the chromatograms, therefore, are likely to be minimal.

Evidence that ethanalamine was liberated by the action of phosphomonoesterase was obtained by paper chromatography of the dinitrophenyl derivatives prepared from the hydrolysate. Only one derivative appeared in the hydrolysate but not in the control and it behaved just like pure dinitrophenylethanolamine in the butanol–water system of Mellon et al. (1953). Dinitrophenylserine was absent from both preparations.

Electrophoresis. Electrophoresis (barbitone buffer, pH 8.6, I 0.05, 0.4 mA/cm. width, Whatman no. 4 paper) of fraction A prepared as above from goat placenta revealed the presence of a phosphoric acid ester fraction that moved towards the anode at a rate of about 0.46 relative to inorganic orthophosphate (1-0). Most of the phosphorus-containing material in fraction B moved at the same rate as did a mixture of phosphylethanolamine and phosphorylcholine under the same conditions. Determination of the total phosphorus on the paper after electrophoresis showed that 59-1 % of the total phosphorus present in fraction A from goat placenta was present in the region of relative rate 0.46. Since fraction B accounts for 71-4 % of the total phosphorus in fraction A, and phosphorylcholine and phosphylethanolamine account for 80-3 % of the total phosphorus in fraction B, the two esters account for 57-4 % of the total phosphorus in fraction A. This figure is compatible with that obtained by quantitative electrophoresis described above.

DISCUSSION

It is reasonable to conclude from these results that phosphorylcholine and phosphorylthanolamine are quantitatively important constituents of the phosphoric acid derivatives extractable from goat placenta by perchloric acid. From the nature of the extract analysed, as outlined in the introduction to this addendum, it is clear that the absence of the glyceryl derivatives of phosphorylcholine and phosphorylthanolamine does not exclude their presence in goat-placental tissue. All four of these derivatives have been shown to occur in human placenta (Porcellati, Curti & Luciani, 1959a, b).

SUMMARY

1. Phosphorylcholine and phosphorylthanolamine are shown to be quantitatively important constituents of the phosphoric acid derivatives present in goat placenta.

I wish to thank Mrs Marian Horner for expert technical assistance.

REFERENCES


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The Electrophoresis of Histones and Histone Fractions on Starch Gel

BY E. W. JOHNS, D. M. P. PHILLIPS, P. SIMSON AND J. A. V. BUTLER.
Chester Beatty Research Institute, Institute of Cancer Research: Royal Cancer Hospital, London, S.W. 3

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Histones can be divided into a number of fractions by the procedures of extraction, electrophoresis, sedimentation or chromatography (for review, see Butler, 1958). Chromatography on carboxymethylcellulose, as developed by Johns, Phillips, Simson & Butler (1960), gave three well-characterized fractions, a lysine-rich fraction (F1), a moderately lysine-rich fraction (F2) and an arginine-rich fraction (F3). Two of these, F2 and F3, are reasonably homogeneous with respect to their N-terminal groups.

These fractions have now been studied by the method of electrophoresis in starch gel introduced by Smithies (1955). Neelin & Connell (1959) re-
ported the fractionation in starch gel of chicken-
erythrocyte histone into 16 bands at pH 4.1 and
4.9. They confirmed that these bands were not
artifacts peculiar to the method by submitting
them to a second electrophoresis, when the bands
retained their homogeneity and relative mobilities.
Neelin & Neelin (1960) further reported the separa-
tion of calf-thymus histone into 18 bands at pH 4.9
and 15 bands at pH 3.9, and by submitting these
fractions to a second electrophoresis they again
confirmed the consistency of the resolution, and
substantiated the complexity of histone.
Sautiêre (1959), using gel at pH 4.1, also examined
two fractions obtained from carboxymethyl-
cellulose columns and found at least 14 bands in
each case. The possibility of some aggregation
occurring and giving extra bands at pH 4 must,
however, be considered. For this reason, the
experiments reported below have been confined to
the separation of histone fractions at approx. pH 2.

