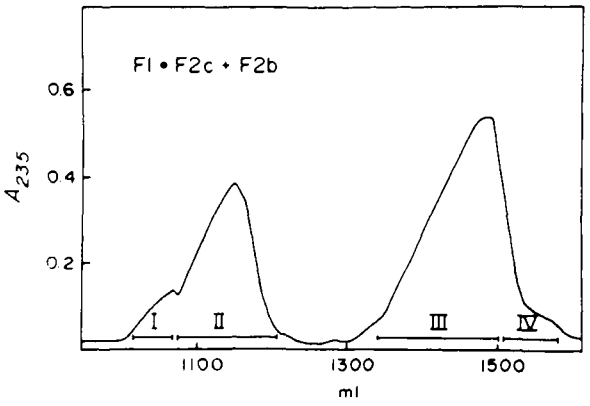


FIGURE 6: Gel filtration of F1 + F2c + F2b histones. F1 + F2c + F2b histones were isolated and oxidized as described under Materials and Methods prior to gel filtration on Sephadex G-100. Column fractions were pooled as indicated.

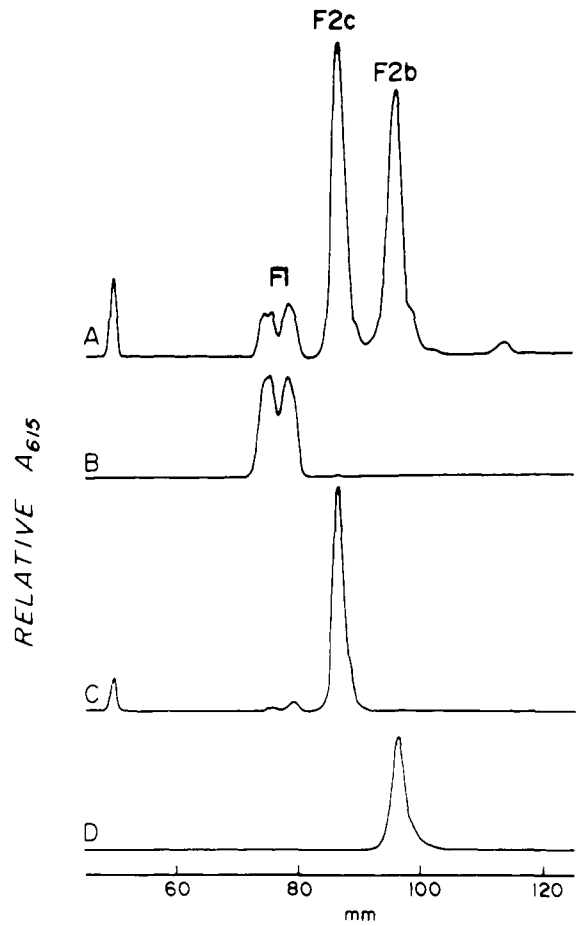


FIGURE 7: Electrophoresis of various F1, F2c, and F2b histone samples. (A) Oxidized F1 + F2c + F2b. (B) Peak I of Figure 6. (C) Peak II of Figure 6. (D) Peak III of Figure 6.

0.25 N HCl and oxidation at pH 8.3, histones F1, F2b, and F2c were resolved by gel filtration on Sephadex G-100 as shown in Figures 6 and 7. It is interesting to note that in spite of a large difference in molecular weight between the F3 dimer (mol wt 30,000–33,000, Marzluff *et al.*, 1972) and the erythrocyte-specific F2c (mol wt 16,000–19,000, Huang and Cieplinski, 1972) these two histones cochromatographed on Sephadex G-100. This finding indicates that in spite of the large molecular weight difference, the hydrodynamic radii

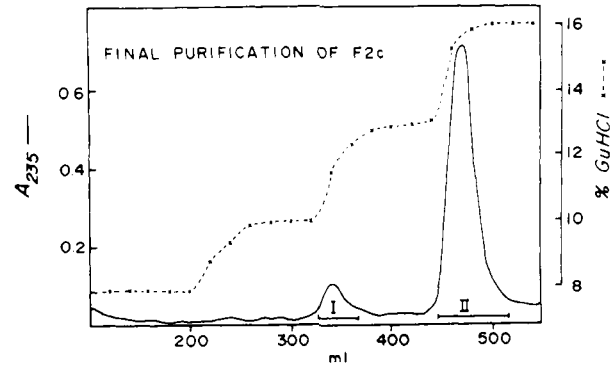


FIGURE 8: Ion-exchange chromatography of peak II of Figure 6 on Bio-Rex-70 resin. Histones were eluted with a stepwise gradient of Gdn·HCl as shown.

