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Depolymerization of Ribonucleic Acid by Enzymes in Basic Proteins from Liver Nuclei and Ribosomes

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Recent studies on the proteins associated with nucleic acids have revealed the composite nature of histones and the basic proteins of ribosomes. Nuclear histones from a variety of tissues have been separated into three main fractions by chromatography on resins (Crampton, Moore & Stein, 1955) and on carboxymethylcellulose (Davison, 1957; Phillips & Johns, 1959; Davis & Busch, 1959; Johns, Phillips, Simson & Butler, 1960). Multiple bands can be produced from these histones and from histone fractions when they are subjected to starch-gel electrophoresis (Neelin & Connell, 1959; Neelin & Neelin, 1960; Setterfield, Neelin, Neelin & Bayley, 1960; Johns & Butler, 1962). The amino acid composition of ribosomal proteins from bacterial, plant and animal cells is reported to be similar in certain respects to the composition of histone protein (Ts'O, Bonner & Dintzis, 1958; Crampton & Petermann, 1959; Butler, Cohn & Simson, 1960).

Pirie (1950) reported the presence of latent ribonucleases in ribonucleoprotein of tobacco leaf, and recent work has suggested that this enzyme is an integral part of the ribonucleoprotein particles of animal, plant and bacteria cells (Elson, 1958; Tashiro, 1958; Ts'O, Bonner & Vinograd, 1958; Siekewitz & Palade, 1958; Dickman & Trupin, 1958; Roth, 1960; Spahr & Hollingworth, 1961; Zillig, Schachtschabel, McCorquodale, Krone & Horne, 1960). The latent nucleases of rat-liver ribosomes have been extracted by dilute acids (Tashiro, 1958; Roth, 1960), and it is possible that they are components of those ribosomal proteins that are similar in chemical composition to histone proteins (Butler *et al.* 1960). The possibility that latent nucleases act as gene regulators in metazoan cells has been proposed by Leslie (1961).

Basic proteins of guinea-pig-liver nuclei and ribosomes have been isolated and examined for RNA-depolymerase activity. Depolymerase activity has been found and the conditions for optimum activity of the preparations are described. The distribution of depolymerases within the complex of basic proteins has been investigated by electrophoresis on starch gel.

METHODS

Isolation of nuclei. Fresh guinea-pig livers (60 g.) were perfused in 0.9% NaCl and the cells broken in a solution containing sucrose (0.25M), CaCl₂ (3 mM) and tris (0.035M), at pH 7.1, by homogenization for 20 sec. in a M.S.E. blade homogenizer, at a 1:1 (w/v) concentration. All operations were carried out at 0–4°. The nuclei were collected by centrifuging at 1000 rev./min. for 5 min. after filtering through nylon gauze and diluting the homogenate to a 1:10 (w/v) concentration. The nuclear pellets were combined and washed by resuspension in 100 ml. of a solution of sucrose (0.25M), citric acid (0.01M) in a Potter-Elvehjem-type homogenizer and were then centrifuged. The total time of the washing procedure was kept to a minimum. Nuclei were stored at –20°.

Nuclei were isolated in the following manner from two strains of human cells. HEP 1 cells were derived from an epitheloid cervical carcinoma (Fjelde, 1955), and HLM cells from the liver of a 20-week human foetus (Leslie, Fulton & Sinclair, 1956). Approx. 5 g. of cells was suspended in 20 ml. of sucrose–CaCl₂–tris solution and 5 ml. volumes were treated initially with a M.S.E. Mullard ultrasonic oscillator (output 60 w, and tuned to 20 kcyc./sec.) by using a stainless-steel probe of 1.9 cm. diam. and glass tubes of 2.3 cm. internal diam., immersed in ice-cold water. This treatment was limited to periods of 15 sec. to reduce to the minimum the destruction of liberated nuclei, and the extent of cell disruption was checked by examination with an interference microscope. When necessary, a second 15 sec. treatment was applied with the oscillator. The

nuclear pellet was collected by centrifuging at 1000 rev./min. for 5 min. and washed twice with sucrose-citric acid in a Potter-Elvehjem homogenizer to complete the release of nuclei from cytoplasmic material.

