reproducible. (It may be mentioned that the chromatogram developed from the homogenized unfertilized eggs with the same solvent, gives a much better resolution and shows a different pattern, Figure 1.) The chromatograms developed from hydrolysed muscle tissues gave a clearer resolution of the spots (Figure 2). Although specific tests for amino acids were not carried out, comparison with standard amino acids suggests the presence of sarcosine, taurine, glycine, and glutamic acid as the most intense spots. Taurine, glycine, and glutamic acid were also compared in another solvent system, namely methanol:water:pyridine (20:5:1). Two-dimensional chromatograms (*n*-butanol:acetic acid:water and phenol) of the material and the material plus sarcosine and taurine and only sarcosine and taurine were compared. Although resolution was poor there were enhanced intensities in the proper places[12].

Zusammenfassung. Nach den vorliegenden Befunden scheinen Taurin und/oder Sarcosin, Glycin und/oder Glutaminsäure die wesentlichen Aminosäuren der *Ciona*-Muskulatur zu sein. Das charakteristische Chromatogramm lässt sich in nahezu gleicher Intensität auch in den Extrakten von ausgehungerten Versuchstieren nachweisen.

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Indian Statistical Institute, Calcutta (India), October 5, 1964.

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The Fractionation of Arginine-Rich Histones from Calf Thymus

Histones from calf thymus and other mammalian tissues can be divided into two major groups – arginine- and lysine-rich histones. The arginine-rich histones are heterogeneous as was shown by JOHNS and BUTLER[1] who, on the basis of solubility in absolute ethanol obtained two arginine-rich fractions coded as F2a and F3[2]. The F3 histones are electrophoretically slow (in starch gel electrophoresis) and contain alanine as the sole NH_2 terminal amino acid. The F2a histones are fast in starch gel electrophoresis and do not possess any definite NH_2 terminal amino acid. Proline, alanine and glycine are usually recovered in small amounts, and PHILLIPS[3] has shown recently that the NH_2 terminal amino acids in this fraction are acetylated to a great extent. Because the starch electrophoresis of the F2a and F3 histones shows the presence of at least two distinct bands in each of the two fractions, attempts were made to fractionate the arginine-rich histones.

The arginine-rich histones were prepared from ethanol washed crude calf thymus nucleohistone[4] by extraction with a mixture of absolute ethanol and 1.25 *N* HCl[5,6]. Extract containing the F2a and F3 histones was precipitated with 6 Vol of cold acetone and the precipitated protein was recovered by centrifugation, washed with acetone and with ether and dried in vacuo. In another series of experiments, the ethanol-HCl extract was evaporated in dialysis bags in a stream of cold air approximately to $^1/_3$ of the original volume and dialyzed against distilled water. The small amount of precipitate which formed during evaporation and dialysis was removed by centrifugation and the F2a and F3 histones were then precipitated with trichloroacetic acid (5% final concentration), converted to hydrochlorides and dried[5].

The precipitate formed during evaporation and dialysis was washed with cold acetone, then by ether, and dried. Part of the material became soluble in acetone and after removal of acetone by flash evaporation a brown lipid-like material was obtained. This material contained no protein or amino acids and no further characterization was attempted. The protein part of the precipitate was hydrolyzed in constant boiling HCl (5.7 *N*) and analyzed for amino acid composition, which is shown in the Table under 'lipo'.

The arginine-rich histones F2a and F3 were separated by filtration on Sephadex G 75 (Fine, in bead form) columns. The swollen gel suspended in 0.01 *N* HCl was poured into columns 50 × 1600 mm, 1500 mg of the F2aF3 histones were dissolved in 15 ml of 0.01 *N* HCl and applied to the column. The fractions were eluted with 0.01 *N* HCl saturated with chloroform. A flow rate of 48 ml/h was maintained and fractions were collected every 10 min. The elution pattern obtained is shown in Figure 1. The F3 histones were eluted from the column first, followed by the F2a fraction. Fractions comprising the peaks were pooled as indicated by arrows in Figure 1, and the proteins were recovered by dialysis against distilled water and by lyophilization.

The F2a histones obtained in this way were further fractionated. 3.0 g of the F2a fraction were dissolved in

[1] E. W. JOHNS and J. A. V. BUTLER, Biochem. J. *82*, 15 (1962).
[2] The symbols used in this paper were originated by Professor J. A. V. BUTLER's group and their original meaning was the order in which these fractions emerged from a carboxymethylcellulose column. Since then the methods for preparation of histone fractions have changed and the symbols lost their original significance.
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1500 ml of 0.2 *N* HCl and dialyzed against an equal volume of *n*-propanol overnight. During dialysis the volume of histone solution was reduced approximately by 75%[7]. The dialysis bags were then transferred into 4000 ml of a mixture of 25% of 2 *N* HCl and 75% of *n*-propanol (2000 ml) and 96% ethanol (2000 ml). After 12 h dialysis the diffusate was replaced by 4000 ml of a mixture of 96% ethanol and *n*-propanol (3:1, v/v) and dialyzed for another 12 h. During this time a heavy white precipitate formed in the bags and was collected by centrifugation, washed with acetone, then with ether, and dried in vacuo ($F2a_{II}$). The supernatant fluid was mixed with 5 Vol of acetone; the precipitate which formed instantly was collected by centrifugation and was dried with acetone and ether ($F2a_I$). 1627.4 mg of the $F2a_I$ and 1322.7 mg of the $F2a_{II}$ were recovered. All preparative work was performed at 4°C.