TABLE I: Amino Acid Analysis of Duck Erythrocyte Histone Fractions.[a]

Amino Acids	Total Amino Acid Content (mole %)					
	F1	F2c	F2b	F2a2	F2a1	F3
Lysine	25.9	24.0	14.5	10.8	9.8	9.7
Histidine	0.4	1.9	2.4	2.3	2.5	1.5
Arginine	2.9	11.6	7.2	9.2	12.9	13.3
Aspartic acid	2.0	1.8	5.1	6.2	5.5	3.8
Threonine	5.3	2.1	6.6	4.3	6.0	7.0
Serine	6.6	9.4	9.3	4.9	3.0	5.3
Glutamic acid	4.4	5.1	8.7	9.4	7.0	10.3
Proline	9.7	7.9	5.0	4.3	1.7	5.1
Glycine	6.8	5.6	6.7	9.2	14.6	4.9
Alanine	24.0	17.1	10.2	12.9	8.6	13.0
Half-cystine	0	0	0	0	0	0.6
Valine	4.3	3.2	6.4	6.0	8.0	4.6
Methionine	0	0.8	1.6	0.4	0.9	1.4
Isoleucine	1.2	3.0	5.1	4.4	4.9	5.3
Leucine	4.8	4.1	6.0	11.9	8.8	9.3
Tyrosine	0.7	2.2	3.4	2.5	3.7	2.2
Phenylalanine	0.7	0.2	1.4	1.2	2.1	2.8

[a] Sources of each of the purified histones are the same as in Figure 10. Amino acid analyses were performed as described under Materials and Methods.

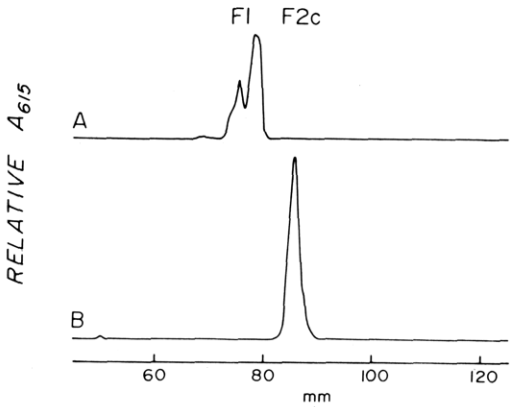


FIGURE 9: Electrophoresis of F1 and F2c histones. (A) Peak I of Figure 8. (B) Peak II of Figure 8.

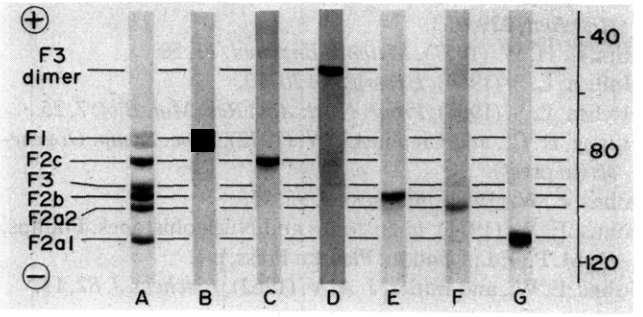


FIGURE 10: Disc gels of purified histone fractions. (A) Erythrocyte total histone. (B) F1 (peak I, Figure 6). (C) F2c (peak II, Figure 8). (D) F3 dimer (peak I, Figure 2). (E) F2b (peak III, Figure 6). (F) F2a2 (peak II, Figure 2). (G) F2a1 (pool II, Figure 4). The scale at the right gives migration distances in millimeters.

of the F3 dimer and F2c must be very close to each other in 0.01 N HCl. (Although not presented here, gel electrophoresis of peak IV (Figure 6) revealed the presence of the small amounts of contaminating histones F2a1 and F2a2.)