Isolation of ribosomes. Nuclear debris and mitochondria were removed from the initial supernatant by refrigerated centrifuging at 10000g for 20 min. The microsome pellets were suspended in 50 ml. of a solution containing sucrose (0.25M), $MgCl_2$ (0.05M), tris (0.035M), sodium deoxycholate (1%, w/v) and Lubrol W (Imperial Chemical Industries Ltd.) (0.5%), at pH 7.8. This solution was overlaid on 6 ml. of a solution containing sucrose (0.35M), KCl (0.6M), $MgCl_2$ (0.01M) and tris (0.35M), at pH 7.8, and the ribosomes were collected by centrifuging at 105000g for 2 hr. (Rendi & Hultin, 1960).

Extraction of basic proteins. Proteins were extracted from nuclei by the method devised by Ui (1957). The extraction was repeated three times with 0.5N- H_2SO_4 and the proteins were precipitated from the acidic supernatant by the addition of 1 vol. of ethanol. In certain preparations (see Table 1) protein that remained soluble in 50% (v/v) ethanol was concentrated in dialysis sacs by a cold-air current and dialysed against water to remove the sulphuric acid. The protein was dissolved in 0.01N-acetic acid, precipitated by ethanol, and dissolved in 0.05M-acetate buffer, pH 4.2, and stored at -20° .

Basic proteins of the ribosomes were also extracted by the above method, and similar results were obtained when the ribosomes were extracted in HCl according to the method of Davison, James, Shooter & Butler (1954).

Carboxymethylcellulose chromatography. Columns of carboxymethylcellulose (Whatman CM 70) were prepared by pouring a slurry of the powder in 0.05M-sodium acetate buffer, pH 4.2, at room temperature into 30 cm. $\times$ 0.9 cm. columns. The powder was allowed to settle under gravity and the columns were washed with buffer until the extinction of the eluate at 278 m μ was zero.

Two methods of elution were used: (1) Gradient elution (Davison, 1957). A sodium chloride gradient was produced by addition of 0.9M-NaCl in 0.05M-acetate buffer, pH 4.2, to a constant-volume (50 ml.) mixing reservoir, giving a sigmoid-shaped salt gradient. The procedure separated the nuclear basic protein into three fractions. (2) Stepwise elution (Johns *et al.* 1960). The concentration of solutions required to elute peaks was determined by pilot runs on the gradient system. Three protein peaks were removed from the column by elution with 0.3M- or 0.4M-NaCl, 0.7M-NaCl, and 0.02N-HCl.

Fractions of 2.5 or 5.0 ml. were collected and the presence of protein was determined by measuring the extinction at 278 m μ on a Unicam spectrophotometer. The protein in the pooled fractions was determined by the method of Lowry, Rosebrough, Farr & Randall (1951) after dialysis against water and freeze-drying.

Purification of commercial ribonucleic acid. Commercial RNA (Nutritional Biochemicals Corp.) was purified by the method of RNA preparation devised by Laskov, Margolis, Littauer & Eisenberg (1959). RNA (5 g.) was dissolved in 90 ml. of 0.1M-EDTA, 90 ml. of 90% (w/v) phenol-water was added and the suspension was homogenized thoroughly with a Teflon pestle. After vigorous mechanical stirring for 1 hr. at room temperature the suspension was centrifuged for 1 hr. at 0° at 2500 rev./min. The lower phenol layer was extracted four times with

0.1M-EDTA and the top aqueous layers were combined. The aqueous solution of RNA was made 2% (w/v) with respect to potassium acetate and 70% (v/v) with respect to ethanol and left at -20° overnight. The resulting white precipitate was centrifuged down, dissolved in 50 ml. of 0.1M-EDTA, made 0.3M with respect to NaCl and left for 3 days at 0° . The purified high-polymer RNA was collected by centrifuging at 10000g for 30 min. (M.S.E. high-speed model 17). The RNA was dissolved in 100 ml. of 0.1M-EDTA, dialysed at 4° against water and freeze-dried. The dried preparation was stored at -20° in a desiccator.

