Studies on the Tissue Specificity of the High-Mobility-Group Non-Histone Chromosomal Proteins from Calf

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(Received 29 November 1977)

The high-mobility-group (HMG) non-histone chromosomal proteins from calf thymus, liver, spleen and kidney were extracted, and fractionated by CM-Sephadex chromatography and trichloroacetic acid precipitation. The isolated proteins HMG 1, HMG 2 and HMG 17 from the tissues were compared by polyacrylamide-gel electrophoresis, isoelectric focusing and amino acid analysis. The results show that the three proteins are very similar in the tissues studied, implying a lack of tissue specificity.

It is now generally accepted that four of the five main histone fractions are involved in the structure of nucleosomes (Kornberg, 1974; Thomas & Kornberg, 1975), the core particle being composed of two each of the histones H3, H4, H2A and H2B (Bradbury, 1975). Histone H1, however, is thought to be involved in the internucleosome regions and probably has some function in cross-linking higher-order structures. As the histones are thought to be non-specific gene repressors, acting by causing these higher-order structures, much attention has been paid to the non-histone chromosomal proteins in a search for specific gene de-repressors (MacGillivray *et al.*, 1972; Stein *et al.*, 1974; Elgin & Weintraub, 1975). However, among these non-histone chromosomal proteins there are also many enzymes and other structural proteins, and the separation and identification of these proteins is being carried out in many laboratories at the present time (MacGillivray, 1976, 1977).

One such group of proteins, designated the high-mobility-group (HMG) proteins, has been isolated (Goodwin & Johns, 1973; Goodwin *et al.*, 1975). These proteins can be extracted from chromatin either with 0.35M-NaCl (Goodwin *et al.*, 1975) or with 5% (w/v) HClO₄, together with histone H1 (Sanders & Johns, 1974). They are relatively low-molecular-weight proteins (30000 or less) with unusual amino acid compositions. They contain approx. 25% basic amino acids and 30% acidic amino acids (Johns *et al.*, 1975). It is thought that these proteins are structural proteins, since they are still present at about 10⁶ molecules per nucleus, which is about 1–5% of the histone content (Walker *et al.*, 1976). The HMG proteins from calf thymus have been fractionated, and four main proteins, HMG 1, HMG 2, HMG 14 and HMG 17, have been isolated in a pure form and characterized (Goodwin

et al., 1975; Walker *et al.*, 1977; Goodwin *et al.*, 1977b).

It has also been shown that proteins HMG 1 and HMG 2 have a form of microheterogeneity which may be similar to that first demonstrated for histone H1 by Kinkade & Cole (1966). Protein HMG 2 can be subfractionated by isoelectric focusing (Walker *et al.*, 1976) or by column chromatography (Goodwin *et al.*, 1976), but no significant differences have been found between the amino acid analyses of the subfractions.

To date, work on the HMG proteins has been concerned primarily with proteins isolated from calf thymus, but little is known about their occurrence in other tissues. Although proteins with similar electrophoretic mobilities have been detected in HClO₄ extracts of other calf tissues (Sanders, 1975), it has not yet been conclusively demonstrated that these HMG proteins exist in tissues other than thymus. We have therefore carried out a detailed investigation of the tissue specificity of HMG proteins in several calf tissues, and the present paper reports the isolation and fractionation of HMG proteins from calf thymus, spleen, liver and kidney and compares their electrophoretic mobilities, amino acid analyses and isoelectric-focusing patterns.

Materials and Methods

Isolation of total HMG proteins

Calf thymus total HMG proteins were extracted from chromatin as described previously (Goodwin *et al.*, 1975). Spleen, liver and kidney proteins were isolated by HClO₄ extraction of purified nuclei as follows: 1 kg of fresh tissue was minced and divided into four portions. Each portion was blended in 800 ml of 0.25M-sucrose in buffer A (2.5mM-KCl,