EXPERIMENTAL

Preparation of whole histone. The calf-thymus histone
used for these experiments was prepared by the method of
Davison, James, Shooter & Butler (1954), with the follow-
ing modifications. The nucleoprotein was isolated by wash-
ing with acidified NaCl (Phillips & Johns, 1959) and
extracted three times with twice its volume of 0.25 N HCl.
After clarification the histone was dialysed against 1 mN
HCl and freeze-dried. The analysis of the whole histone
is given in Table 1.

Preparation of histone fractions. The histone fractions
were prepared by column chromatography on carboxy-
methylcellulose (Johns et al. 1960) and isolated by precipi-
tation with trichloroacetic acid at a final concentration
of 0.3 M, or 0.9 M with the lysine-rich fraction F1. The precipi-
tates were dissolved in 40 ml of ethanol and 0.5 ml of
conc. HCl was then added, followed by 100 ml of acetone.
The precipitates were washed in ether and dried under
vacuum. The white precipitates were all readily soluble in
water to give clear solutions. The analyses of these frac-
tions are given in Table 1.

Amino acid analyses. Amino acids and N-terminal groups
were determined as described by Phillips (1958) and Phillips &
Johns (1959).

Starch-gel electrophoresis. The method used was essentially
the same as that described by Smithies (1955). Unbuffered
0.01 N HCl was used for filling the electrode vessels and for
making the gels. This gave the lowest possible conductivity
at pH 2 and so minimized the heating effects. Platinum
electrodes were used in conjunction with 2.5 l. capacity
electrode vessels. The 0.01 N HCl was renewed after each run.

The pH of the gel was 2.3, and at this pH the resistance of a
gel of dimensions 2 cm. × 0.5 cm. × 26 cm. was only
12 000 ohms. This low resistance limited the potential which
could be applied to 100v, because the heating became
excessive at about 1w. The sample (1-5 mg. of whole
histone and 0.5 mg. of the fractions) was applied in 0.1 ml.
of 0.01 N HCl with starch powder as a supporting medium.
No adsorption of histone to the starch grains was observed
on staining the gel strips after the runs. The voltage
gradient was 4v/cm. and was applied for 18 hr. All runs
were carried out at room temperature.

The histone in the gel was located by staining with naph-
thalene black. Permanent laboratory records were made by
the contact-printing method of Johns (1960). These contact
prints do not reproduce satisfactorily and for this purpose
are replaced by photographs of the stained gel (Pl. 1) and
by drawings showing the position of the bands (Fig. 1).

Table 1. Composition of histone and histone fractions

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. The proportions of N-terminal groups are molar percentages of all such groups
found.

