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ETHANOL PRECIPITATION ANALYSIS OF THYMUS HISTONE

P. BIJVOET

Laboratory for Physiological Chemistry, University of Utrecht (The Netherlands)

Though thymus histone, and occasionally also the histone prepared from other tissues, has been subjected to electrophoresis and ultracentrifugation and various fractions have been obtained from it by preparative procedures, no certainty has been obtained about the number of components bound to deoxyribonucleic acid in the nucleus. In our opinion one of the reasons for this uncertainty is the fact that not all workers in this field have been careful enough to avoid the possibilities of denaturation and aggregation in the course of the isolation of the total histone and during the storage of the product obtained, before the number of components was determined. With the ultimate aim of isolating the total histone from various tissues under the mildest well-controlled conditions, necessary for a comparison of the histone from various sources, we have thoroughly examined two well-known methods of isolation of thymus histone. These methods, described by BUTLER and associates[1], and CRAMPTON, LIPSHITZ AND CHARGAFF[2], respectively, belong to the group of methods that start with the preparation of nucleohistone from the total gland. We preferred to test these methods, and not those that begin with the isolation of the nuclei, since we are not convinced that the isolation of sufficient amounts of nuclei is possible without loss of chromosomal protein.

We have chosen an analytical ethanol precipitation technique at —5° C for the

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characterisation of the products obtained, as we expected that such a method would give a more detailed picture of the composition of the total histone than electrophoresis or ultracentrifugation. This procedure was carried out along similar lines as the salting-out technique of DERRIEN[3]. Compared with the latter it has the advantage that the protein precipitates can be removed by centrifugation instead of filtration. Therefore, it will be possible also to develop a micro-modification suitable for the analysis of the total histone extracted from very small amounts of tissue.

When it seemed desirable to correlate some observations made by the ethanol precipitation method with aggregate formation, use was also made of ultracentrifugation.

EXPERIMENTAL

Procedure of the ethanol precipitation analysis

Taking all the precautions recommended by DERRIEN[3] for preparing the mixtures of salt solution and water used in his salting-out method, mixtures of decreasing volumes of water and increasing volumes of 70% ethanol to a total volume of 8 ml were placed in a series of test tubes. The increment of the successive volumes of 70% ethanol was 0.2 ml. The tubes were then placed in a thermostat filled with an ethanol water mixture at $-5 \pm 0.1°$ C.

5 vol. % of a 3.5 M phosphate solution, pH 6.5, was then added to the histone solution precooled to 0° C. Two hours after the tubes with ethanol-water mixtures had been placed in the thermostat at $-5°$ C, 2 ml samples of the histone-phosphate solution were added. Thus the ethanol concentration increments of the successive final mixtures were 1.4%.

After about 14 hours'* incubation at $-5°$ C the precipitates formed were spun down by centrifuging for 30 minutes at $-5°$ C and 1200 $\times$ g. The supernatants were carefully siphoned off and transferred to a second series of precooled test tubes. After all supernatants had been collected in this way—up to this point all manipulations had been performed in the cold room at about 0° C—they were all placed in water of room temperature in order to raise their temperature to that of the spectrophotometer (Beckman, model E) within 5 minutes. The extinctions at 275 mμ were then immediately read**. (If the temperature of the ethanol-containing supernatants is allowed to rise slowly or the extinction measurements are carried out with some delay after room temperature has been reached, a slight opalescence, which disturbs the exact measurement of the extinction, is often observed, in particular at the higher ethanol concentrations.)

The protein contents of the supernatants can also be determined by means of the Kjeldahl method. However, the destruction of the relatively large amounts of ethanol present requires considerable time and causes in general much trouble. Therefore, when we had to perform Kjeldahl determinations besides extinction measurements, we preferred to remove the supernatants as completely as possible from the sediment. It was found that the latter dissolved easily in ice-cold 0.1 M phosphate solution, pH 6.5, an indication that no denaturation had occurred during the incubation in the presence of ethanol at $-5°$ C. These solutions could then be used without any difficulty for the Kjeldahl determinations. In principle the results obtained in this way are not quite as accurate as those obtained by Kjeldahl determinations in the supernatants, as a consequence of the small amount of supernatant with which a sediment is inbibed. In our hands, however, this error proved to be negligible compared to the sum of the errors inherent in the manipulations of which the whole procedure consists.

