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Observations on the Species and Tissue Specificity of Histones

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The heterogeneity of the histones is now well established. A number of fractionation methods involving extraction procedures (Davison & Butler, 1954; Daly & Mirsky, 1955; Crampton, Stein & Moore, 1957; Ui, 1957), fractionations of previously isolated histones (Stedman & Stedman, 1951; Davison & Shooter, 1956; Cruft, Mauritzen & Stedman, 1958) or chromatography (Crampton, Moore & Stein, 1955; Davison, 1957; Luck, Rasmussen, Satake & Tsvetikov, 1958; Davis & Busch, 1959) have been proposed. Recently it has been shown that by a combination of two previous chromatographic methods (Davison, 1957; Phillips & Johns, 1959) it is possible to resolve calf-thymus histone into three main groups, namely, f1, a very lysine-rich fraction; f2, a slightly lysine-rich fraction and f3, an arginine-rich fraction (Johns, Phillips, Simson & Butler, 1960), but starch-gel electrophoresis showed that these were also complex (Johns, Phillips, Simson & Butler, 1961). A method of extracting f3 in quantity from calf thymus was given by the same authors.

Few extensive characterizations of histones prepared from other tissues of the calf or from similar tissues in different species have been made. Crampton *et al.* (1957) found that two main fractions of histones prepared from calf thymus, liver, kidney and from guinea-pig testis were each nearly identical in their amino acid composition and the chromatographic patterns of tryptic hydrolysates.

Vendrely, Knobloch & Matsudaira (1958) made qualitative chromatographic analyses of the hydrolysates of deoxyribonucleoproteins from different tissues of calf and from the erythrocytes of carp, trout, pike, tench, frog, fowl and duck, and found in all cases similar chromatographic patterns of amino acids.

On the other hand, Mauritzen & Stedman (1959, 1960) claim that their analytical results provide evidence of the tissue as well as species specificity of the arginine-rich β-histones. They also claim differences of electrophoretic mobility of similar histones derived from different sources (Cruft, Mauritzen & Stedman, 1954; Mauritzen & Stedman, 1959, 1960).

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Neelin & Butler (1959, 1961) and Neelin & Connell (1959) described some differences in the Amberlite IRC-50 elution and starch-gel electrophoresis patterns for the histones from calf thymus and chicken erythrocytes and also differences in histones from several chicken tissues. Similarly, Davis & Busch (1959) and Starbuck & Busch (1960) found differences in carboxymethylcellulose chromatography patterns and in incorporation of ^{14}C-labelled amino acids for histones prepared from various normal and neoplastic tissues.

In an attempt to obtain more information on this question a comparative analytical study was carried out with histones from spleen and liver of normal and leukaemic rats, from spleen and liver of calves and from Ehrlich ascites tumour.

MATERIALS AND METHODS

An attempt to resolve histones prepared from tissues other than calf thymus into the three main fractions as described by Johns *et al.* (1960), by using columns of carboxymethylcellulose as prepared by Peterson & Sober (1956), failed to give a complete separation into these groups. Although a three-peak elution pattern was obtained, each fraction was found to be contaminated by one or both of the other fractions, e.g. in f1 it was found by starch-gel electrophoresis that all three components were present and in f2 and f3 a mixture of both. Even rechromatography of the material recovered from each peak did not produce a complete fractionation.

In some cases a fraction was not recovered at all on rechromatography, perhaps because the action of proteolytic enzymes is much higher in tissues other than calf thymus (Phillips & Johns, 1959). For neoplastic tissues, with the exception of leukaemic tissue, the preparation of good samples of f1 and f2 fractions was impossible, probably for the same reason. Possibly as a result of the action of these enzymes some degraded histone eluting from the columns as a forerunner was observed in these cases. Fig. 1 shows the general scheme of fractionation which was finally used.

The method of extraction of the arginine-rich fraction as described by Johns *et al.* (1960) for calf thymus was applied to the deoxyribonucleoprotein, derived from preparations of saline-washed nuclei. In all cases the arginine-rich histone (C) obtained was contaminated by a substantial amount of the slightly lysine-rich one and further chromatographic purification was necessary. Ba and Bb both belonged to the slightly lysine-rich group and as they contained similar electrophoretic components in different proportions they were united to give the whole electrophoretic group B. The methods described in detail below gave satisfactory fractions.