Final Purification of Histone F2c. As demonstrated in Figures 8 and 9, the final purification of histone F2c was achieved by ion-exchange chromatography on Bio-Rex-70 resin in order to remove the small amounts of F1 and F3 dimer still present after gel filtration on Sephadex G-100 (Figure 7C). As shown, histone F1 was eluted from the column at a lower ionic strength than was F2c. The F3 dimer present had the greatest affinity for the column but was readily eluted by 40% Gdn·HCl (w/v).

Criteria of Purity of the Isolated Histones. Disk gels of the final purified F2c along with the other purified histone fractions are shown in Figure 10.

Amino acid compositions of each of the purified histone fractions are presented in Table I. These amino acid compositions are in close agreement with those of the corresponding histone fractions present in calf thymus (Panyim *et al.*, 1971).

In view of the amino acid compositions reported above in conjunction with the use of polyacrylamide gel electrophoresis as a criterion of purity, it is apparent that the fractionation procedures reported here for avian erythrocyte histones provide several advantages over earlier procedures, especially in the isolation and fractionation of the arginine-rich histones. For example, the arginine-rich histone F2a1 fraction as isolated previously was apparently highly contaminated with histone F3 as indicated by the reported Gly/Ala ratio of 0.92 (Vidali and Neelin, 1968) as compared to a Gly/Ala ratio of 1.70 reported here and a Gly/Ala ratio of 1.93 reported for purified calf thymus F2a1 histone (Panyim *et al.*, 1971). Furthermore, when fractionated previously by another method (Adams and Neelin, 1969) avian erythrocyte histones F2a1 and F3 were apparently obtained in very poor yield relative to histone F2c.

In addition to avoiding the above problems, the procedure reported here has two major advantages. (1) In the initial histone fractions obtained by selective extraction of chromatin, there is sufficient separation of bands via polyacrylamide gel electrophoresis to permit accurate determination of specific activities on small amounts (10–40 μg) of histone following *in vitro* labeling studies. (2) The procedure reported here appears to give identical fractionation of the histones from avian reticulocytes as well as mature erythrocytes. A following paper (Sanders *et al.*, 1972) employs the procedure reported here in a comparative study of histone acetylation and deacetylation in avian reticulocytes and erythrocytes.

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Purification and Characterization of a Glycoprotein from the Intimal Region of Porcine Aorta†

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ABSTRACT: A relatively simple procedure was devised for the isolation and purification of a glycoprotein from the intimal region (150 μ in thickness). This procedure included the extraction of the tissue with buffer at neutral pH and fractionation of the extract with ammonium sulfate followed by column chromatography on diethylaminoethylcellulose. A highly purified glycoprotein obtained by above methods appeared to be homogeneous by ultracentrifugation and polyacrylamide gel electrophoresis. The $s_{25,w}$ value was found to be 4.86 S. The weight-average molecular weight of the glycoprotein determined by sedimentation equilibrium method was 71,000. The glycoprotein did not contain hexuronic acid nor sulfate. The amino acid and carbohydrate composition of the glycoprotein was determined. The carbohydrate moiety consisted of 1 mole each of fucose and sialic acid, 2 moles of mannose, 3 moles each of glucose and galactose, and 4 moles of *N*-acetylhexosamine per molecule of the glycoprotein. Due to the presence of equimolar amounts of glucose and galactose and the absence of hydroxylysine in the molecule, it is suggested that this glycoprotein is unique in its characteristics and hence is of a new type.

Normally, intima may be defined as that layer extending from the endothelium to the internal elastic lamellae of the arteries. It has been recognized that the histochemical, ultrastructural and biochemical properties of the intima are distinctly different from those of the rest of the arterial wall (Lazzarini-Robertson, 1963).

The occurrence of glycoproteins in the mammalian arterial wall has been demonstrated histologically (Bertelsen, 1963), chemically by the presence of sialic acid and of those neutral sugars known to be unassociated with mucopolysaccharides (Murata and Kirk, 1962; Seng *et al.*, 1965), and by direct isolation of these macromolecules from this tissue (Radhakrishnamurthy *et al.*, 1964; Barnes and Partridge, 1968; Moczar and Robert, 1970). In these studies the entire aortic

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