1.5mM-MgCl₂, 3mM-CaCl₂ and 1mM-Tris/HCl, pH7.4) for 1 min at top speed and 4 min at half speed in a Kenwood blender. The homogenates were passed through a nylon sieve and centrifuged for 30 min at 2000 g. The pellet was dispersed in 2.4M-sucrose in buffer A to give a final sucrose concentration of 2M. The solution was homogenized for 2 min at half speed in the blender and then centrifuged at 30000 g for 1 h. The nuclei were washed with at least 20 vol. of buffer A by using a Dounce homogenizer followed by centrifugation for 10 min at 2000 g. HMG proteins, together with histone H1, were extracted from the nuclei by extraction with 3 × 3 vol. of 5% HClO₄ as described previously (Sanders & Johns, 1974). The combined extracts were filtered through a no. 4 sintered-glass funnel, then 0.03 vol. of conc. HCl was added, followed by 3.5 vol. of acetone to precipitate most of the histone H1. The histone H1 was then removed by centrifugation for 15 min at 2000 g and the supernatant clarified by filtering through a no. 4 sintered-glass funnel. To the filtrate 2.5 vol. of acetone was added and the HMG proteins that precipitated were collected, washed once with acetone/0.1M-HCl (6:1, v/v), then three times with acetone, before drying under vacuum.

The yields of total HMG proteins from thymus, spleen, liver and kidney, determined by weighing, were 1 g, 100 mg, 40 mg and 40 mg respectively per kg of starting tissue.

Fractionation of total HMG proteins by column chromatography

HMG proteins were fractionated on a CM-Sephadex C-25 column as described by Goodwin *et al.* (1975), with some modifications as follows. The total HMG proteins obtained from the above procedure were dissolved in 3–4 ml of 7.5mM-sodium borate buffer (pH8.8) and titrated to pH8.8 with 1M-NaOH. The solutions were dialysed overnight against 2 litres of 0.15M-NaCl in borate buffer. The samples were centrifuged at 2000 g for 15 min to remove aggregates (very small amount of HMG proteins, mostly HMG 1 and HMG 2) and the supernatants were applied to a CM-Sephadex C-25 column (2.5 cm × 27 cm) previously equilibrated with 7.5mM-borate buffer (pH8.8). Then 150 ml of 0.15M-NaCl in the same buffer was pumped through the column at a flow rate of 0.75 ml/min, followed by a 600 ml salt gradient (0.15–1.25M-NaCl) at the same flow rate. Fractions (5 ml) were collected and the *A*₂₂₀ was measured. The NaCl concentrations of the fractions were determined from the conductivity of the solutions at 25°C. Fractions were pooled as indicated by the elution profiles and the proteins isolated by acidifying to 0.1M-HCl and adding 6 vol. of acetone.

Further purification of proteins HMG 2 and HMG 17 from liver and kidney

Fraction C from the two tissues (see the elution profile in Fig. 2) were dissolved in water at concentrations of 1 mg/ml and clarified by centrifugation at 2000 g for 10 min. The supernatants were cooled to 4°C, then made 0.2M in β-mercaptoethanol and 10% in trichloroacetic acid by adding 100% trichloroacetic acid. Precipitates of protein HMG 2 were collected by centrifugation for 10 min at 2000 g, washed once with 10% trichloroacetic acid, twice with acetone/0.1M-HCl (6:1, v/v) and three times with acetone, and dried.

Protein HMG 17 from liver and kidney fraction D (Fig. 2) was redissolved in water and the impurities were precipitated with 18% trichloroacetic acid (final concn.) by adding 100% trichloroacetic acid and centrifuging at 2000 g for 5 min. The supernatants, which contained partially pure protein HMG 17, were acidified to 0.2M-HCl and 6 vol. of acetone was added. The precipitates were collected and washed as described above.

Polyacrylamide-gel electrophoresis

Polyacrylamide-gel electrophoresis of HMG proteins was carried out as described previously (Johns, 1969) by using 20% polyacrylamide gels (0.6 cm × 8 cm) at pH2.4. The gels were run for 3 h at 180 V, stained with 0.2% Procion Navy in 7% (v/v) acetic acid and destained in 40% ethanol at 50°C.

Isoelectric focusing

Thin-layer isoelectric focusing between pH3.5 and 9.5 was carried out as described previously (Walker *et al.*, 1976) by using the LKB Multiphor isoelectric-focusing apparatus. Proteins were dissolved in water at a concentration of 5 mg/ml and 10 μl (50 μg) of each sample was applied to the gel. Total electrophoresis time was 2 h. Albumin with a recognized isoelectric point (pH~5.0) was run as a control. The pH gradient of the gel was measured on 5 mm sections removed along the length of the gel. They were immersed in 0.5 ml of 10mM-NaCl solution and equilibrated overnight at room temperature (21–24°C) before measurement of the pH. The gel was stained for 2 h in 0.05% (w/v) Coomassie Brilliant Blue dissolved in ethanol/acetic acid/water (5:2:13, by vol.) containing 0.1% (w/v) CuSO₄, then overnight in 0.01% (w/v) Coomassie Blue in the same solution. The gel was destained with ethanol/acetic acid/water (5:2:13, by vol.).