In accordance with DERRIEN the results were expressed by means of two types of curves, *viz.* the "direct" ethanol precipitation curve, obtained by plotting the extinction at 275 mμ or the nitrogen content of the supernatants against the ethanol concentration, and the so-called "derived" curve, obtained by plotting the negative value of the increment of the extinction or of the nitrogen content of the supernatants per increment of the ethanol concentration ($-\Delta E/\Delta C$ or $-\Delta N/\Delta C$, respectively) against the ethanol concentration. If the amount of precipitate does not augment upon increasing the ethanol concentration the derived curve descends to the abscissa;

* After 4 hours of incubation equilibrium had not yet been reached; after 14 hours, however, the amount of precipitate formed was not less than after 24 hours. For practical reasons 14 hours was adopted as the time of incubation, without examining if the equilibrium had already been reached somewhere between 4 and 14 hours.

** If the histone solutions had contained nucleic acid or nucleotides this method would have been inadequate for protein determination. They gave, however, a negative Dische reaction, while the light absorption curve had only one small peak, situated at 275 mμ.

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a peak in the derived curve indicates the precipitation of a protein, or of a mixture of proteins with identical solubilities, in the corresponding region of ethanol concentrations. Thus, when we are speaking about "components" of the total histone, indicated by peaks in the derived curves, this does not imply that we believe that we have proved them to be homogeneous proteins.

Results of the ethanol precipitation analysis

According to the method of BUTLER *et al.*[1] fresh calf thymus is homogenized in 0.14*M* NaCl in the Waring blendor and centrifuged. The sediment is washed with 0.14*M* NaCl. In the sediment then obtained two layers can be distinguished, a fibrous lower layer and a creamy upper layer which contains the nucleohistone. This layer is repeatedly washed with 0.14*M* NaCl until the supernatant is quite clear. Then the nucleohistone is dissociated in a medium containing 0.2*N* HCl by grinding the acidified creamy mass in a ball-mill for at least one hour. The nucleic acid remains precipitated while the histone dissolves. The histone solution is dialysed against distilled water and concentrated by freeze-drying.

From the beginning of our experiments we replaced the freeze-drying by ultrafiltration. Later on we obtained histone solutions of sufficiently high concentration without ultrafiltration.

According to the method of CRAMPTON *et al.*[2] fresh calf thymus is homogenized in a solution containing 0.1*M* NaCl and 0.05*M* Na citrate (pH 7.0). The sediment obtained by centrifugation is first washed four times with this solution and then three times with water, and brought to pH 7.0 by adding a little Na bicarbonate. During these final washings the sediment gradually swells to a gelatinous mass of nucleohistone, which after stirring in the Waring blendor with water (pH 7.0) and spinning down, is washed with 0.15*M* NaCl and finally dissolved in water (pH 7.0). The solution is then brought to 2.6*M* NaCl concentration. The nucleic acid dissociated from the histone is precipitated after half an hour by adding two volumes of 95% ethanol at about 0° C. The alcoholic histone solution is concentrated by vacuum destillation below 40° C and dialysed against distilled water.

The products obtained by both methods did not differ essentially from each other when subjected to ethanol precipitation analysis (Fig. 1). In this connection it should be pointed out that we dialysed the histone solution, prepared according to BUTLER

