Some Researches on Histones

J. A. V. BUTLER

From the Chester Beatty Research Institute, Institute of Cancer Research, Royal Cancer Hospital, London, England

ABSTRACT The histone extracted from calf thymus glands is a complex system of proteins, which can be fractionated by chromatography on carboxymethyl cellulose columns into three principal fractions (1) very lysine-rich, (2) moderately lysine-rich, (3) arginine-rich. When examined by starch gel chromatography each of these gives more than one band. Methods have been devised for further separation of the components in some cases. The components show characteristic differences in end groups and certain amino acids as well as in their basic character. Histones extracted from various rat tissues can be separated into similar fractions, of which the amino acid analyses are similar to those derived from calf thymus, within the experimental error. To this extent, no species or tissue specificity of the fractionated histones was observed. Although all the histone fractions contain approximately one basic amino acid to three non-basic amino acids their structure is not regular, as Phillips has shown that in certain fractions the number of non-basic groups between two basic groups may vary from 0 to seven or more. The possible functions of histones are discussed.

INTRODUCTION

Basic proteins were first extracted from cell nuclei by Miescher in 1870–1874 (1), who isolated the type known as protamines from salmon sperm. The name, histone, was originally given to a basic protein extracted from chicken erythrocytes by Kossel in 1884 (2). Although a considerable amount of work was done in succeeding years on the simpler protamines by Miescher, Kossel, Felix, and others, comparatively little was learned about histones until modern methods of protein analysis became available. Preparations from a number of tissues and the first attempt at fractionation were made by Stedman and Stedman (3), who distinguished what they then called the main histone and the subsidiary histones, the latter having about half the arginine content of the former. They also obtained a fraction rich in lysine, although a quantitative analysis was not given.

Fractionation Procedures

During the 1950's numerous attempts at further fractionation were made, particularly on the histone of calf thymus. On the one hand Davison and

Butler (4), Crampton, Moore, and Stein (5), and Daly and Mirsky (6) all obtained very lysine-rich histones with over 25 mols per cent lysine and 1 to 4 per cent of arginine, while other procedures gave rise to two other fractions one rich in arginine (lys/arg = 0.8) and the other somewhat richer in lysine (lys/arg = 1.1–1.5). Some of these procedures are listed in Table I.

Methods of fractionation by chromatography were developed with partial success by Crampton, Moore, and Stein (5), by Davison (7), and by Luck *et al.* (8).

An improved method of chromatography on carboxymethyl cellulose (CMC) developed by Phillips and Johns (10) and by Johns, Phillips, Simson, and Butler (11) has permitted the separation of the calf thymus histone into

TABLE I

LYSINE-ARGININE RATIOS OF SOME HISTONE FRACTIONS

Method	Fraction	Lysine/arginine	Authors
Citric acid extraction	Extract	18	Davison and Butler (4)
Isolectric precipitation at pH 10.6	Precipitate	1.01	Daly and Mirsky (6)
Isoelectric precipitation at pH 10.6	Supernate	11	Daly and Mirsky (6)
Salt-NH_3	Precipitate	0.7	Phillips and Johns (10)
Salt-NH_3	Supernate	2.3	Phillips and Johns (10)
Chromatography amberlite	A	15.7	Crampton *et al.* (5)
Chromatography amberlite	B	1.13	Crampton *et al.* (5)
Aggregation	β	0.74	Cruft *et al.* (9)
Chromatography CMC	f1A	9.0	Johns *et al.* (11)
Chromatography CMC	f2	1.34	Johns *et al.* (11)
Chromatography CMC	f3	0.78	Johns *et al.* (11)

three distinct fractions (see Fig. 1). The first (f1) eluted by sodium acetate contains (in two peaks) the very lysine-rich histones; the second (f2) eluted by 0.01 N HCl consists of moderately lysine-rich histones and the third, eluted by 0.02 N HCl the arginine-rich histones. Amino acid analyses of these fractions are shown in Table II.