Total and N-terminal amino acid analyses

Total amino acids were determined by using a Rank Hilger amino acid analyser. Samples were

hydrolysed for 24h in 6M-HCl at 110°C. No corrections were made for hydrolytic losses.

N-Terminal amino acids were determined by the dansyl method as described by Hartley (1970).

Results

The polyacrylamide-gel-electrophoretic pattern of total HMG proteins from calf thymus is quite complex, about 17 bands being visible on overloaded gels (Johns *et al.*, 1975). However, we have subsequently found that some of these are degradation products, and in a total HMG-protein preparation in which proteolysis has been completely inhibited there are only four main HMG proteins, HMG 1, HMG 2, HMG 14 and HMG 17 (Goodwin *et al.*, 1978). The polyacrylamide-gel-electrophoresis patterns of the total HMG proteins from the four calf tissues (thymus, spleen, liver and kidney) are shown in Fig. 1. The HMG proteins from thymus were extracted from chromatin prepared by washing this tissue with saline/EDTA as described previously (Goodwin *et al.*, 1975). In the case of the other three tissues, it was necessary to isolate nuclei first to minimize contamination. Although with thymus we know there is no quantitative or qualitative difference in HMG proteins prepared from chromatin or nuclei, it is not known whether this is true for liver, kidney and spleen also. The four main HMG proteins, HMG 1, HMG 2, HMG 14 and HMG 17, are present in all four tissues, though in calf thymus protein HMG 14 is only barely visible on gels and this is due to the rapid degradation of this protein in this particular tissue (Goodwin *et al.*, 1978). The amounts of the total HMG proteins in the HClO₄ extracts of the four tissues, thymus, spleen, liver and kidney, are respectively 20%, 27%, 16% and 10% of the histone H1 in the extracts. Per weight of tissue the amounts of the total HMG proteins obtainable from liver, spleen and kidney are much smaller than that obtained from thymus, and in order to fractionate 30–50mg of protein it was necessary to modify the CM-Sephadex chromatographic procedure previously described for the fractionation of the large quantities of calf thymus HMG proteins (Goodwin *et al.*, 1975). A smaller column was used, and β -mercaptoethanol was omitted so that the A₂₂₀ of the fractions could be read. The elution profiles of the total HMG proteins from the four tissues are shown in Fig. 2. The elution profile for calf thymus proteins (Fig. 2a) is as described previously (Goodwin *et al.*, 1975). Peak B is protein HMG 1, peak C is protein HMG 2, peak D contains protein HMG 17. Protein HMG 14 is eluted between proteins HMG 2 and HMG 17 in peak X and is the subject of a separate paper (Goodwin *et al.*, 1977b). The proteins eluted after fraction D are uncharacterized proteins and will not be considered here. The

Fig. 1. *Comparative polyacrylamide-gel electrophoresis of calf thymus, spleen, liver, and kidney HMG proteins* HMG proteins were isolated from spleen, liver and kidney nuclei with HClO₄ as described in the Materials and Methods section. Calf thymus HMG proteins were isolated from chromatin as described previously (Goodwin *et al.*, 1975). On the left of each gel is calf thymus total HMG proteins; on the right are spleen (a), liver (b), and kidney (c) HMG proteins.

electrophoretic analysis of the corresponding fractions from the spleen, liver and kidney HMG-protein elution profiles is shown in Fig. 3. Some aggregation of liver and spleen proteins HMG 1 and HMG 2 takes place, and this aggregate is eluted between pure proteins HMG 1 (peak B) and HMG 2 (peak C), in peak C'. The electrophoretic analyses show that the purified HMG 1 and HMG 2 proteins from peaks B and C contain some high-molecular-weight material running near the tops of the gels. This is in fact caused by disulphide-bridge formation between the two cysteine residues in each protein. These high-molecular-weight bands can be removed by reduction with 40% (w/v) mercaptoacetic acid (Walker *et al.*, 1976). This is shown in Fig. 4, which is a polyacrylamide-gel-electrophoretic analysis of

Fig. 2. *CM-Sephadex ion-exchange chromatography of HMG proteins from (a) thymus, (b) spleen, (c) liver and (d) kidney*

Total HMG proteins from the four tissues were fractionated by *CM-Sephadex* column chromatography at pH 8.8 as described in the Materials and Methods section. Peak B in each case is protein HMG 1; peak C' is protein HMG 1 plus protein HMG 2; peak C is protein HMG 2; peak X contains protein HMG 14 and peak D contains protein HMG 17. —, A_{220} ; ----, NaCl gradient.