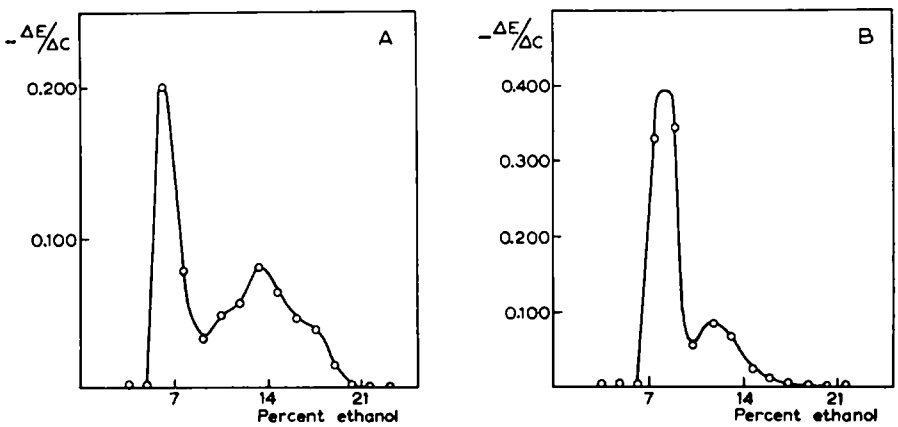

Fig. 1. Derived ethanol precipitation curves at pH 6.5. A: histone prepared according to BUTLER *et al.*, B: histone prepared according to CRAMPTON *et al.*

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et al., against 0.035 *M* phosphate buffer, pH 6.5, instead of water. As will become clear from the results described below, it is also important to remark that the analysis was carried out immediately after the products had been dialysed.

The weak points in the BUTLER *et al.* method are the prolonged treatment with HCl in the ball-mill and the prolonged dialysis, the weak points in the CRAMPTON *et al.* method the prolonged contact of the histone with concentrated NaCl solution (possibility of aggregate formation!), and the destillation at temperatures up to 40° C in order to remove the ethanol. As the dissociation of the nucleohistone by concentrated NaCl is essential in the latter procedure it would be difficult to find means of preventing aggregate formation. We have therefore given special attention to the BUTLER *et al.* method in view of the possibility of changing the ethanol fractionation curve by introducing alterations.

1. The prolonged treatment of the nucleohistone with hydrochloric acid in the ball-mill was replaced by a few minutes' gentle stirring by means of a common stirring motor. The dissociation of the nucleohistone appeared to be practically complete in a few minutes. When 1 volume 0.8*N* HCl was added to a mixture of 1 volume of "creamy layer" (nucleohistone) and 1 volume of water, practically no more histone could be extracted by a second treatment with HCl (Table I). Hence this very simple method was adopted for the subsequent experiments.

TABLE I

NITROGEN CONTENT OF EXTRACTS OBTAINED WITH VARIOUS HCl CONCENTRATIONS

1st extraction: 1 vol. "creamy layer" + 1 vol. H_2O + 1 vol. hydrochloric acid. 2nd extraction: sediment after 1st extraction + 2 vol. hydrochloric acid.

1st extraction			*2nd extraction*		
HCl added	*pH*	*mg N/ml extr.*	*HCl added*	*pH*	*mg N ml extr.*
0.2 *N*	1.6	1.06	0.1 *N*	1.0	0.76
0.4 *N*	1.0	1.75	0.2 *N*	0.8	0.39
0.8 *N*	0.6	2.14	0.4 *N*	0.6	0.18
1.6 *N*	0.4	2.33	0.8 *N*	0.2	0.17

2. The dialysis was omitted and replaced by neutralisation to pH 6.5.

If the histone was prepared under these modified conditions and the ethanol precipitation analysis carried out immediately after neutralisation, in order to avoid alterations of the histone during storage, the derived curve shown in Fig. 2 was obtained. The difference with Fig. 1 is striking. While the derived curves in Fig. 1 have two peaks, a sharp one at 7–8% ethanol and a broad one at 12–13% ethanol, the derived curve represented in Fig. 2 has only one sharp peak at 13% ethanol. If the histone solution of pH 6.5 was, however, stored for a few days at 0° C a second peak at 8% ethanol concentration appeared, the curve (Fig. 3) showing some resemblance to the curves of Fig. 1. The CRAMPTON *et al.* product, to which Fig. 1B refers, had been analysed immediately after preparation. It is therefore evident that an alteration of the histone is brought about during dialysis at pH 6.5 or by storage of the BUTLER *et al.* product at pH 6.5, an alteration which also occurs in the course of the CRAMPTON *et al.* method.

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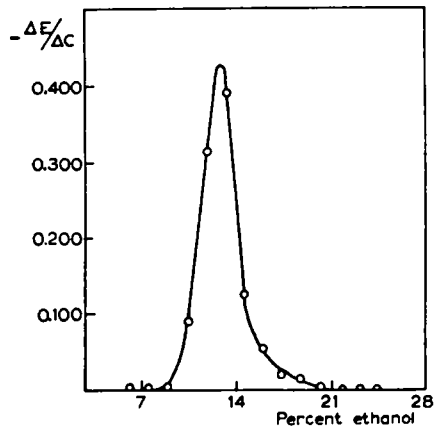

Fig. 2. Derived ethanol precipitation curve at pH 6.5 of histone, prepared according to the modified BUTLER *et al.* procedure and analysed immediately after neutralisation of the acid solution.