The characterization of these fractions has been greatly assisted by the use of starch gel electrophoresis, developed by Smithies (12) and applied to histones by Neelin and Connell (13) and Neelin and Butler (14). We have preferred to use very acid starch gels (pH 2.3) to avoid aggregation artefacts and typical pictures obtained with the whole histone from calf thymus and some fractions are shown in Fig. 2. When this method was employed with the histone fractions described above, it was possible to identify the distribution of the electrophoretic bands between the different groups, as shown in Fig. 3 (Johns *et al.* (15)). It is evident that the groups isolated by the chromato-

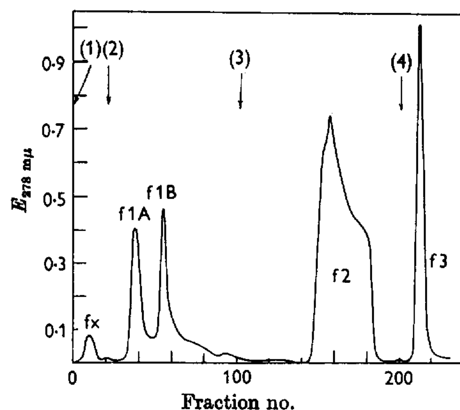

FIGURE 1. Chromatography of calf thymus histone on a carboxymethyl-cellulose column (11). Eluting solutions: (1) 0.1 M sodium acetate buffer, pH 4.2; (2) 0.17 M sodium acetate buffer, pH 4.2; (3) 0.01 N hydrochloric acid; (4) 0.02 N hydrochloric acid (from *Biochem. J.*, 1960, *77*, 632).

TABLE II

AMINO ACID ANALYSES OF SOME FRACTIONS FROM CALF THYMUS HISTONE (11)

Values given are moles per 100 moles of all amino acids.

Fraction	fIA	fIB*	f2	f2(A)‡	f3 Chroma-tography	f3 Acid-ethanol extract
Aspartic acid	8.0 (Asp + Glu)	3.7	14.3 (Asp + Glu)	6.4	16.9 (Asp + Glu)	4.2
Glutamic acid		6.7		9.0		12.0
Glycine	7.2	7.0	10.3	11.8	6.6	6.1
Alanine	23.6	21.1	11.1	10.3	13.0	13.7
Valine	4.8	4.9	7.5	6.5	5.2	5.7
Leucine + isoleucine	5.4	5.2	12.7	14.9	13.8	13.6
Phenylalanine	0.7	1.3	1.5	1.7	2.9	2.4
Tyrosine	0.5	0.8	2.7	3.0	0.5	1.3
Serine	6.1	6.0	5.2	3.2	4.4	4.2
Threonine	5.6	4.8	5.4	5.7	6.2	6.9
Proline	8.9	9.1	3.6	3.2	4.9	4.7
Arginine	2.9	4.1	9.8	10.9	13.2	14.0
Histidine	0.4	0.5	2.7	1.9	2.2	2.2
Lysine	25.9	24.5	13.1	11.4	10.3	9.0
Lysine/arginine	9.0	6.0	1.34	1.05	0.78	0.64
Basic/acidic *N*-terminal groups	3.6	2.8	1.79	1.55	1.52	1.54
Alanine	—	—	10	—	69	96
Proline	—	—	70	—	14	3
Others	—	—	20	—	17	1

* Unpublished determination by D. M. Phillips and E. W. Johns.

‡ Analysis by E. W. Johns and J. A. V. Butler (19).