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Whole histone</th>
<th>Arginine-rich (F3)</th>
<th>Lysine-rich (F1)</th>
<th>Slightly lysine-rich (F2)</th>
<th>Groups extracted from gel</th>
<th>Arginine-rich extraction method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspartic acid</td>
<td>5·1</td>
<td>16·9</td>
<td>8·0</td>
<td>14·3</td>
<td>19·2</td>
<td>[3·5]</td>
</tr>
<tr>
<td>Glutamic acid</td>
<td>8·5</td>
<td>6·6</td>
<td>6·2</td>
<td>10·3</td>
<td>6·4</td>
<td>[3·5]</td>
</tr>
<tr>
<td>Glycine</td>
<td>7·9</td>
<td>13·0</td>
<td>23·6</td>
<td>11·1</td>
<td>12·5</td>
<td>20·5</td>
</tr>
<tr>
<td>Alanine</td>
<td>15·0</td>
<td>5·2</td>
<td>4·3</td>
<td>7·5</td>
<td>5·3</td>
<td>4·8</td>
</tr>
<tr>
<td>Valine</td>
<td>6·5</td>
<td>13·8</td>
<td>5·4</td>
<td>12·7</td>
<td>10·0</td>
<td>7·2</td>
</tr>
<tr>
<td>Leucine + isoleucine</td>
<td>11·5</td>
<td>13·8</td>
<td>5·4</td>
<td>12·7</td>
<td>10·0</td>
<td>7·2</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>1·7</td>
<td>2·9</td>
<td>0·7</td>
<td>2·7</td>
<td>1·2</td>
<td>2·3</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>5·7</td>
<td>4·4</td>
<td>6·1</td>
<td>5·2</td>
<td>6·8</td>
<td>6·0</td>
</tr>
<tr>
<td>Serine</td>
<td>6·0</td>
<td>6·2</td>
<td>5·6</td>
<td>5·4</td>
<td>6·1</td>
<td>6·0</td>
</tr>
<tr>
<td>Threonine</td>
<td>6·0</td>
<td>6·2</td>
<td>5·6</td>
<td>5·4</td>
<td>6·1</td>
<td>6·0</td>
</tr>
<tr>
<td>Proline</td>
<td>5·6</td>
<td>4·9</td>
<td>8·9</td>
<td>3·6</td>
<td>5·3</td>
<td>8·1</td>
</tr>
<tr>
<td>Histidine</td>
<td>1·8</td>
<td>2·2</td>
<td>0·4</td>
<td>2·7</td>
<td>4·3</td>
<td>1·5</td>
</tr>
<tr>
<td>Lysine</td>
<td>16·4</td>
<td>10·3</td>
<td>25·9</td>
<td>13·1</td>
<td>11·0</td>
<td>23·1</td>
</tr>
<tr>
<td>Arginine</td>
<td>8·4</td>
<td>13·2</td>
<td>2·9</td>
<td>9·8</td>
<td>10·0</td>
<td>4·1</td>
</tr>
<tr>
<td>Lysine/arginine</td>
<td>2·0</td>
<td>0·78</td>
<td>8·9</td>
<td>1·34</td>
<td>1·1</td>
<td>5·7</td>
</tr>
</tbody>
</table>

N-Terminal groups

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Whole histone</th>
<th>Arginine-rich</th>
<th>Lysine-rich</th>
<th>Slightly lysine-rich</th>
<th>Groups extracted from gel</th>
<th>Arginine-rich extraction method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alanine</td>
<td>39</td>
<td>69</td>
<td>—</td>
<td>10</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Proline</td>
<td>49</td>
<td>14</td>
<td>—</td>
<td>70</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Others</td>
<td>12</td>
<td>17</td>
<td>—</td>
<td>20</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>
Starch-gel-electrophoresis patterns of whole histone and histone chromatographic fractions. (a) Arginine-rich histone F3; (b) slightly lysine-rich histone F2; (c) whole histone; (d) lysine-rich histone F1; (e) F1, F2 and F3 recombined; (f) arginine-rich histone made by the ethanol-HCl extraction procedure.

E. W. JOHNS, D. M. P. PHILLIPS, P. SIMSON AND J. A. V. BUTLER

(Facing p. 190)
RESULTS

Some preliminary runs were carried out with whole histone in which the concentrations of acid in the gel and in the applied histone solutions were varied. The concentration of the acid in the gel is critical, as can be seen from Fig. 1a. In 0.01 N-HCl resolution into many bands occurred, but in 0.02 N-HCl only two bands appeared. Increasing

the acid concentration in the applied histone solutions had no effect on the resolution of the bands but did slightly increase their mobilities (see Fig. 1b). In all subsequent runs 0.01 N-HCl was used for making the gel and for the sample application.

Under these conditions whole histone gave rise to at least 10 bands, falling into three main groups designated E1, E2, E3 in order of decreasing mobility, as shown in Fig. 1c. Group E1 had five distinct bands, group E2 at least three bands and group E3 two bands. Similar patterns have been obtained from four different preparations of the whole histone of calf thymus.

The histone fractions prepared by the methods described above have been submitted to electrophoresis under the same conditions and compared with the original histone (see Pl. 1). It can be seen that the arginine-rich fraction F3 gives rise to the electrophoretic group E3 of the whole histone; the moderately lysine-rich fraction F2 corresponds to group E1 and the lysine-rich fraction F1 corresponds to group E2. The fraction F3 used in this case was slightly contaminated with other proteins, possibly by degradation products, and the faint band with the higher mobility was not apparent in other arginine-rich fractions which were run.