The protein samples were hydrolyzed in constant boiling HCl (5.7 *N*) in sealed evacuated tubes and analyzed with the aid of automatic amino acid analyzer. The NH_2 terminal amino acids were determined by reacting the samples with 1-fluoro-2, 4-dinitrobenzene[8]. The starch gel electrophoresis was performed in horizontal arrangement as described previously[4].

The amino acid composition of the F2a and F3 histones as shown in the Table, suggests that a good fractionation was achieved by chromatography on Sephadex G 75. Starch gel electrophoretic patterns of the recovered proteins further proved that the fractions are virtually free of contamination (Figure 2). To eliminate possible contamination caused by overlapping of the elution peaks, a small portion of the effluent (indicated in Figure 1 by the width of the central arrow) was excluded. The amino acid analytical data shown for the F2a and F3 histones in the Table are in good agreement with the composition of similar fractions reported by JOHNS and BUTLER[1]. These authors utilized differential solubility of the arginine-rich histone fractions in absolute ethanol in order to separate the F2a and F3 histones. To achieve about the same degree of homogeneity as reported in this paper, several repeated purification steps in absolute ethanol were required, resulting in substantial losses during the procedure. The described chromatographic method is much simpler and superior in the almost quantitative recovery of the fractionated histones.

Amino acid composition of arginine-rich histones

The amino acids are expressed as moles per 100 moles of all amino acids found. A correction (10%) was made for the hydrolytic losses of serine. The values are averages of three to five determinations carried out for each fraction. Hydrolysis in 5.7 *N* HCl was carried out at 110°C for 22 h

Amino acid	Histone fraction				
	F3	F2a	$F2a_I$	$F2a_{II}$	'Lipo'
Lysine	9.0	11.1	10.6	12.6	8.0
Histidine	2.8	2.6	2.1	2.8	1.3
Arginine	12.4	12.1	13.3	10.4	8.9
Aspartic acid	4.4	5.7	5.1	5.6	5.9
Threonine	6.5	5.4	6.3	4.8	5.8
Serine	4.1	3.5	3.2	3.7	14.9
Glutamic acid	10.8	8.4	7.0	8.7	9.8
Proline	4.3	3.2	2.0	4.1	4.5
Glycine	6.6	11.6	14.0	10.1	8.5
Alanine	12.5	11.4	9.1	13.4	9.1
Valine	4.9	6.3	7.4	5.6	5.2
Methionine	1.2	0.4	0.5	0.1	0.2
Isoleucine	5.2	4.7	5.2	4.1	4.4
Leucine	10.1	10.4	8.4	11.3	8.6
Tyrosine	2.1	2.4	3.2	1.9	1.9
Phenylalanine	2.5	1.2	2.0	0.7	2.6

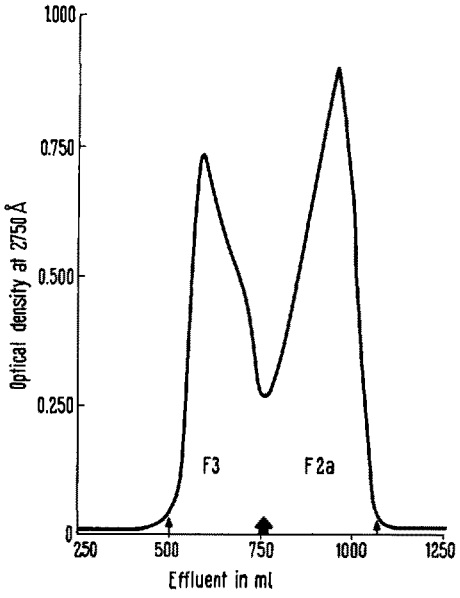

Fig. 1. Elution pattern of 1500 mg of arginine-rich histones F2aF3 when chromatographed on Sephadex G 75 column 50 × 1600 mm. Arrows indicate points where the fractions were pooled for recovery of histones.

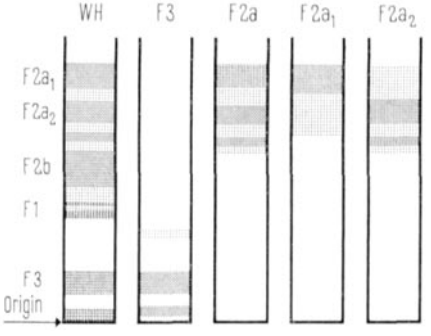

Fig. 2. Drawing of the calf thymus histone fractions separated by electrophoresis in starch gel. WH is the unfractionated whole histone containing all the Fractions (F3, arginine rich; F1, very lysine rich; F2b, moderately lysine rich; and F2a, arginine rich). The F3 is arginine-rich histone as recovered from the first peak after chromatography on Sephadex G 75, and F2a is arginine-rich histone recovered from the second peak of the same chromatographic run (Figure 1). $F2a_{II}$ is the precipitate obtained by dialysis against a mixture of *n*-propanol and ethanol and $F2a_I$ is the protein recovered from the, supernatant fluid after such dialysis (see text). The proteins were stained with Amido Black 10 B.