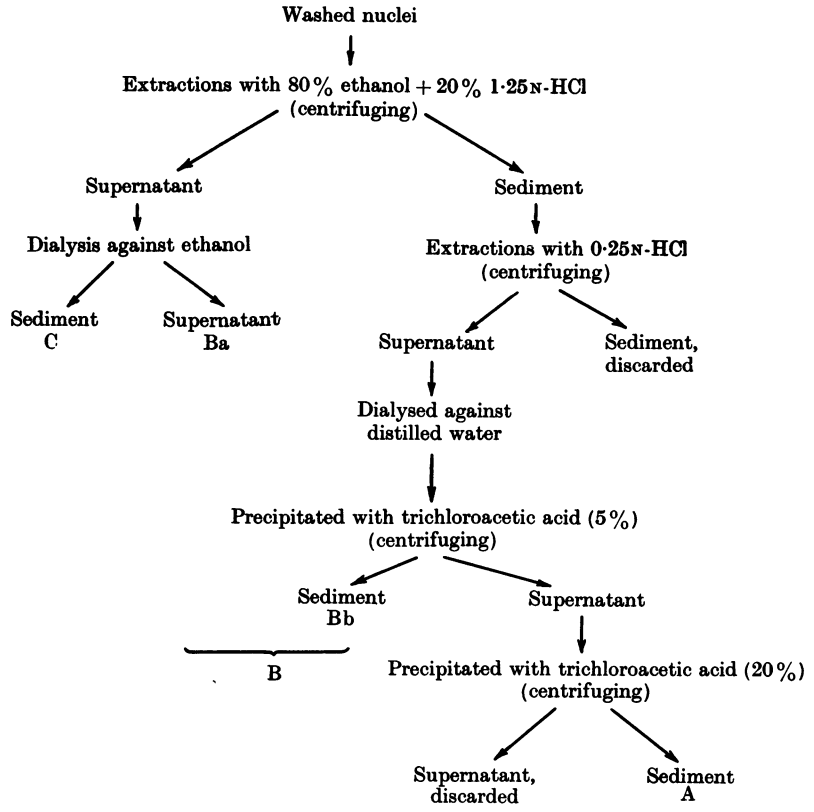

Fig. 1. Scheme of isolation of histone fractions.

Isolation of nuclei. Fresh tissues or tissues frozen and stored over solid CO_2 were used for all preparative work. The following method for isolation of nuclei was used and resulted in nuclei with only slight cytoplasmic contamination.

The sliced tissue was mixed with 5 vol. of 0·25 M-sucrose containing 5 mM-$CaCl_2$ and homogenized in the Potter–Elvehjem homogenizer for a short time. The homogenate was filtered through one layer of lint and the filtrate was then resuspended in the homogenizer and given approximately 20 strokes with a suitable plunger. The final homogenate was underlaid with an approximately equal volume of 0·34 M-sucrose containing 0·2 mM-$CaCl_2$ and centrifuged at 1700*g* for 10 min. Then the isolation scheme described by Schneider (1958) was followed. After four successive washings the final sediment was rehomogenized in 20 vol. of 2·2 M-sucrose according to Chaveau, Moulé & Rouiller (1956) and centrifuged for 1 hr. at 40000*g*. The final sediment contained the nuclei.

Isolation of histones. Nuclei prepared in this way were homogenized in about 30 vol. of 0·14 M-NaCl containing 0·01 M-trisodium citrate. The homogenate was centrifuged for 10 min. at 2000*g*; the supernatant was discarded and the sediment washed twice more in the same manner. The sediment was washed twice by homogenization in 70% ethanol and centrifuging. This procedure yielded crude deoxyribonucleoprotein in the form of a white sediment from the last centrifuging. This was extracted with 0·25 N-HCl in 80% (v/v) ethanol as described by Johns *et al.* (1960). The arginine-rich fraction obtained in this way requires further purification when applied to tissues other than calf thymus.

On dialysis against ethanol, a precipitate of the arginine-rich histone was obtained, which was redissolved in 80% ethanol containing 0·25 N-HCl and redialysed against ethanol. The white precipitate was washed with acetone and ether and dried *in vacuo* giving the crude fraction of arginine-rich histone (C).