Assay of ribonucleic acid-depolymerase activity. The assays were carried out by incubating the RNA with the protein preparations at 37° for 1 hr., the undegraded RNA then being precipitated and the percentage degradation estimated from the extinction at 260 m μ of the supernatants. Blank assays were carried out by the immediate addition of ethanol-acetate to unincubated digests. Enzymic activity was confirmed by complete inactivation of the enzymes by autoclaving at 15 lb./in.² for 3 hr. Care was taken to avoid contamination of glassware by skin nucleases (Holley, Apgar & Merrill, 1961), and RNA preparations were checked for non-specific degradation under assay conditions in the absence of added protein.

Each digest consisted of equal volumes of histone, buffer and RNA solutions (total volume 1.5 or 3 ml.). The details of the buffers used are given with the Tables and Figures, and the final concentrations of the buffers are quoted. All assays were carried out in duplicate and the assay conditions are reported in the Table captions. After incubation, undegraded RNA was precipitated by the addition of 4 or 8 ml. of 1% (w/v) potassium acetate in 85% (v/v) ethanol and the tubes were left at -20° overnight. The precipitate of RNA was removed by centrifuging at 2000 rev./min. for 15 min.

The unit of ribonuclease activity is defined as that activity which produces an increase in extinction of 0.100 at 260 m μ , and the specific activities are expressed in units/ μ g. of protein nitrogen. The amount of depolymerization was calculated from the difference in extinction at 260 m μ of the supernatant of incubated and unincubated digests, on the basis of an extinction of 0.322 for 1 μ g. of RNA phosphorus/ml., which we determined on our preparations.

Starch-gel electrophoresis. Starch gels were made by the method described by Smithies (1955). A solution of 16% (w/v) starch (British Drug Houses Ltd.) in 0.03M-acetate buffer, pH 5.4, containing urea (4M) was used to form gels in a Perspex trough (18 cm. $\times$ 3 cm. $\times$ 0.6 cm.). Electrode connexions were made of gauze strips. 0.3M-Acetate buffer, pH 5.4, was used in electrode vessels of 800 ml. capacity. Histone samples (600 μ g. of protein) were applied in a starch-grain slurry to a 2 mm. slit. Electrophoresis was carried out at 3v/cm. for 18 hr. in a cold room.

Histone bands were located by staining one half of the gel with Amido Black (Gurr Ltd.). Nuclease activity was detected on the other half of the gel by a modification of the method described by Daoust & Amano (1960). A layer of 1% (w/v) RNA prepared as described above in 0.1M-acetate buffer, pH 5.4, containing NaCl (0.15M) was spread on the gel and incubated at 37° for 2 hr. The gel was stained in aq. 2% (w/v) toluidine blue. Destaining in water for 48 hr. revealed the areas of depolymerization.

RESULTS

Fractionation of basic proteins on carboxymethylcellulose columns, and on starch gels. In the initial stages of the investigation basic protein extracted from nuclei with 0.5N-sulphuric acid was fractionated into four peaks by elution from carboxymethylcellulose columns by the gradient system (Leslie, 1961). The first peak (Fx) was not retained on the column, did not contain protein and had a higher extinction at 260 m μ than at 278 m μ , suggesting the presence of polynucleotides. It was found that the three histone peaks, F1, F2 and F3, could be more conveniently separated by stepwise elution (Fig. 1a) with 0.3M-sodium chloride, 0.7M-sodium chloride and 0.02N-hydrochloric acid (see the Methods section). The nomenclature adopted is similar to that of Johns *et al.* (1960).