The three histone chromatographic fractions were mixed together in the same proportions as they were obtained from the column and submitted to electrophoresis. The pattern obtained (see Pl. 1) was that of the original histone. Some attempts have been made to extract the histones of the different groups from the starch gel. The three groups from a run with whole histone were isolated by cutting the gel into portions and then submitting them to the freezing and thawing method described by Smithies (1955), 0.1 N-HCl being used as the solvent. A maximum of only 30% of the histone present was recovered in this way. These protein solutions were hydrolysed in 6 N-HCl at 110° and analysed in the usual way. If charring occurred owing to the residual starch in solution, a considerable loss of arginine and smaller losses of other amino acids were observed. Although no visible charring occurred in the analyses reported, it is still possible that the low arginine figure for E3 is due to this cause.

DISCUSSION

The experiments described above show that the recombined chromatographic fractions gave the same electrophoretic pattern as the unfractionated histone. This demonstrates that extra bands have not been produced by the electrophoresis or by changes during chromatography. On the other hand, it is clearly indicated that all the fractions obtained from the column are complex.
Table 2. Correlation of histone fractions obtained by chromatography and electrophoresis

<table>
<thead>
<tr>
<th>Chromatographic fraction</th>
<th>Lysine-rich</th>
<th>Slightly lysine-rich</th>
<th>Arginine-rich</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td></td>
<td>F2</td>
<td>F3</td>
</tr>
<tr>
<td>F2</td>
<td></td>
<td>F1</td>
<td>E1</td>
</tr>
<tr>
<td>E2</td>
<td>F3</td>
<td>E1</td>
<td>E3</td>
</tr>
<tr>
<td>N-Terminal groups</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>High proline content</td>
<td>High glycine content</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low acidic amino acids</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fractions of Cruft, Hindley, Mauritten &amp; Stedman (1957)</td>
<td>α</td>
<td>γ (?)</td>
<td>β</td>
</tr>
</tbody>
</table>

The arginine-rich fraction F3 (electrophoretic group E3, the slowest migrating group of bands) contains at least two proteins. The fraction used for these experiments had 69% of alanine N-terminal groups (Table 1, column 3), but other arginine-rich fractions prepared by an extraction procedure (Johns et al. 1960) having up to 96% of alanine N-terminal groups (Table 1, column 9) give the same two major bands on electrophoresis (Pl. 1f). It seems likely therefore that these two major constituents of group E3 both have alanine as their N-terminal group. The lysine/arginine ratio of the proteins extracted from the gel is higher than that of F3. This may be due to some loss of arginine on hydrolysis, or to fractionation during extraction from the gel. However, another definite characteristic of the arginine-rich histones, namely, the high content of acidic amino acids, is apparent in the analysis (Table 1).

The lysine-rich fraction (F1), which corresponds to electrophoretic group E2, gives a pattern of three bands, which may correspond to the three α-fractions of Cruft, Hindley, Mauritten & Stedman (1957). The analysis of this group, extracted from a gel after electrophoresis of the whole histone, gave 25-5% of lysine, thus confirming group E2 as the lysine-rich histone. Other confirmation comes from the high proline content and low content of acidic amino acids and leucine and isoleucine which are characteristic of this group (see Table 1).

The slightly lysine-rich fraction (F2) from the carboxymethylcellulose column, which has proline as its main N-terminal group, corresponds to electrophoretic group E1. One characteristic of this fraction is the very high glycine content of nearly 10%. It can be seen from Table 1 that group E1 is the only group with such a high value.

Groups E2 and E3 correspond both in order of electrophoretic mobilities and approximately in composition to electrophoretic fractions α and β obtained by Cruft, Mauritten & Stedman (1957) and Cruft et al. (1957). The fractions labelled γ by Cruft et al. are possibly included in our group E1, but this cannot be decided until the subgroups can be isolated and analysed. The various correlations are given in Table 2.