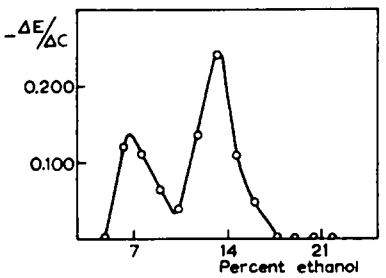

Fig. 3. Derived ethanol precipitation curve at pH 6.5 of histone, prepared according to the modified BUTLER *et al.* procedure and analysed after 5 days' storage at 0° C of the neutralized solution.

In Fig. 4 the curves A and B are the direct ethanol precipitation curves, representing the protein contents of the supernatants determined by measurement of the extinction at 275 mμ and by nitrogen determination according to Kjeldahl, respectively, of the product before storage at pH 6.5 (Fig. 2 shows the curve derived from curve A in Fig. 4). When the component I, represented by the peak at 13% ethanol in Fig. 2, has been completely precipitated (at about 17% ethanol) these curves have not yet reached the basis lines (the broken horizontal line is the basis line of the extinction measurements, *viz.* the line representing the extinctions of the phosphate–water–ethanol mixtures; the abscissa is the basis line for the Kjeldahl determinations). Obviously at 17% ethanol concentration a considerable amount of nitrogen (about 20%) is still in solution, corresponding to an extinction of about 12% of the extinction observed before precipitation of component I. Moreover, it can be seen that with still higher ethanol concentrations the extinction of the supernatants does not decrease, while their nitrogen content diminishes only very slowly. Indeed at about 56% ethanol concentration 8–10% of the original nitrogen had still remained in solution. It was moreover impossible to precipitate the nitrogen present in the supernatant at 20% ethanol completely by adding an equal volume of 10% trichloroacetic acid solution; all nitrogen was however precipitated by the Folin-Denis Reagent (phospho-molybdo-tungstic acid). From these observations we concluded that a second component occurs in the freshly prepared histone, *viz.* a protein of very low molecular weight (component II).

A further peculiarity is that in the region of component I, with the chosen dimensions of abscissa and ordinate, the extinction curve falls more steeply than the nitrogen curve, whereas in the region of component II the extinction line parallel to the abscissa is situated below the nitrogen line. This means that component II has a lower content of aromatic amino acids than component I. The whole behaviour of component II in ethanol precipitation agrees with the presumption that this component is a protein of very low molecular weight.

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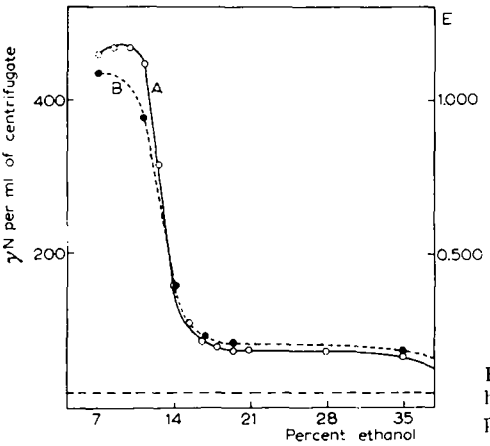

Fig. 4. Direct ethanol precipitation curves at pH 6.5 of histone, prepared according to the modified BUTLER *et al.* procedure and analysed immediately after neutralisation of the acid solution. Curve A: extinction measurements (the curve of Fig. 2 is derived from this curve). Curve B: Kjeldahl determinations. The broken horizontal line is the basis line of the extinction measurements.

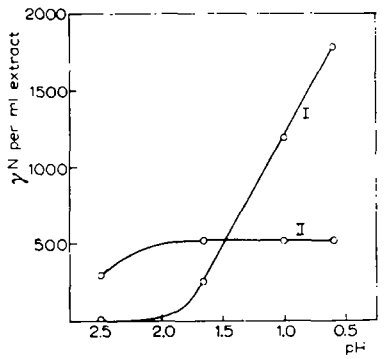

Fig. 5. Liberation of histones from nucleohistone at various pH's. Curve I: Histone component I. Curve II: Histone component II.