The demonstration that histone could be extracted from thymus nuclei by ethanol-hydrochloric acid (Johns & Butler, 1962) indicated that some protein might remain in the 50% (v/v) ethanol-sulphuric acid supernatants after the bulk of the histone had been precipitated by the addition of ethanol. The supernatant was concentrated by evaporation in the cold to remove ethanol and dialysed to remove sulphuric acid. Of the total protein extracted, nearly 10% was soluble in 50% (v/v) ethanol-0.25N-sulphuric acid. Stepwise carboxymethylcellulose chromatography showed the presence of all three histone fractions. Comparison of the amounts of protein present in the ethanol-precipitated and ethanol-soluble histone fractions (Table 1) indicates that there is a larger proportion of F1 in the latter.

When the ribosomal basic protein was chromatographed under the same conditions as above, two protein peaks, designated R1 and R2, were obtained. They correspond in the elution stages to the F1 and F3 fractions of nuclear histone (Fig. 1b). Although the ribosomal and nuclear proteins behave similarly on carboxymethylcellulose chromatography, starch-gel electrophoresis showed that

Fig. 1. Fractionation of basic proteins of guinea-pig-liver nuclei (a) and ribosomes (b) by stepwise elution from 30 cm. columns of carboxymethylcellulose (Whatman CM 70). Fx and Rx material was washed through with 0.05M-acetate buffer, pH 4.2. The other fractions were eluted as indicated in Table 1, and the amount of protein in the serial tubes was determined by the extinction at 278 m μ .

Table 1. *Fractionation of basic proteins obtained from guinea-pig-liver nuclei and ribosomes*

The proteins were extracted with ice-cold 0.5N-H₂SO₄, precipitated at 50% (v/v) ethanol (see the Methods section) and fractionated by stepwise elution from carboxymethylcellulose columns. Protein equivalent to 8.5 mg. of protein nitrogen, determined by the method of Lowry *et al.* (1951), was applied to the column. The percentage of protein in each fraction is given in parentheses.

Origin of protein	Solubility in 50% (v/v) ethanol-0.25N-H ₂ SO ₄	Percentage eluted from column	Protein nitrogen eluted (mg.)		
			With 0.3M-NaCl	With 0.7M-NaCl	With 0.02N-HCl
Nuclei	Insoluble	50	0.04 (1)	1.71 (42)	2.31 (57)
	Soluble	70	0.30 (5)	0.96 (17)	4.42 (78)
Ribosomes	Insoluble	90	2.15 (27)*	—	5.85 (73)

* Eluted with 0.4M-NaCl.

Table 2. *Specific activities of fractions of basic proteins*

All values were determined after 1 hr. of incubation with RNA (30 μ g. of phosphorus) at 37° in buffers containing 0.15M-NaCl. F1, F2 and F3 (nuclear) and R1 and R2 (ribosomal) are fractions obtained by chromatography on carboxymethylcellulose (see Table 1). The pH at which the assay was carried out was the optimum for the fraction.

Origin of protein	Solubility in 50% (v/v) ethanol-0.25N-H ₂ SO ₄	Fraction	pH of buffer	10 ³ × Specific activity
Nuclei	Insoluble	*	7.3	11.7
	Soluble	*	7.3	62
	Insoluble	F1	6.6	411
	Soluble	F1	6.6	458
	Insoluble	F2	7.3	16
	Insoluble	F3	6.3	8.6
Ribosomes	—	R1	6.4	340
	—	R2	6.8	95

* These are the soluble and insoluble fractions prepared from acid extract of nuclei without chromatography on carboxymethylcellulose.

more bands are obtained from the ribosomal basic proteins (Figs. 3*b* and 3*c*).