the purified and reduced proteins HMG 1 and HMG 2 from the four tissues. The results show that the HMG 1 proteins from the four tissues and spleen HMG 2 protein are pure, giving single bands on the

Fig. 3. *Polyacrylamide-gel-electrophoretic analyses of the CM-Sephadex fractions (Fig. 2)*

(a) A, calf thymus total HMG proteins; B, C', C, X and D, spleen HMG protein fractions of Fig. 2. (b) B, C', C, X and D are the liver HMG protein fractions of Fig. 2. (c) A, calf thymus total HMG proteins; B, C, D and X, kidney HMG protein fractions of Fig. 2.

Fig. 4. Comparative polyacrylamide-gel-electrophoretic analyses of the HMG 1 and HMG 2 proteins after reduction with 40% (v/v) mercaptoacetic acid

Proteins HMG 1 and HMG 2 isolated by CM-Sephadex chromatography (Fig. 2) were reduced with 40% mercaptoacetic acid (see the Materials and Methods section) before electrophoresis on polyacrylamide gels. On the right of each gel are thymus total HMG proteins; on the left are: (a) thymus HMG 1 protein; (b) spleen HMG 1 protein; (c) liver HMG 1 protein; (d) kidney HMG 1 protein; (e) thymus HMG 2 protein; (f) spleen HMG 2 protein; (g) liver HMG 2 protein; (h) kidney HMG 2 protein.

gels. Liver and kidney HMG 2 proteins show some contamination. The impurities could be removed from the kidney HMG 2 protein by precipitating the protein with 10% (w/v) trichloroacetic acid (Fig. 5). This was not effective for further purification of the liver HMG 2 protein. Recycling the liver HMG 2 protein through CM-Sephadex chromatography also failed to remove the contaminations.

Fraction D of the elution profiles contains protein HMG 17 (Fig. 3). Most of the other contaminants in this fraction can be removed by precipitation with 18% (w/v) trichloroacetic acid, and the electrophoretic analyses of the HMG 17 proteins partially purified in this way from liver and kidney are shown in Fig. 6.

In Fig. 3(a) the spleen protein HMG 17 (gel D) does not appear to coincide with the thymus marker in this particular electrophoretic analysis. This is probably due to unequal expansion of the gels during staining and destaining, since when spleen fraction D was run on the same gel with total thymus HMG proteins the two HMG 17 bands had the same mobilities (results not shown).

The total and *N*-terminal amino acid analyses of proteins HMG 1 and HMG 2 from the various tissues

are given in Table 1. As noted above, liver HMG 2 protein is not completely pure and has two *N*-terminals, glycine and lysine. It is apparent from the total amino acid analyses that the proteins HMG 1 and HMG 2 are similar in the different tissues.

Protein HMG 2 from calf thymus has a number of subfractions which have different isoelectric points in the pH 7–9 range (Walker *et al.*, 1976). It was decided to investigate whether this microheterogeneity was tissue-specific, as has been found for histone H1. The isoelectric-focusing patterns of the various HMG 2 proteins are shown in Figs. 7(a) and 7(b). The calf thymus HMG 2 protein has four main bands focusing between pH 7 and 8.5 (Fig. 7a, gel A). These four proteins have been isolated and characterized previously (Goodwin *et al.*, 1976). In addition, two minor bands (pH 6–7) are occasionally seen. This is probably not contamination with protein HMG 1, since protein HMG 1 does not normally give discrete bands in this region, and these two, more acidic, bands are possibly two additional HMG 2 subfractions which have not yet been identified. Spleen and liver HMG 2 proteins are eluted from the CM-Sephadex column in two peaks, C and C' (Fig. 2). The isoelectric focusing of proteins from these two

Fig. 5. Polyacrylamide-gel electrophoresis of calf thymus total HMG proteins and calf kidney protein HMG 2. Protein HMG 2 from kidney was prepared by CM-Sephadex chromatography and fractional trichloroacetic acid precipitation, and was reduced with 40% mercaptoacetic acid (see the Materials and Methods section) before electrophoresis.