The supernatants obtained after precipitation of the C fraction during dialysis were collected and mixed together, and the volume was reduced to one-tenth of the original by evaporation in the cold room, in Visking tubing. The reduced volume was then dialysed against distilled water, slightly acidified with HCl. After dialysis, trichloroacetic acid, 1 g./ml., was added to a concentration of 5%. A precipitate that formed overnight was collected by centrifuging, washed with acetone containing 1% of HCl, then with acetone only and finally with ether, and then dried *in vacuo* (Ba). No further precipitate was obtained from the supernatant by increasing the trichloroacetic acid concentration up to 20%.

The residue left after extraction of the nuclei with 80% ethanol–HCl was extracted three times with aqueous 0·25 N-HCl; the mixed extracts were dialysed against distilled water until the pH of the dialysis residue was approximately 3·0 (12 hr.). The protein was then precipitated with

trichloroacetic acid, to a final concentration of 5%. The precipitate which formed overnight was collected, washed and dried in the same way as described for Ba (Bb). The supernatant contains practically the whole of the very lysine-rich fraction, which is soluble in 5% trichloroacetic acid. This fraction was obtained by increasing the trichloroacetic acid concentration to 20%. The precipitate that formed overnight was denser than the voluminous trichloroacetic acid precipitates of C, Ba and Bb. It was collected, redissolved in a minimal amount of 5% trichloroacetic acid and clarified by centrifuging. The reprecipitation with trichloroacetic acid was repeated until the sediment, dissolved in 5% trichloroacetic acid, gave a clear solution. The final precipitate was washed and dried as described above. The very lysine-rich fraction (A) resulting from this procedure was pure as shown by starch electrophoresis, and no further purification was necessary. The general scheme of this fractionation is shown in Fig. 1.

Purification of the B and C fractions. The arginine-rich histone (C) was dissolved in distilled water, clarified by centrifuging and chromatographed on the carboxymethylcellulose column as below. The slightly lysine-rich histones (Ba and Bb) were mixed and dissolved in distilled water, clarified by centrifuging and reprecipitated with trichloroacetic acid (5% final concentration) to remove traces of the very lysine-rich component (A). The trichloroacetic acid precipitate (B) collected by centrifuging was washed and dried as above and dissolved in distilled water for chromatography on carboxymethylcellulose.

Chromatography. The carboxymethylcellulose was prepared according to Peterson & Sober (1956) and was used as described by Phillips & Johns (1959). The slightly lysine-rich fraction (B) was obtained with 0·01 N-HCl as eluent, the arginine-rich histone (C) with 0·02 N-HCl.

The portion of the arginine-rich fraction resulting from the chromatography of B was mixed with C, and the whole neutralized carefully and rechromatographed with steps of 0·01 N-, 0·012 N- and 0·02 N-HCl, dialysed against distilled water for 6 hr. and freeze-dried.

Isolation of histones from leukaemic tissues. For leukaemic rat liver or spleen the following method, similar to that applied by Butler, Davison, James & Shooter (1954) to calf thymus, was found to be satisfactory. The spleens or livers from rats bearing acute myeloid leukaemia (Hlavayová, 1957) heavily infiltrated with leukaemic cells were homogenized in 0·14 M-NaCl containing 0·01 M-trisodium citrate and given at least six successive washings in the same solution. The final sediment of crude deoxyribonucleoprotein was extracted three times with aqueous 0·25 N-HCl; the mixed extracts, clarified by centrifuging, were dialysed against distilled water and freeze-dried.

To a solution of the whole histone so obtained, trichloroacetic acid was added to a final concentration of 5%. The very lysine-rich fraction was obtained from the supernatant as described above by increasing the trichloroacetic acid concentration to 20%. The precipitate was resolved into the B and C fractions as above on carboxymethylcellulose columns. This method was not satisfactory with the other tissues investigated. All preparations of the histones from leukaemic rat spleen and liver were made by Dr V. Holoubek in Bratislava.

Starch-gel electrophoresis. The method used was essentially the same as that described by Smithies (1955). Unbuffered 0·01 N-HCl was used in place of the borate buffer for the electrode vessels and for making the gel, as described in more detail by Johns *et al.* (1961).