Starch-gel electrophoresis, although a powerful means of resolution of histones, does not give patterns which are directly comparable with those obtained on free electrophoresis. It is possible, however, that a reversible type of adsorption is taking place and that the process is in fact electrochromatography (Svensson, 1956).

SUMMARY

1. Electrophoretic patterns on starch gel at pH 2.3 have been obtained from whole calf-thymus histone and from the three fractions obtained by chromatography on carboxymethylcellulose.

2. The recombined chromatographic fractions, when run on starch gel, produced the original pattern of whole histone.

3. Correlations have been made between the chromatographic fractions and the electrophoretic groups, and other characteristics of these groups are given.

We wish to thank Mr D. Power for technical assistance.

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REFERENCES


Chromatographic Methods for the Study of Amines from Biological Material

By K. BLAU

Biochemistry Department, University of London King's College, Strand, London, W.C. 2

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Amines occur in most biological fluids and extracts, and considerable work has been done on their isolation, in particular where the amines are of physiological interest, as are the catechol group, and 5-hydroxytryptamine and histamine. The methods used have usually been designed specifically for isolating the amine or group of amines in question, rather than for general application to all amines. The procedures presented here were chosen for their ability to deal with bases in general and for their relative mildness; they are not applicable to amino acids, which have already received adequate attention.

Since biological material usually contains amines in relatively low concentration, chromatography has been widely used to study them. Some of the methods have been adapted from those developed for amino acids; others have been worked out specially for amines (on paper: Bremner & Kenten, 1951; Schwzyzer, 1952; Herbst, Keister & Weaver, 1958; on ion-exchange resins: Weber, 1951; Gardell, 1953; Kirshner & Goodall, 1957). Not all of the standard amino acid techniques can be applied to amines: thus electrolytic desalting only works with neutral compounds or ampholytes, and sulphonated polystyrene resins retain amines too firmly for convenient separation, so that rather powerful eluents are needed (Wall, 1953). Weakly acidic resins proved to have the necessary properties to overcome both these difficulties.

In the present work the amines were concentrated from a dilute solution or extract by ion-exchange resins used in the sequence described by Weber (1951), and the mixture of bases, after separation from most of the inorganic material, was resolved by column chromatography on buffered, weakly acidic resin. The fractions were analysed for amines, and those containing the peaks were then desalted on another column of resin in the free acid form. The resulting solution on evaporation to dryness yielded the crystalline amine hydrochlorides. Paper chromatography in five different solvents and paper electrophoresis were used to compare the products thus isolated with known compounds.

MATERIALS AND METHODS

Buffer solutions. Except where otherwise stated, buffer solutions are those of McIlvaine (1921).

Ion-exchange resins. De-Acidite FFX (100-mesh beads, chloride form; Permutit Co. Ltd.). This resin was specially made for the present work, and differs from the commercially available De-Acidite FF by improved chemical stability. It was regenerated by stirring with twice its volume of 5% NaOH, filtering on a sintered funnel, and repeated washing on the filter with 5% NaOH until the filtrate was free of chloride. The resin was then washed with boiled (i.e. CO₂-free) and cooled distilled water until free of alkali. Columns were poured from a slurry of this resin in CO₂-free water. If not used at once, the column was rinsed again before use, since residual traces of alkali and of tertiary amine were leached from the resin on prolonged standing in the free-base form.

Zeo-Karb 225 (50 μ beads, hydrogen form; Permutit Co. Ltd.) was treated as described for the similar resin Amberlite XE-84 by Hirs, Moore & Stein (1953).

Zeo-Karb 225 (100-mesh beads, hydrogen form: Permutit Co. Ltd.) was graded by backwashing (Hamilton, 1958). Coarse and fine fractions, of about 10% of the total amount of resin each, were rejected, and the main fraction was then treated like the 50 μ bead resin. It was buffered by stirring with twice its volume of the appropriate buffer, and solid A.R. NaOH was then added to the stirred suspension of resin until the pH of the supernatant remained constant at the