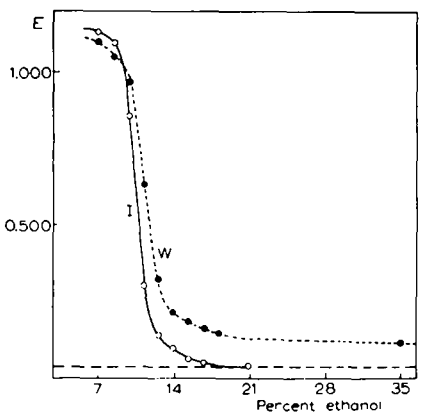

Fig. 6. Direct ethanol precipitation curves at pH 6.5 of histone component I (curve I) and whole histone (curve W).

As shown by Table I the total histone is practically completely extracted from the nucleohistone by adding 1 volume water and 1 volume 0.8 *N* HCl to 1 volume of the repeatedly washed mass of nucleohistone. An experiment in which various amounts of HCl were added, and the amounts of Comp. I and Comp. II in the extracts were determined by precipitation of component I at 20% ethanol, showed that component II is almost completely dissociated from the nucleic acid at pH 2, an H-ion concentration at which component I is just beginning to be liberated (see Fig. 5). These observations which rule out the possibility that component II is an artefact produced by the action of a relatively strong acid solution on a postulated complex of components I and II, might indicate a method for preparing components I and II on a larger scale. We preferred, however, to prepare greater amounts of both components by first extracting the total histone and then precipitating component I by adding 20% ethanol, as it might be expected that in this way purer fractions would be obtained.

The first attempts in which 95% ethanol, precooled to —5° C, was slowly added to the precooled histone solution of pH 6.5 to a final ethanol concentration of 20%, failed, chiefly as a consequence of the relatively high salt concentration of the neutralized histone solution, which caused incomplete precipitation of component I at 20% ethanol concentration. Moreover, upon ethanol precipitation analysis of the

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precipitate obtained this appeared to contain a component giving a peak in the derived curve at 8% ethanol, which points to alteration of component I.

For preparative purposes we were thus compelled to work along lines similar to those of the ethanol precipitation analysis*. First the ethanol–water mixture was made and precooled to —5° C and then the cooled histone solution added. Both fractions were then separated by centrifugation.

The sediment (component I) could easily be redissolved in ice-cold phosphate buffer, pH 6.5. Upon ethanol fractionation analysis of the solution obtained the derived curve was identical with the curve reproduced in Fig. 2, having only one sharp peak at 13% ethanol, while the direct curve descended to the base line (Fig. 6), thus indicating that component II was absent.

The supernatant (containing component II) was freeze-dried. The dry mass was dissolved in water and dialysed against water for 24 hours at 0° C in cellophane tubing manufactured by Kalle and Co, Wiesbaden (Germany). No nitrogen loss was observed.

Comparison of ethanol precipitation analysis and ultracentrifugation

In this section the question is studied, whether the peak situated at about 7% ethanol in the derived ethanol precipitation curves, reproduced in Figs. 1A, 1B and 3, represents the formation of aggregates from one or both histone components, designated by I and II in these curves. Therefore whole histone, prepared according to the modified BUTLER *et al.* method, and both components, prepared as described above, were studied (either fresh or dialysed or stored for various times) in the ultracentrifuge.

As can be seen from Fig. 7A (example) and Table II every whole histone solution of pH 6.5 centrifuged for about 3 hours at 56,100 r.p.m. in the Spinco type E ultra-

TABLE II

SEDIMENTATION CONSTANTS AFTER DIALYSIS OR STANDING AT 0° C AND pH 6.5

Runs of about 3 h at 56,100 r.p.m. and pH 6.5.