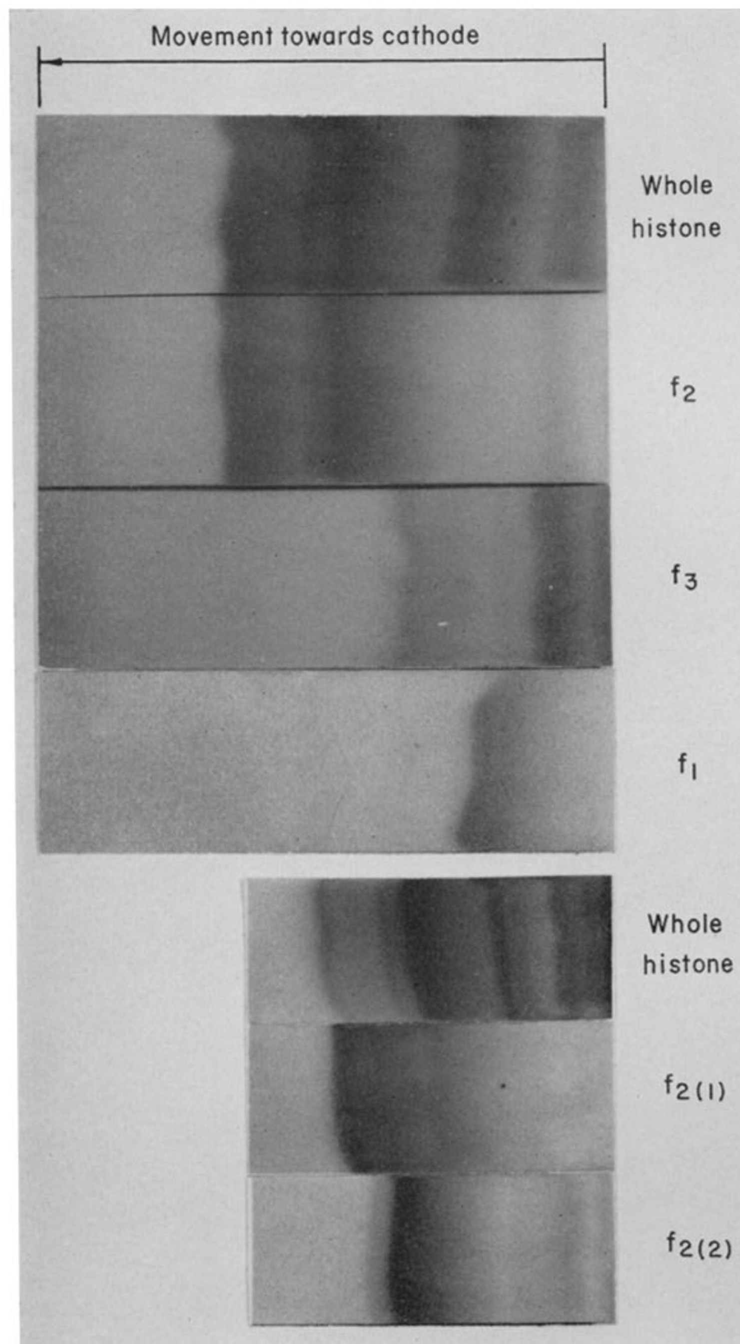

FIGURE 2. Starch gel electrophoresis pictures of the whole histone from calf thymus and various fractions obtained therefrom (18, 19).

graphic process are still complex and a complete resolution of the components of the histone has not been achieved.

The characterization of the histones has also been helped by the study of the *N*-terminal groups of the different fractions. With whole histone, it was found by Phillips (16) that the principal *N*-terminals are proline and alanine, with small amounts of several others, which tended to diminish when precautions were taken to avoid proteolytic degradation during the preparation. The examination of the fractions mentioned above further showed that the *N*-terminal of fraction f2 was mainly proline and that of f3 mainly alanine. The very lysine-rich fraction f1 had as *N*-terminals proline, lysine, and valine, but the amount collected was much smaller than that expected from the molecular weight. Phillips (17) has since shown that acetyl groups are obtained by hydrolysis of this fraction where they are presumably present as the *N*-terminal.