Neelin & Neelin (1960) have reported that the mobilities of histone and histone fractions are dependent on the amounts of the samples applied. This has been confirmed by our work and seems to indicate that some type of adsorption is playing an important part in the separation. It is therefore necessary when making comparisons to take the same weight of a given histone fraction as that present in the sample of whole histone used. The actual weights (mg.) of protein applied to the gel (2 cm. × 0·5 cm. × 26 cm.) were as follows: whole histone, 2·0; lysine-rich fraction, 0·4; slightly lysine-rich fraction, 1·1; arginine-rich fraction, 0·5. All samples were dissolved in 0·1 ml. of 0·01 N-HCl and applied to the gel with starch grains as a supporting medium. The voltage gradient was 4 v/cm. and was applied for 18 hr. All runs were carried out at room temperature.

The relative mobilities of the principal bands of each fraction were measured by the distance travelled by the leading edge of the main bands, in each case relative to the fastest band of the whole histone from calf thymus in the same run. These determinations were carried out in trays with six channels, to which were applied five samples to be examined and a sample of whole histone from calf thymus as standard. The mobilities of the different bands of the whole histone of calf thymus, relative to the fastest band, which is taken as 1·0, are given in the last row of Table 1.

Amino acid and N-terminal group analyses. These were determined as previously described (Phillips, 1958; Phillips & Johns, 1959) by a modification of the fluorodinitrobenzene method of Sanger (1945).

All analyses were performed in duplicate, and the values given represent the averages obtained with two or three distinct preparations.

As a rule only one determination of the *N*-terminal group was made for each fraction.

RESULTS

The starch-gel electrophoretic method was applied primarily to establish the substantial purity of the fractions examined, as judged from the non-appearance of the bands of other fractions. The lysine-rich fractions (A) appeared to be free from contamination of any kind; but the other fractions showed weak components derived from other groups. The slightly lysine-rich fractions (B) all contain a small proportion of a slow-moving component, whose relative mobility is similar to that of the arginine-rich component. The arginine-rich fractions (C) are more heavily contaminated and show minor bands which do not correspond closely to any of the other groups. However, most of them come within the range of the slightly lysine-rich group (B) (relative mobility 0·6–1·00) but they probably do not belong to this group as the content of *N*-terminal proline in these fractions is very small. As the *N*-terminal groups other than proline and alanine found in the C fractions are so much greater than in the corresponding fraction of calf thymus, it is probable that some proteolytic

degradation has occurred in these fractions. The general conclusion to be drawn from inspection of the starch-electrophoresis patterns is that groups B and C as isolated are mainly the component indicated although they contain minor amounts of other components. We are unable to determine the amounts of these impurities, but it is unlikely that they will have much effect on the total amino acid analyses of these fractions, e.g. 10% of the slightly lysine-rich fraction (containing 9–10% of arginine) will only bring the arginine content of the arginine-rich fraction down from 14 to 13·5% and this is within the experimental error.

The relative mobilities of the leading bands of these fractions are given in Table 1. For the slightly lysine-rich histone, the relative mobility of the slowest band is also given.

It is evident that, with one or two exceptions, the mobilities of the bands are approximately the same for all the tissues examined. It cannot be claimed on this basis that component histones from different sources are identical, but, apart from minor bands due to impurities or incomplete separation, all the tissues examined give similar sets of bands with approximately the same mobilities.

The amino acid analysis of the different fractions and the *N*-terminal groups found, when determined, are given in Tables 2–4.

DISCUSSION

The fractions obtained by the methods applied are all composite and similar to the fractions obtained by somewhat similar methods from calf thymus by Johns *et al.* (1961). Fraction A consists of two components both very rich in lysine, B of three or four moderately lysine-rich components, and C of two arginine-rich components. No significant differences in the gel-electrophoresis band structure of the fractions isolated were noted in the

Table 1. *Relative mobilities of fractions from different tissues*

Figures in parentheses refer to minor bands observed which do not properly belong to the group under consideration.