When starch-gel electrophoresis was applied to the individual fractions F1, F2 and F3 isolated from liver nuclei, it became evident that chromatography on carboxymethylcellulose columns failed to give complete separation into pure fractions. This agrees with the reports of Agrell & Christenson (1961) and Hnilica, Johns & Butler (1962) that histones prepared from tissue other than calf thymus are not well separated by chromatography on carboxymethylcellulose.

Nuclease activity in basic proteins from liver. The specific activities of RNA nuclease in various fractions of basic proteins are shown in Table 2. The specific activity of pancreatic ribonuclease assayed under similar conditions is about 17-fold the activity in the most active nuclear fraction (F1, soluble) and nearly 23 times more active than ribosomal fraction R1. Nuclear F1 protein is 25 times as active as F2, and 48 times as active as F3. The activity of the histone soluble in 50% (v/v) ethanol-acid is 4 times the activity in the precipitated histone. It was subsequently found that 92% of the nuclease activity could be precipitated from acid solution by 90% (v/v) ethanol.

The effect of heat treatment on the activity in the different fractions was investigated (Fig. 2). In all fractions, nuclease activity is present after heating for 1 hr. at 80° in water. The profiles of F2, F3 and R2 are slightly different from those of F1 and R1. With the former fractions, higher concentrations of protein were required for the assays and the depolymerase activities were low. The consistent feature of these experiments was the resistance of enzyme protein to inactivation by heat, a feature that Elson (1959) and Spahr & Hollingworth (1961) found with ribonuclease of *Escherichia coli* ribosomes.

Fig. 2. Effect of heat on the depolymerase activity of basic proteins from liver nuclei and ribosomes. ●, Percentage of RNA degraded by nuclear proteins; ○, percentage of RNA degraded by ribosomal proteins. The nuclear fractions F1 (1 μ g.), F2 (10 μ g.) and F3 (10 μ g.), and ribosomal fractions R1 (0.2 μ g.) and R2 (2 μ g.), were heated at 20, 40, 60, 80, 100 and 120° (autoclaved) for 1 hr. The assays of depolymerase activity were carried out at 37° for 1 hr. in the presence of RNA equivalent to 30 μ g. of RNA phosphorus, and buffered at the pH indicated for each fraction in Table 2.

Nuclease activity was located in the multiple bands of histones and ribosomal basic proteins obtained by starch-gel electrophoresis. As described in the Methods section, toluidine blue could be

dissolved out of the gel in regions of nuclease activity, and the resulting pattern of staining compared with the protein bands located in the other half of the gel by Amido Black staining (Figs. 3*a*–3*d*).

Nuclear histone has three active regions; the ribosomal basic proteins show only one. In the former, it is significant that the region in which the least degradation occurs is occupied by the arginine-rich basic proteins (Johns & Butler, 1962).

Latency of nuclease activity. An important feature of the nuclease activity present in ribosomes is that it can be detected only when the normal structural combination of RNA and protein is altered (Tashiro, 1958; Elson, 1959; Roth, 1960; Spahr & Hollingworth, 1961). It was therefore necessary to find the extent to which the nuclease activity of the various basic proteins, fractionated on carboxymethylcellulose, could be stimulated by the procedure employed to release the latent ribonuclease of ribosomes.

Table 3 shows the percentage depolymerization of RNA incubated for 1 hr. at 37° in the presence of basic proteins from guinea-pig-liver nuclei and ribosomes and three reagents used to disrupt ribosomes, 0.15M-sodium chloride, 0.01M-EDTA and 4M-urea. The effect of incubating in the presence of 0.1M-magnesium chloride was also investigated.