Fig. 6. Polyacrylamide-gel-electrophoretic analysis of partially purified HMG 17 proteins from (a) liver and (b) kidney

Liver and kidney proteins HMG 17 were isolated from fraction D of the CM-Sephadex chromatography fractionation (Fig. 2) by fractional precipitation with trichloroacetic acid.

peaks from spleen is shown in Fig. 7(a), gels B and C, and shows that the protein HMG 2 subfractions are the same as the thymus protein HMG 2 subfractions. Similarly, liver protein HMG 2 in peaks C and C' has the same protein HMG 2 subfractions (Fig. 7a, gels D and E). Kidney protein HMG 2 (Fig. 7b) has four subfractions the same as the four main calf thymus protein HMG 2 subfractions. Thus the four main subfractions focusing between pH 7 and 8.5 are common to all four tissues. In liver and spleen there has been a partial fractionation of the protein HMG 2 subfractions in peaks C and C' by the ion-exchange chromatography. In fact, the subfractions can be almost completely separated by the higher-resolution CM-cellulose ion-exchange chromatography described previously (Goodwin *et al.*, 1976).

Isoelectric focusing of the HMG 1 proteins from the four tissues (results not shown) gave similar

complex patterns of bands and smears in the pH 6–8 range in all.

Protein HMG 17 has only been isolated in a pure form from calf thymus. Only partially purified proteins were obtained from liver and kidney by further trichloroacetic acid fractionation of peak D of the CM-Sephadex chromatography (Fig. 6). However, the analyses of these two partially purified proteins (Table 2) are presented to demonstrate their similarity to the calf thymus protein HMG 17 (Walker *et al.*, 1977). Thus the proteins have the characteristically high contents of proline, glycine and alanine as well as the typically high contents of acidic and basic residues. In addition, the quantities of the aromatic amino acids are low. We have not yet been able to analyse protein HMG 17 by isoelectric focusing, primarily because of the problem of fixing it in the 4% polyacrylamide gel.

Table 1. Total amino acid (mol%) and N-terminal amino acid analyses of HMG 1 and HMG 2 proteins from thymus, spleen, liver and kidney

Proteins HMG 1 and HMG 2 were prepared from the four tissues by CM-Sephadex ion-exchange chromatography (Fig. 2) as described in the Materials and Methods section, and in the case of HMG 2 proteins from liver and kidney, these were further purified by fractional precipitation with 10% (w/v) trichloroacetic acid.

Amino acid	HMG 1				HMG 2			
	Thymus	Spleen	Liver	Kidney	Thymus	Spleen	Liver	Kidney
Asp	11.0	10.5	11.6	10.9	9.6	9.7	10.6	10.1
Thr	3.0	2.7	2.6	2.9	2.7	2.5	2.3	3.3
Ser	5.3	5.1	5.3	5.3	7.5	7.2	6.1	7.6
Glu	17.8	17.1	16.5	16.6	17.1	16.9	16.1	16.6
Pro	5.2	6.0	6.3	6.5	7.7	7.5	5.7	6.9
Gly	5.2	6.3	5.7	6.2	6.3	6.9	6.5	7.7
Ala	8.2	8.2	8.9	8.9	7.3	7.0	11.6	8.3
Val	2.3	2.4	2.3	2.1	2.1	2.0	3.3	2.6
Cys	3.0	1.8	1.4	1.3	1.6	1.6	—	—
Met	1.1	0.8	1.7	0.7	1.2	1.2	0.2	1.2
Ile	1.9	1.9	2.0	2.0	1.4	1.6	1.8	1.7
Leu	2.2	2.4	2.6	2.4	2.1	2.4	2.4	3.0
Tyr	2.8	3.1	3.2	3.1	2.6	2.9	3.6	2.4
Phe	5.6	5.4	4.7	4.3	4.2	4.1	4.0	4.4
Lys	16.8	18.8	18.7	19.9	19.1	18.4	18.7	16.8
His	1.7	1.5	1.7	1.5	1.7	2.0	1.8	1.8
Arg	5.5	4.6	4.3	3.9	5.1	4.8	5.5	5.2
N-Terminal amino acid	Gly	Gly	Gly	Gly	Gly	Gly	Gly Lys	Gly

Fig. 7. Isoelectric focusing of HMG 2 proteins from thymus, spleen, liver and kidney
HMG 2 protein fractions C and C' from the CM-Sephadex column (Fig. 2) were analysed by isoelectric focusing, pH 3.5–9.5, by using an LKB Multiphor apparatus. (a) Isoelectric-focusing patterns of: (A) thymus HMG 2 protein; (B) spleen HMG 2 protein (fraction C); (C) spleen HMG 2 protein (fraction C'); (D) liver HMG 2 protein (fraction C); (E) liver HMG 2 protein (fraction C'). (b) Isoelectric-focusing patterns of (A) protein HMG 2 from thymus and (B) HMG 2 protein from kidney.