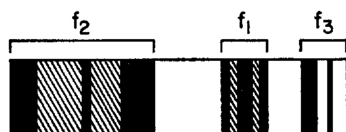

FIGURE 3. Diagrammatic representation of bands of calf thymus whole histone, showing their identification. The diagram shows features which are not visible in the photographs.

Further fractionation methods have been devised, which enable the preparation of some components in appreciable quantities. Thus Johns *et al.* (18) found that certain components only are extracted from calf thymus tissue (or nuclei) by acid in 80 per cent ethanol.

On dialyzing the extract against 100 per cent ethanol the arginine-rich histones are obtained as a precipitate, while one component of the moderately lysine-rich groups (f2, 1) remains in the solution (19). The very lysine-rich group can also be extracted quantitatively from the tissue by 5 per cent perchloric acid (19). A scheme of fractionation which makes use of these facts is shown in Fig. 4.

Species or Tissue Specificity of Histones

It can thus be only a matter of time before the principal histone components have been isolated from favorable tissues. It is perhaps a little premature to consider the question of species or tissue specificity, but some observations have been made by a number of authors. Mauritzen and Stedman (20) prepared and analyzed the arginine-rich histone (termed β) from three different fowl cell types and three bovine tissues (as well as calf thymus). The corresponding values for most amino acids were nearly equal but differences in certain amino acids were found which were regarded as being outside the analytical and other errors. However, no criteria of the purity of the prepara-

tions apart from the aggregation property were given. Neelin and Butler (21) also compared the starch electrophoretic pictures of histones from certain cells and found significant differences.

Hnilica *et al.* (22) have prepared three corresponding histone fractions from a number of rat tissues by methods similar to those mentioned above. With tissues other than calf thymus these methods did not give a complete separation, and additional purification steps had to be applied. The fractions separated were examined by starch electrophoresis and shown not to contain significant amounts of the other fractions. The analyses obtained of corresponding fractions were in nearly all cases remarkably independent of the

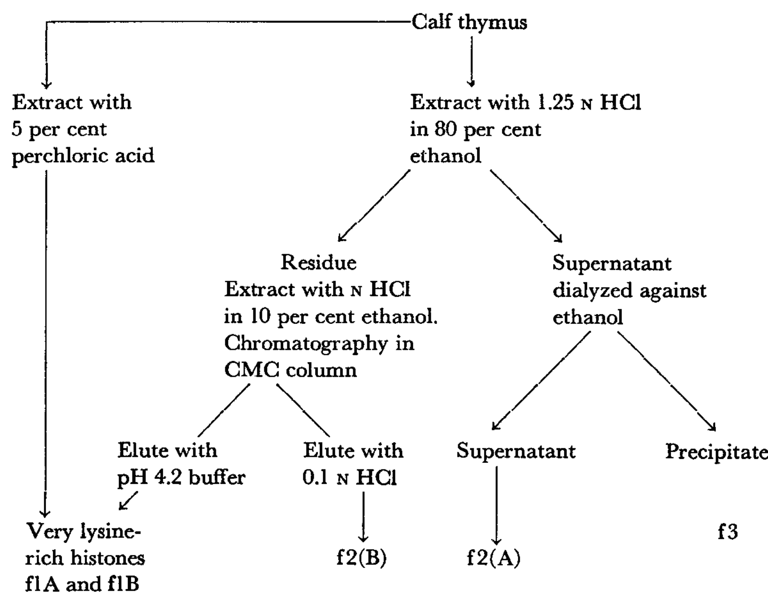

FIGURE 4. Scheme of fractionation of some constituents of calf thymus histone (19).

tissue from which they were obtained and the characteristic differences of composition of the fractions were preserved irrespective of the origin. It cannot, however, be assumed until histones from more diverse organisms have been examined that all somatic cells have a similar complement of histones irrespective of the species or cell type. The methods so far available also do not give an accurate estimate of the relative proportions of different histones in any type of cell.