[7] It is convenient to open the bags and pool the contents. To avoid losses of the F2a histones, the emptied bags should be washed with the mixture of *n*-propanol-2 *N* HCl-ethanol used for the next dialysis (volume approximately equal to the amount of removed fluid). If the fraction 2a was contaminated with F3 histones, these will precipitate during dialysis against *n*-propanol-2 *N* HCl-ethanol and must be removed before the dialysis against *n*-propanol-ethanol is started.

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As was mentioned, the starch gel electrophoresis of both F2a and F3 histones shows the presence of at least two electrophoretic components in each of the fractions. The described dialysis against *n*-propanol and ethanol can fractionate the F2a histones into two components: the $F2a_I$ which is electrophoretically faster and the $F2a_{II}$ with slightly lower electrophoretic mobility in starch gel (Figure 2). These two fractions differ significantly in their content of several amino acids (e.g. arginine, lysine, alanine, glycine, etc.). The electrophoretically slower and still composite $F2a_{II}$ histone has, in addition, a higher ratio of leucine to isoleucine (Table).

The NH_2 terminal amino acid of the F3 histones is alanine (95%); proline, alanine glycine, and lysine were found in various amounts in the F2a fraction. In general, the recovery of DNP amino acids was very low in the F2a histones, indicating the inaccessibility of the NH_2 terminal for dinitrophenylation[3]. The same was found for the two subfractions $F2a_I$ and $F2a_{II}$.

The discovery of a lipid-bound histone fraction is not entirely new. BAKAY et al.[9] mentioned the presence of a small amount of lipid-like material in nucleohistone, but no composition was reported. A lipid material persistently appeared in the X-ray diffraction patterns of native calf thymus nucleohistones and could not be removed by purification of the nucleohistone by dissolving in water and precipitating with 0.14 *M* NaCl (the 60 Å spot). This indicates that the lipid is firmly attached to nucleohistone[10], and WILKINS suggested the possible presence of sphingomyelins in nucleohistones[11]. The phosphate groups of such lipids could easily combine with the seryl residues found in the 'lipo' histone (Table). WILKINS, on the basis of X-ray diffraction, estimated the amount of sphingomyelin-like lipid in calf thymus nucleohistone as 3%. This value is very close to the observed yield of the 'lipo' histone described in this paper. The amount of this material varies significantly in nucleohistones of different origin, with liver being about the richest source of such protein[12].

Zusammenfassung. Die argininreiche Fraktion des Kalbsthymushistons (Fraktion 2a) wurde in zwei Komponenten zerlegt. Zusammensetzung und elektrophoretische Trennung der Komponenten ($F2a_I$ und $F2a_{II}$), ebenso die Eigenschaften eines neuen, lipidgebundenen, serinreichen Histons wurden beschrieben.

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Phase Contrast Microscope Observations on the Pancreas of Fasting Rats

The morphological and histochemical changes observed in the pancreas of rats kept under conditions of prolonged fasting have been reported in previous papers[1,2]. They can be summed up as severe cytoplasmic damage, nuclear lesions, swelling and progressive disappearance of the mitochondria, together with a sharp reduction in the DNA and RNA content.

It has been previously indicated, however, that these changes are not accompanied by a notable reduction in the zymogen granules, which are present even after prolonged fasting (150–160 h). All these results have been obtained using classic histological and histochemical methods, such as Heidenhain's technique with iron-hematoxilin and Brachet's with methyl green-pyronine. The present study has been undertaken to try to resolve the problem of the persistence of the zymogen granules after 160 h fast.

Ultra-thin sections of rat pancreas were subjected to phase contrast microscopy, some of the specimens being previously subjected to silver impregnation.

Materials and methods. Twenty albino rats (Sprague-Dawley strain) weighing about 200–250 g, were kept under conditions of complete fasting, apart from ad libitum availability of water, for 160 h.

Another ten rats were kept on a standard diet as control. The animals were killed by decapitation, and small pieces of the pancreas were fixed immediately in 2% osmium tetroxide buffered at pH 7.2–7.4 (after Palade), dehydrated with ethyl alcohol and embedded in butyl-methyl-metacrilate. Sections of 1000–2000 Å thickness were cut, using a Porter-Blum microtome equipped with a glass knife. The sections, distended with chloroform vapours, were placed on cover-slips and observed in phase contrast under a Leitz Ortholux microscope equipped with objectives for high contrast. Some specimens were subjected to silver impregnation, according to JONES' technique[3] as modified by MARINOZZI[4], and following precautions reported elsewhere[5].

Results and discussion. The use of this technique confirms some data already provided by morphological and histochemical studies and also gives some new information.

Nuclear damage is visible, with optically empty nuclei. The nucleoli are often missing or very much reduced in

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