When the RNA and the nuclear basic proteins are incubated in buffer alone at pH 6.6, only the F1 protein produces significant depolymerization. This is augmented by 0.15M-sodium chloride, 0.01M-EDTA or 4M-urea, and inhibited by Mg^{2+} ions. The nuclease activity associated with the R1 and R2 fractions of ribosomes responds in a similar manner to the four reagents. With both the F1 and R1 fractions, the enzyme is not entirely latent in the absence of salt, nor is it completely inhibited by the presence of Mg^{2+} ions. No depolymerase

Fig. 3. Location of basic proteins and nuclease activity, after electrophoresis on starch gel. Details of the procedure are given in the Methods section. (a) and (b): Electrophoresis of ribosomal basic protein. (a) Slice of gel coated with 1% (w/v) RNA solution, incubated at 37° for 2 hr., and RNA stained with aq. 2% (w/v) toluidine blue. Destaining of the gel in water occurs in the area in which RNA has been degraded. (b) Slice of same gel stained with Amido Black to show location of basic proteins. (c) and (d): Electrophoresis of nuclear histones. (c) Slice of gel stained with Amido Black to show location of histones. (d) Slice of same gel treated as for (a). Three areas of nuclease activity can be seen.

Table 3. *Latency of nuclease activity*

Incubation of nuclear and ribosomal protein fractions with RNA (30 μ g. of phosphorus) for 1 hr. at 37° in 0.03M-cacodylate-HCl buffer, pH 6.6, and in this buffer containing NaCl (0.15M), EDTA (0.01M), urea (4M) and $MgCl_2$ (0.1M). F1, F2 and F3 (nuclear) and R1 and R2 (ribosomal) are fractions obtained by chromatography on carboxymethylcellulose (see Table 1). The values of specific activity are means of closely agreeing duplicates. The experiment was repeated with different preparations of basic proteins and similar results were obtained.

Origin of protein	Fraction	$10^3 \times$ Specific activity				
		With no addition	With 0.15M-NaCl	With 0.01M-EDTA	With 4M-urea	With 0.01M- $MgCl_2$
Guinea-pig liver	F1	228	390	405	390	75
	F2	1.5	6.6	2.0	2.5	0
	F3	2.2	3.2	2.5	0.5	0
	R1	110	310	300	320	60
	R2	25	87	90	40	0
HLM cells	F1	36	134	81	137	18
HEP 1 cells	F1	70	93	117	119	14

activity can be detected in F2, F3 or R2 fractions in the presence of 0.1M-magnesium chloride.

The nuclei of two human cell strains (HLM and HEP 1), which have been maintained for many years in tissue culture, were isolated and their soluble proteins fractionated on carboxymethyl-cellulose columns. The F1 fraction from each strain contained RNA-depolymerase activity with similar characteristics to those of the activity in the F1 fraction of liver nuclei.

Effect of pH on depolymerization. The percentage depolymerization of RNA produced by the different fractions was determined over the pH range 4.4–8.5. The buffers employed are specified in the legend of Fig. 4, and dissociation of RNA and protein was obtained by including 0.15M-sodium chloride in the medium. Four pH optima were detected in the nuclear fractions. With F1 protein, depolymerization was maximal at pH 5.7 and 6.5, with F2 proteins, at 7.2, and with F3 protein, at

pH 5.5 and 6.3. The ribosomal fraction R1 showed optimum activity at pH 5.2 and 6.4; R2 protein gave only one peak at 6.8.

There is, of course, a significant difference in the extent of degradation of RNA produced by the various fractions. With proteins F2, F3 and R2, only about 10% of RNA is degraded at the pH optima in 1 hr. as compared with 20–40% degradation during incubation with the F1 and R1 fractions.

DISCUSSION

The main purpose of the investigation was to obtain information about the ribonuclease activity associated with acid extracts of nuclei and ribosomes. At the outset, it was known that: (1) the composition of the basic proteins of ribosomes has certain general similarities to the composition of histone proteins (cf. Phillips, 1962); (2) there is present in ribosomes a latent nuclease activity, which appears to be associated with their basic proteins (Tashiro, 1958; Roth, 1960); (3) the ribonuclease of pancreatic tissues can be most effectively extracted with 0.25N-sulphuric acid (Dickman & Morrill, 1959). The last fact raises the possibility that such acid extracts of subcellular components may contain ribonuclease as a contaminant.