Structure of Nucleoprotein

In the nucleus, histones are found in combination with DNA. Careful analyses of the relative amounts of basic amino acids and of phosphate groups in the nucleoprotein (23, 24) have shown that the ratio is nearly unity. In some

cases a deficit of basic groups of about 10 per cent, as compared with phosphate, has been observed (23, 5) but this may be due to loss of some histone during the extraction and washing of the nucleoprotein. In most histones the amount of basic groups is about 25 per cent of all the amino acids present although this is exceeded in the very lysine-rich group. If the arrangement of the amino acids were a regular one, we should in most cases find that basic amino acids were separated by three non-basic groups. However, there is strong evidence that the arrangement is not a regular one as Phillips (25) has found from the study of the peptides present in trypsin digests of the arginine-rich fraction f3, that the number of non-basic groups between two basic groups may vary from 2 to 7 or more. One of the first products of trypsin digestion is a precipitate which has a much lower ratio of basic amino acids than the average, indicating the presence in the histone molecules of tracts of relatively non-basic character.

Function of Histones

Many hypotheses of the functions of histones have been put forward. As part of the deoxyribonucleoprotein complex they must contribute to the structure of the chromosome. They are combined with the phosphate groups by a salt-like bond, which is easily dissociated in concentrated salt solutions. This combination will undoubtedly help to prevent the DNA from forming complexes with other proteins which may be present in the nucleus. The question of other proteins in the nucleus is a very difficult one. The nucleoprotein as isolated usually contains a small percentage of non-histone proteins of an acidic character. The amount of these varies much from one tissue to another, but also depends on the preparative procedures. Their relation to the histone is therefore uncertain. There is no *a priori* reason why the chromosome should not contain proteins other than histone—particularly in the case of banded chromosomes, such as those of *Drosophila* salivary glands, which contain visible regions with variable ratios of DNA and protein.

It was suggested by Stedman (26) that the histones also act as gene inhibitors, *i.e.* they render the genes, carried by the DNA, ineffective until the appropriate cell division has occurred. They would thus control differentiation. This would imply that particular histones are missing in each type of cell, and therefore the histones found would be cell-specific. As pointed out it has been difficult to demonstrate any cell specificity of histones, sufficiently great to show up in the over-all analyses of amino acids.

A modified and complicated version of this has been proposed by Leslie (27) who suggests that the histone attached to each DNA molecule acts as a specific ribonuclease for the RNA which is formed on the DNA template and thus prevents the gene expression except in appropriate circumstances.

The ten or more distinct species of histones in mammalian cells with characteristically differing composition, which are repeated in at least a variety of tissues, must have distinct functions. It does not seem at present that there is any evidence of any relation between the number of kinds of histone present in a cell and the number of chromosomes. The fact that the different types of histones have distinctive end groups may be important; *e.g.*, they might serve to protect the histone from proteolytic action by specific endopeptidases until required.

It is also possible that histones may have active functions in cell metabolism. It has been shown that the turnover rate of amino acids in histones is greater than can be accounted for if histones are merely synthesized in connection with the replication of chromosomes (28–30). The purpose of this turnover has not been detected. It would obviously be of great interest if it could be shown that the histones take some part in the transfer of genic information from the DNA to the cytoplasm. Basic proteins somewhat similar to histones, but apparently not identical with them, have been extracted from an RNA-rich fraction of ribosomes (31), but so far no clear indication has been obtained that the nuclear histones are precursors of these compounds (32).

It has been shown that in tumors histones are more active relative to the protein of the cytoplasm of the same cells, than in normal tissues (33, 30), as indicated by the relative incorporation of amino acids under *in vivo* conditions. This indicates a greater degree of metabolism of the histones in actively dividing tissues; but the actual amount of incorporation is greater than is necessary for replication. In these experiments the possibility that the protein metabolism in the cytoplasm of the normal tissues is abnormally low has not been excluded.

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