Fraction	Lysine-rich (main band)	Moderately lysine-rich (two main bands)	Arginine-rich (main band)
Tissue			
Calf thymus	0·39	0·60, 1·0 (0·11)	0·19 (0·51)
Ox liver	0·40	0·67, 0·97 (0·14)	0·19 (0·71, 0·84)
Ox spleen	0·39	0·69, 1·0 (0·14)	0·13 (0·63)
Rat liver	0·37	0·77, 0·98 (0·08)	0·19 (0·65)
Leukaemic rat liver	0·40	0·70, 0·95 (0·11)	0·16 (0·47, 0·57)
Rat spleen	0·39	0·75, 1·0 (0·11)	0·19 (0·63)
Leukaemic rat spleen	0 43	0·67, 1·0 (0·11)	0·22 (0·49, 1·0)
Ehrlich ascites	0·40	Degraded	0·31 (0·80)
Whole histone* (calf thymus)	0·42	0·71, 1·0	0·18

* Average of ten results.

Table 2. *Composition of very lysine-rich histones (A)*

The amino acids are expressed as moles/100 moles of all amino acids found and no correction has been made for the hydrolytic losses of amino acids. Amide was not determined.

Origin of histone fraction ...		Calf thymus	Calf liver	Calf spleen	Rat liver	Leukaemic rat liver	Rat spleen	Leukaemic rat spleen	Ehrlich ascites
Amino acid									
Aspartic acid	Acidic	2·9	2·6	2·6	2·5	2·5	2·7	2·4	2·4
Glutamic acid	Acidic	3·9	3·9	3·8	3·7	3·6	3·6	3·2	3·8
Glycine		6·2	6·3	6·8	6·8	6·5	6·7	6·4	7·0
Alanine		24·7	24·9	25·0	24·8	25·0	24·6	24·8	24·9
Valine		5·6	5·6	5·4	5·2	5·6	5·5	5·6	5·4
Leucines		5·8	5·4	5·4	5·1	5·0	5·4	5·4	5·1
Phenylalanine		0·8	0·8	0·8	0·8	0·9	0·9	0·8	0·8
Tyrosine		0·2	0·3	0·3	0·3	0·4	0·3	0·5	0·2
Serine		5·7	5·7	5·8	5·9	5·8	6·0	5·7	5·8
Threonine		5·6	5·9	5·9	5·8	6·2	5·9	6·0	5·7
Proline		9·0	8·4	8·4	9·0	8·8	8·7	9·0	8·7
Histidine	Basic	0·4	0·4	0·3	0·4	0·4	0·3	0·4	0·4
Lysine	Basic	26·6	26·9	27·0	26·9	26·6	26·7	27·0	27·0
Arginine	Basic	2·6	2·9	2·6	2·8	2·7	2·7	2·8	2·8
Lysine:arginine		10·23	9·27	10·38	9·60	9·85	9·88	9·64	9·64
Basic:acidic		4·35	4·64	4·67	4·85	4·86	4·71	5·29	4·87
N-terminal groups					Not determined				

different preparations although the method is not at present a quantitative one.

On the basis of this investigation it is clear that there are no major differences in the composition of the corresponding fractions from the different sources examined. Such minor differences as appear may be due either to analytical errors or to contamination by small quantities of the other

Table 3. *Composition of slightly lysine-rich histones (B)*

The amino acids are expressed as moles/100 moles of all amino acids found and no correction has been made for the hydrolytic losses of amino acids. Amide was not determined. The proportion of *N*-terminal groups are molar percentages of all such groups found.

Origin of histone fraction ...	Calf thymus	Calf liver	Calf spleen	Rat liver	Leukaemic rat liver	Rat spleen	Leukaemic rat spleen	Ehrlich ascites
Amino acid								
Aspartic acid } Acidic	5·9	5·7	5·6	6·4	5·2	5·2	5·3	5·8
Glutamic acid } Acidic	8·0	7·2	7·7	7·1	6·8	6·9	6·8	8·0
Glycine	10·4	11·0	10·0	11·1	10·5	10·9	11·0	10·2
Alanine	12·0	11·3	12·4	11·5	11·6	11·6	11·8	11·7
Valine	7·6	7·9	8·0	7·7	7·6	7·7	8·0	7·6
Leucines	13·4	13·1	12·6	14·0	13·0	13·1	13·9	14·0
Phenylalanine	1·8	1·8	2·0	1·5	1·8	1·9	2·0	1·9
Tyrosine	2·1	2·0	2·0	1·8	2·0	2·0	1·9	1·9
Serine	5·4	5·2	5·7	5·4	5·8	5·7	5·1	5·4
Threonine	5·4	5·5	5·5	5·4	5·8	5·8	5·5	5·4
Proline	3·6	3·3	3·9	3·5	3·9	3·9	3·6	4·0
Histidine } Basic	2·2	1·8	1·7	2·0	2·0	1·8	2·1	1·6
Lysine } Basic	12·7	13·8	13·4	12·9	13·4	13·7	12·8	12·6
Arginine } Basic	9·5	10·4	9·5	9·7	10·5	9·8	10·2	9·9
Lysine:arginine	1·33	1·33	1·41	1·32	1·28	1·40	1·25	1·27
Basic:acidic	1·75	2·01	1·85	1·82	2·16	2·09	2·07	1·75
N-Terminal groups								
Alanine	9	8	—	11	40*	7	39*	—
Proline	74	76	—	73	51*	77	53*	—
Others	17	16	—	16	9*	16	8*	—
Wt. (g./mole of *N*-terminal groups)	29 400	32 200	—	28 200	31 600	26 000	36 000	—