Material	*Pretreatment*	S_{20}		% β	*Polydispersity of β*	*Mean temp. of rotor °C*
		α	β			
Whole extract	examined after standing 2 h (salt content: about 0.2 *M* NaCl; 0.035 *M* phosphate buffer, pH 6.5)	1.7 ± 0.1	8 ± 0.5	ca. 5	±	20–25
Whole extract	the same for 40 h	1.3 ± 0.1	3 ± 0.5	ca. 40	++	9–14
Whole extract	dialysed against 0.035 *M* phosphate buffer, pH 6.5, for 18 h	1.7 ± 0.1	5 ± 0.5	ca. 20	+	11–17
Whole extract	the same for 40 h	1.6 ± 0.1	5 ± 0.5	ca. 20	++	5–11
Comp. I	examined 5 h after adding 0.035 *M* phosphate buffer, pH 6.5, to the ethanol-precipitated comp. I	2.3 ± 0.1	4 ± 0.5	ca. 20	--	14–17
Comp. II	examined after 24 h dialysis of the freeze-dried comp. II against 0.035 *M* phosphate buffer, pH 6.5	1.0 ± 0.1	—	--	—	11–17

* Same ratio, *viz.* 8:2, of vol. of ethanol–water and histone soln. as in the analytical procedure.

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centrifuge, seems to contain two components, which we designate as α and β. For component α (presumably ethanol precipitation component I) sedimentation constants S_{20} were calculated varying from 1.3 to 1.7, while for component β (presumably aggregate) S_{20} varied from 3 to 8. If the ultracentrifugation was started after only 2 hours' storage at 0° C, though in this experiment the temperature of the rotor increased from 20 to about 25° C, only 5% of the whole histone was present as component β, while this percentage increased to 20 or 40 after prolonged dialysis or storage. As is further shown by Fig. 7A, component α had not become detached from the meniscus. It therefore seemed probable that after 3 hours of ultracentrifugation a histone of very low molecular weight was still present in component α, probably corresponding to component II observed in the ethanol precipitation analysis.

This was confirmed by an experiment with component I prepared by ethanol fractionation, which appeared to be completely detached from the meniscus after about only two hours (see Fig. 7B). This time component α had the rather higher S_{20} value of 2.3, probably because of the simultaneous absence of the low molecular substance and presence of the high molecular component β, which had a sedimentation constant of 4.

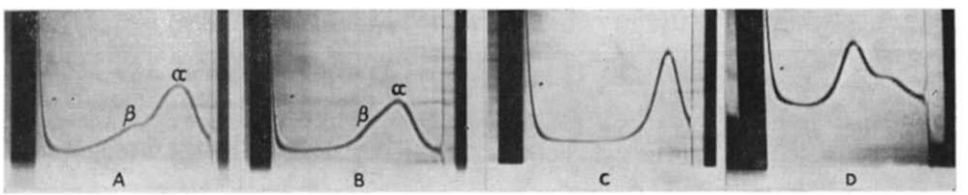

Fig. 7. Ultracentrifuge patterns. 56,100 r.p.m. A: whole histone, pH 6.5, 172 min; B: component I, pH 6.5, 122 min; C: component II, pH 6.5, 170 min; D: whole histone, pH 4.0, 282 min.

When the isolated component II was examined it did not become detached from the meniscus for about three hours. An S_{20} value of 1.0 ± 0.1 was calculated. Component β appeared to be absent (see Fig. 7C).

The great variability of the sedimentation constants calculated for component β and the broad shape of the peaks representing it, indicate pronounced heterogeneity of this component. Altogether it seems probable that the occurrence of the peaks β demonstrates the formation of aggregates during dialysis or storage and even during ultracentrifugation, when a low temperature is not maintained.

As it is known that aggregate formation can be avoided by working at lower pH we next performed ultracentrifuge experiments at pH 4.0 with solutions of whole histone and of component I, separated by ethanol fractionation, without exposing these solutions to the influence of pH 6.5.

Patterns of total histone solutions, dialysed or stored at pH 4.0, showed two partly overlapping boundaries. The solutions had to be centrifuged for 5 hours in order to separate these boundaries sufficiently for the determination of the sedimentation constants (see Fig. 7D). The fastest of these two components had an S_{20} of 1.5 ± 0.1 (probably identical with ethanol precipitation component I), the second (probably identical with ethanol precipitation component II) one of 0.9 ± 0.1 (see Table III). Because of the overlapping of the two boundaries the second component was difficult to measure, while the sedimentation constant of the first component was depressed

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by the presence of the second. Indeed when the first component was run seperately at pH 4.0 it appeared to be homogeneous with $S_{20} = 1.7 \pm 0.1$ (see Table III).