It was, therefore, desirable to find out if latent nucleases were present in the basic proteins of nuclei and ribosomes, and, if they were, to find whether their conditions for optimum activity were similar or dissimilar (cf. Leslie, 1961). The basic proteins extracted from liver ribosomes and nuclei both contain fractions that can be eluted from carboxymethylcellulose columns with 0.3M- or 0.4M-sodium chloride and 0.02N-hydrochloric acid, but the dissimilarity of their electrophoresis patterns on starch gel indicates important differences in their composition. Whether these differences result from the absence of protein corresponding to the nuclear F2 fraction in ribosomes or whether they indicate two distinct populations of protein is not known.

The procedure for localizing RNA depolymerase on starch gel showed three areas of activity with nuclear histone and only one broad band of activity with ribosomal basic proteins. In view of the multiple banding often shown by an enzyme when subjected to electrophoresis on starch gel (cf. Sur, Moss & King, 1962) it cannot be concluded that the results are evidence for the existence of different nucleases in ribosomal and nuclear proteins. The most significant feature is that nuclease activity is not associated with the slowest-migrating band on starch gel which corresponds to the arginine-rich form of histone protein. This is consistent with the observation that fraction F3,

Fig. 4. Effect of pH on the nuclease activity of basic proteins from liver nuclei and ribosomes. ●, Percentage of RNA degraded by nuclear proteins; ○, percentage of RNA degraded by ribosomal proteins. Nuclear fractions F1 (1 μ g.), F2 (10 μ g.) and F3 (10 μ g.), and ribosomal fractions R1 (0.2 μ g.) and R2 (1 μ g.), were incubated for 1 hr. at 37° with RNA equivalent to 30 μ g. of RNA phosphorus. The final concentrations in the digests were 0.03M-buffer and 0.15M-NaCl. The buffers used were acetic acid-NaOH (pH 4.4–5.3), sodium cacodylate-HCl (pH 5.1–7.0) and sodium borate-boric acid (pH 7.2–8.5). The assays were performed in duplicate pH at intervals of 0.2.

prepared by carboxymethylcellulose chromatography, is the least active under assay conditions.

It is significant that the highest percentages of RNA degradation occurred with the nuclear and ribosomal fractions eluted from carboxymethylcellulose with 0.3M- or 0.4M-sodium chloride. Under the conditions of ribonuclease assay the enzyme activity is not entirely latent when incubated in the presence of buffer alone. The stimulation observed on the addition of 0.15M-sodium chloride to the digests is also a feature of ribonuclease of the pancreatic type (Kalnitsky *et al.* 1959). It is possible that activation of nuclease from a state of complete latency can only be observed when increased salt concentration, EDTA or 4M-urea is used to release the enzyme from the ribosomal particles (cf. Elson, 1958). These agents will also alter the secondary structure of RNA and basic proteins, and such alterations may contribute to the observed increases in depolymerase activity.

The existence of peaks of nuclease activity at pH 5.3–5.5, 6.3–6.5 and 7.2 for nuclear preparations F1, F2 and F3, taken in conjunction with the three or four bands of activity detected on starch gel, might be considered to indicate the presence of more than one type of enzyme. Likewise, there are two major peaks of activity at pH 6.4 and 6.8 for ribosomal fractions R1 and R2, which may be associated with the single broad band obtained in the starch-gel test. However, it cannot be excluded that different forms of histone present in the digests interact with a single specific nuclease or with the substrate and produce peaks of activity within the pH range investigated. The origin of the enzyme or enzymes in the preparations of basic proteins is not settled and investigations on the nature of products of RNA degradation are continuing.