* Whole unfractionated histone.

Table 4. *Composition of arginine-rich histones (C)*

The amino acids are expressed as moles/100 moles of all amino acids found and no correction has been made for the hydrolytic losses of amino acids. Amide was not determined. The proportion of *N*-terminal groups are molar percentages of all such groups found.

Origin of histone fraction ...	Calf thymus	Calf liver	Calf spleen	Rat liver	Leukaemic rat liver	Rat spleen	Leukaemic rat spleen	Ehrlich ascites
Amino acid								
Aspartic acid } Acidic	6·3	5·3	4·9	5·2	5·6	5·8	5·4	5·6
Glutamic acid } Acidic	8·4	8·8	8·4	8·4	8·1	7·9	7·8	8·7
Glycine	6·6	7·2	7·0	6·9	6·7	6·9	6·9	8·6
Alanine	13·4	12·2	12·7	12·3	12·6	13·2	12·5	11·3
Valine	6·1	5·9	5·7	5·9	6·1	6·8	6·6	6·2
Leucines	13·4	13·5	13·2	13·6	13·2	13·5	13·3	13·2
Phenylalanine	2·9	2·9	3·0	3·0	2·8	2·6	2·6	2·4
Tyrosine	1·6	2·0	1·8	1·8	1·8	1·8	1·5	2·1
Serine	4·9	4·8	4·7	4·9	5·1	4·9	5·1	5·2
Threonine	6·1	6·9	6·9	7·2	6·5	6·3	6·8	5·9
Proline	4·9	4·5	4·6	4·7	5·2	4·8	5·0	4·1
Histidine } Basic	2·2	2·6	2·6	2·6	2·4	2·4	2·5	2·6
Lysine } Basic	10·2	10·0	10·6	10·0	10·4	9·5	10·0	12·8
Arginine } Basic	13·1	13·4	13·9	13·5	13·5	13·8	14·0	11·3
Lysine:arginine	0·78	0·75	0·76	0·74	0·77	0·69	0·71	1·13
Basic:acidic	1·73	1·84	2·04	1·92	1·92	1·87	2·00	1·88
N-terminal groups								
Alanine	95	84	83	81	—	86	—	56
Proline	2	1	2	2	—	1	—	32
Others	3	15	15	17	—	13	—	12
Wt. (g./mole of *N*-terminal groups)	18 800	13 500	12 000	14 000	—	17 000	—	22 700

proteins. On the other hand the characteristic differences between the fractions are reproduced in all the preparations, e.g. all fractions A contain about 25 % of alanine and 9 % of proline, whereas fractions B contain 11–12 % of alanine and 10–11 % of glycine and all fractions C contain about 13 % of alanine and 6–7 % of glycine.