Table 2. *Amino acid analyses (mol%) of calf thymus, liver and kidney HMG 17 proteins*

Protein HMG 17 from calf thymus was prepared by CM-Sephadex chromatography as described previously (Walker *et al.*, 1977). HMG 17 proteins from calf liver and kidney were prepared by CM-Sephadex chromatography (Fig. 2) followed by fractional trichloroacetic acid precipitation as described in the Materials and Methods section.

Amino acid	HMG 17		
	Thymus	Liver	Kidney
Asp	12.0	11.6	9.6
Thr	1.2	2.3	3.0
Ser	2.3	5.5	4.5
Glu	10.5	12.2	12.8
Pro	12.9	15.7	12.2
Gly	11.2	11.8	10.7
Ala	18.4	11.6	16.3
Val	2.0	3.9	3.0
Cys	—	—	—
Met	—	0.2	0.1
Ile	trace	0.3	0.2
Leu	1.0	1.6	1.5
Tyr	—	0.1	0.1
Phe	trace	0.2	0.1
Lys	24.3	17.6	20.9
His	—	0.3	0.2
Arg	4.1	5.0	4.8

Discussion

From the results in the present paper it is concluded that proteins HMG 1 and HMG 2 are present in calf liver, spleen and kidney, and have very similar properties to the now well-characterized thymus proteins. Thus the electrophoretic mobilities, amino acid analyses and isoelectric-focusing patterns are all very similar. The only difference that has been observed is that the liver and spleen HMG 2 proteins behave somewhat differently in the CM-Sephadex ion-exchange chromatography, the subfractions being partially separated in the two peaks, C and C'. Despite this it is quite clear from the isoelectric-focusing patterns that the four main subfractions of protein HMG 2 are common to the four tissues studied, and demonstrates close similarity between the HMG 2 proteins in these tissues.

Judging from the electrophoretic analyses shown in Figs. 1 and 3, protein HMG 17 appears to be present in all four tissues studied. However, only partially pure proteins were isolated from liver and kidney, a number of other components being visible on the polyacrylamide gels shown in Fig. 6. Further purification of this fraction was not possible because of the limited quantities of protein finally recovered. This exemplifies some of the problems encountered when isolating non-histone proteins from tissues such as liver, where the quantities of protein per

weight of tissue are small and contamination from other non-histone proteins is more likely than with thymus. Nevertheless, the amino acid analysis of the HMG 17 proteins obtained from liver and kidney compares quite well with that of the calf thymus protein and adequately demonstrates the existence of this protein in these two tissues.

The remaining HMG protein, HMG 14, has only recently been purified and has been isolated from thymus and kidney (Goodwin *et al.*, 1977b). Again, judging from the electrophoresis shown in Figs. 1 and 3, it appears to be present in all four tissues examined in this study.

It is thus concluded that the HMG proteins are not thymus-specific, similar, if not identical, proteins being found in three other calf tissues. This lack of tissue specificity of the HMG proteins thus far found does resemble the situation with the histones. This, together with the fact that HMG proteins have been found in nucleosomes (Goodwin *et al.*, 1977a) and that there are fairly large quantities of each protein in the cell nucleus, supports the view that they could be chromatin structural proteins. In addition, it seems that these proteins are not confined to calf tissues, since they have been identified by electrophoretic analysis in tissues of rabbits, rats, pigs, sheep and in chicken erythrocytes (Goodwin *et al.*, 1978). Also, HMG-like proteins have been isolated from trout testis (Watson *et al.*, 1977), *Drosophila melanogaster* (Alfageme *et al.*, 1976) and the fruit fly *Ceratitis capitata* (Franco *et al.*, 1977).

Although recent reports have suggested a possible association of HMG-like proteins with transcriptionally active chromatin in trout testis (Levy *et al.*, 1977) and duck erythrocytes (Vidali *et al.*, 1977), results from this laboratory indicate that this is not so in other tissues such as thymus and liver (Goodwin *et al.*, 1978) and therefore the function of the HMG proteins as yet remains unknown.

We thank Dr. J. M. Walker and Miss Bridget Parker for the amino acid analyses. G. H. G. and E. W. J. are supported by grants to the Chester Beatty Research Institute (Institute of Cancer Research: Royal Cancer Hospital). A. R. acknowledges a grant from the Ministry of Science of Iran.

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