Although most of the experiments in the present investigation were done with basic proteins precipitated from 0.5N-sulphuric acid at 50% (v/v) ethanol concentration, it was found that nearly 10% of the total histone from liver nuclei remained in solution in the ethanol-acid supernatant. When this histone was isolated and subjected to carboxymethylcellulose chromatography, it separated into three fractions (Table 1), the most abundant of which was F3. Comparison of the yields of the ethanol-acid-soluble histones (F1, F2 and F3) with the corresponding fractions of the insoluble portion shows that they contained relatively more F1 protein (over one-third of the total).

In these circumstances, it seems that proteins representative of the total nuclear histones may be soluble in ethanol-acid. However, Johns *et al.* (1960) report that only the arginine-rich and part of the moderately lysine-rich histones of calf thymus are soluble in 70–80% (v/v) ethanol–0.25N-

hydrochloric acid. It is also possible that protease action has promoted the solubility of histone in ethanol-acid in the present experiments, as Dounce & Umana (1962) find that no protein of this type is produced if proteolysis is suppressed during the isolation of histones from nuclei.

SUMMARY

1. Basic proteins extracted from guinea-pig-liver nuclei have been separated by chromatography on carboxymethylcellulose columns into three fractions (F1, F2 and F3). The ribosomal basic proteins, when subjected to the same procedure, separate into only two fractions (R1 and R2), which correspond in position to F1 and F3 respectively.

2. Different electrophoretic patterns are obtained with the basic proteins of nuclei and ribosomes when they are separated on starch gel.

3. The depolymerase activity towards ribonucleic acid of protein fractions obtained from carboxymethylcellulose columns has been investigated. The most active depolymerization is associated with F1 and R1 fractions. The activity in fractions F1, R1 and R2 is stimulated by incubation in the presence of either 0.15M-sodium chloride, 0.01M-ethylenediaminetetra-acetate, or 4M-urea, and is inhibited by 0.1M-magnesium chloride.

4. Variation in depolymerase activity over the pH range 5–8.5 in the presence of 0.15M-sodium chloride was also investigated. Three peaks of optimum activity were found with the nuclear histones, and two major peaks with the ribosomal basic proteins.

5. A technique was developed for localizing the depolymerase activity in the bands of protein produced by electrophoresis on starch gel. Three distinct active regions were obtained with the nuclear histones, but only one active region was obtained with ribosomal basic proteins.

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Properties of the Peptide Chains of Normal and Pathological Human γ -Globulins

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Serum γ -globulins have certain common physico-chemical and immunological characteristics, but are heterogeneous according to several criteria. The main component of human γ -globulin has a molecular weight of about 150 000 and is usually referred to as 7s γ -globulin; the heterogeneity of this fraction is shown by its wide range of electrophoretic mobility and diffuse spread on ion-exchange chromatography as well as by the multiplicity of antibodies which it contains. About 5–10% of γ -globulin consists of protein having a molecular weight of about 1 000 000 and referred to as 19s γ -globulin; this fraction can be identified by immunoelectrophoresis when it is usually called β_2 M- or γ_1 M-globulin. Immunoelectrophoresis has also led to the identification of a third type of γ -globulin known as γ_1 A- or β_2 A-globulin, which is mainly of similar molecular size to 7s γ -

globulin, but, like the 19s fraction, has a relatively high carbohydrate content. In multiple myelomatosis and certain related conditions in man, abnormal γ -globulins distinguished by their relative homogeneity are found in the serum; these pathological proteins may belong to any of the three main γ -globulin types.

Methods that have recently been used in the study of antibody structure provide a means for investigating the relationships between the three types of γ -globulin, and the differences between normal and pathological γ -globulins. These methods involve a preliminary splitting of the protein by enzymic digestion (Porter, 1959) or reduction of disulphide bonds (Edelman, 1959). When human 7s γ -globulin is reduced in the presence of 6M-urea about 15 disulphide bonds/molecule are split and the molecular weight falls from about 150 000 to