Differences of amino acid composition of the arginine-rich histones from different species and tissues have been claimed by Mauritzen & Stedman (1959, 1960), the only authors who appear to have described the arginine-rich histones from tissues other than calf thymus. In the arginine-rich β-histone from ox tissues Mauritzen & Stedman (1960) found differences of content of certain amino acids up to 20 % on the basis of which they claimed cell specificity. However, the analyses given by these authors for the β-histone (arginine-rich) from calf thymus, ox liver and ox spleen differ appreciably from those given above for the C fractions, e.g. all Mauritzen & Stedman's values for alanine, arginine and lysine are lower than the whole range of values obtained by us, whereas with glutamic acid, glycine and leucines the reverse is the case (cf. Tables 4 and 5). Some differences in the values for serine, threonine and tyrosine might be due to the fact that no corrections for decomposition of these amino acids during hydrolysis have been made. The figures given here for the arginine-rich histone from calf thymus agree with other analyses of this fraction (Phillips & Johns, 1959; Johns *et al.* 1960). It would appear then that the fractions obtained by Mauritzen & Stedman using the aggregation method, which differ from our C fractions, may be contaminated by a protein that is low in basic amino acids. Mauritzen & Stedman gave no tests of purity on the β-fractions other than the aggregation effect.

In our own preparations tests of purity have been applied and it was considered that such differences as appeared could be accounted for by the various experimental errors or impurities.

Although no significant differences are found in the analyses of the groups of histones from different tissues given here, it is possible that when the individual histones responsible for the distinct bands of the starch-gel electrophoresis pattern are obtained in a pure state, differences in their composition when derived from different tissues will be found. Such differences could, however, not be large or they would show up in the composite group analyses given here.

Although the fractions obtained from different tissues are very similar to each other, the proportions present in the tissue may differ. We do not have a reliable method of determining these proportions as the yields of the various separations may be variable. At the outset of these investigations some starch-gel-electrophoretic patterns were obtained of the whole histone extracted by dilute acid from various tissues. These were apparently similar on visual inspection, but no quantitative method for comparing the different bands is yet available.

Table 5. *Composition of different histones described by other authors*

The amino acids are expressed as moles/100 moles of all amino acids.

Origin of histone fraction		Calf thymus *f3	Calf thymus †	Ox liver †	Ox spleen †	Calf thymus *f1	Calf thymus ‡A	Calf thymus *f2	Calf thymus ‡B
Amino acid									
Aspartic acid	Acidic	16·9	5·2	5·5	5·8	8·0	2·0	14·3	5·1
Glutamic acid			9·6	9·5	9·4		3·2		8·7
Glycine		6·6	9·3	9·5	8·7	7·2	6·7	10·3	9·1
Alanine		13·0	11·8	11·0	10·5	23·6	26·0	11·1	11·1
Valine		5·2	5·7	6·1	6·4	4·8	5·0	7·5	7·0
Leucines		13·8	14·7	14·9	15·6	5·4	5·0	12·7	13·6
Phenylalanine		2·9	2·6	2·6	2·7	0·7	0·5	1·5	1·8
Tyrosine		0·5	2·7	3·0	3·3	0·5	0·5	2·7	2·9
Serine		4·4	4·1	4·2	4·2	6·1	6·4	5·2	5·4
Threonine		6·2	6·2	6·0	5·9	5·6	5·7	5·4	5·7
Proline		4·9	4·5	4·2	4·5	8·9	9·3	3·6	4·0
Methionine		—	1·0	1·0	1·2	—	—	—	0·3
Histidine		2·2	2·0	2·0	1·8	0·4	—	2·7	2·3
Lysine	Basic	10·3	8·8	8·8	8·1	25·9	28·3	13·1	12·1
Arginine		13·2	11·9	12·0	11·7	2·9	1·7	9·8	10·1
Lysine: arginine		0·78	0·74	0·74	0·69	8·93	16·60	1·34	1·20
Basic: acidic		1·52	1·53	1·53	1·42	3·65	5·77	1·60	1·60

* Fractions described by Johns, Phillips, Simson & Butler (1960) for calf thymus histone.

† β-Histones (arginine-rich) described by Mauritzen & Stedman (1960).

‡ Fractions prepared by Crampton, Stein & Moore (1957); the amino acid values represent the average of figures for different preparations in Tables 2 and 3 (Crampton *et al.* 1957).

SUMMARY

1. Methods of preparation of three distinct fractions of histones have been described and applied to the thymus, spleen and liver of calves and to the liver and spleen of normal and leukaemic rats.

2. No differences in amino acid composition or in N-terminal groups of the same fractions from the different sources were found, outside the probable experimental errors. On the other hand the characteristic analytical differences between the fractions were apparent in all the preparations.

3. The purity of the fractions was examined by starch-gel electrophoresis. Apart from minor bands observed in some cases and probably due to unremoved impurities, no differences of the patterns or of the mobilities were observed which could be ascribed to tissue or species specificity.

4. Some qualification of the above statements are necessary for fractions derived from the Ehrlich ascites tumour. A complete resolution into the fractions was not achieved in this case.

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REFERENCES

Butler, J. A. V., Davison, P. F., James, D. W. F. & Shooter, K. V. (1954). *Biochim. biophys. Acta*, **13**, 224.
Chaveau, J., Moulé, Y. & Rouiller, C. (1956). *Exp. Cell Res.* **11**, 317.
Crampton, C. F., Moore, S. & Stein, W. H. (1955). *J. biol. Chem.* **215**, 787.
Crampton, C. F., Stein, W. H. & Moore, S. (1957). *J. biol. Chem.* **225**, 363.
Cruft, H. J., Mauritzen, C. M. & Stedman, E. (1954). *Nature, Lond.*, **174**, 580.
Cruft, H. J., Mauritzen, C. M. & Stedman, E. (1958). *Proc. Roy. Soc.* B, **149**, 21.
Daly, M. M. & Mirsky, A. E. (1955). *J. gen. Physiol.* **38**, 405.
Davis, J. R. & Busch, H. (1959). *Cancer Res.* **19**, 1957.
Davison, P. F. (1957). *Biochem. J.* **66**, 708.
Davison, P. F. & Butler, J. A. V. (1954). *Biochim. biophys. Acta*, **15**, 439.
Davison, P. F. & Shooter, K. V. (1956). *Bull. Soc. chim. Belg.* **65**, 85.
Hlavayová, E. (1957). *Neoplasma*, **4**, 10.
Johns, E. W., Phillips, D. M. P., Simson, P. & Butler, J. A. V. (1960). *Biochem. J.* **77**, 631.
Johns, E. W., Phillips, D. M. P., Simson, P. & Butler, J. A. V. (1961). *Biochem. J.* **80**, 189.
Luck, J. M., Rasmussen, P. S., Satake, K. & Tsvetikov, A. N. (1958). *J. biol. Chem.* **233**, 1407.
Mauritzen, C. M. & Stedman, E. (1959). *Proc. Roy. Soc.* B, **150**, 299.
Mauritzen, C. M. & Stedman, E. (1960). *Proc. Roy. Soc.* B, **153**, 80.
Neelin, J. M. & Butler, G. C. (1959). *Canad. J. Biochem. Physiol.* **37**, 843.
Neelin, J. M. & Butler, G. C. (1961). *Canad. J. Biochem. Physiol.* **39**, 485.
Neelin, J. M. & Connell, G. E. (1959). *Biochim. biophys. Acta*, **31**, 539.
Neelin, J. M. & Neelin, E. M. (1960). *Canad. J. Biochem. Physiol.* **38**, 355.
Peterson, E. A. & Sober, H. A. (1956). *J. Amer. chem. Soc.* **78**, 751.
Phillips, D. M. P. (1958). *Biochem. J.* **68**, 35.
Phillips, D. M. P. & Johns, E. W. (1959). *Biochem. J.* **72**, 538.
Sanger, F. (1945). *Biochem. J.* **39**, 507.
Schneider, J. H. (1958). *J. biol. Chem.* **233**, 154.
Smithies, O. (1955). *Biochem. J.* **61**, 629.
Starbuck, W. C. & Busch, H. (1960). *Cancer Res.* **20**, 891.
Stedman, E. & Stedman, E. (1951). *Phil. Trans.* B, **235**, 565.
Ui, N. (1957). *Biochim. biophys. Acta*, **25**, 493.
Vendrely, R., Knobloch, A. & Matsudaira, H. (1958). *Nature, Lond.*, **181**, 343.

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The Metabolism of 2-Fluoroethanol

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2-Fluoroethanol was first synthesized by Swarts (1914), but there was no report of its toxicity until its use as a rodenticide was patented in 1935 in Germany (Schrader, 1935). 2-Fluoroethanol has about the same toxicity as fluoroacetate to the rat and its structural relationship to this compound makes it likely that it is toxic for the same reason, namely as a substrate for a lethal synthesis of fluorocitrate (McCombie & Saunders, 1946; Saunders, Stacy & Wilding, 1949; Peters, 1952; Bartlett,