It appears, therefore, that peak a, observed in the ultracentrifuge at pH 6.5, represents the ethanol precipitation components I and II together, while the variation of the sedimentation constants calculated from this peak is caused by the varying amounts of component I removed from the mixture by aggregate formation.

TABLE III

SEDIMENTATION CONSTANTS AFTER DIALYSIS OR STANDING AT 0° C AND pH 4.0

Runs of about 5 h at 56,100 r.p.m. and pH 4.0.

Material	*Pretreatment*	S_{20} *comp. I*	S_{20} *comp. II*	*Mean temp. of rotor °C*
Whole extract	dialysed against MILLER AND GOLDER buffer[4], pH 4.0, $\mu = 0.2$, for 18 h	1.5 ± 0.1	0.8 ± 0.1	5–12
Whole extract	after standing 40 hours (salt-content: about 0.2 M Na citrate, pH 4.0)	1.5 ± 0.1	0.9 ± 0.1	9–36
Comp. I	about 10 h after adding MILLER AND GOLDER buffer, pH 4.0, $\mu = 0.2$, to the ethanol-precipitated comp. I	1.7 ± 0.1	—	8–12

In neither of the diagrams made at pH 4.0 was a trace of a component with S_{20} between 3 and 8 observed. Thus aggregate formation was indeed prevented under these conditions.

In order to try to correlate these findings with ethanol precipitation curves, ethanol precipitation analysis was carried out at pH 6.5 with solutions of whole histone, stored for 5 days at 0° C and pH 0.6, 4.0, and 6.5 respectively. The derived curves are shown in Fig. 8. Indeed only after storage at pH 6.5 does the peak at about 7% appear to be present. We thus come to the final conclusion that the protein precipitated at about 7% ethanol consists of aggregates formed from histone component I. The formation of aggregate can hardly be prevented in ultracentrifugation at pH 6.5.

That component II is not concerned with aggregate formation is supported by the observation that upon ultracentrifugation of this component at pH 6.5 no aggregate formation was observed (see Fig. 7C).

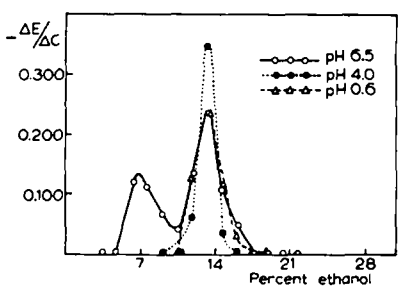

Fig. 8. Derived ethanol precipitation curves at pH 6.5 of component I, stored for 5 days at 0° C and pH 0.6, 4.0 and 6.5, respectively.

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DISCUSSION

The results of our ultracentrifuge experiments are in complete concordance with the results of several other authors[1,2,5,6], who reported that the histones have a marked tendency to aggregate, especially when stored at room temperature or higher temperatures or at pH's above the neutral point. In particular they confirm the work of UI[5], which was published in a preliminary note after our work on the ethanol-precipitation analysis had been completed. This author also separated two components by ethanol fractionation below 0° C. He obtained an arginine-rich fraction I of mol. weight 37,000, which precipitated at 20% ethanol and showed a pronounced tendency to aggregate, and a lysine-rich component II of molecular weight 8,400, which did not precipitate at 20% ethanol and did not show aggregation. Indeed our component I appeared to contain 12% arginine and 12% lysine, and our component II about 2% arginine and 30% lysine. Hence our fractions appear to be identical with those obtained by UI.

The method of BUTLER *et al.* is suitable for the preparation of non-aggregated histone when prolonged dialysis up to neutral reaction is avoided. It may, however, be considerably simplified by replacing the treatment with HCl in the ball-mill by a few minutes stirring with a glass rod. Aggregate formation cannot be prevented in the method of CRAMPTON *et al.* Therefore, in order to obtain a well-defined product the method of BUTLER *et al.* is to be preferred to the method of CRAMPTON *et al.* It is not permissible to conclude that the non-aggregated component I occurs as such in thymus. The question whether the non-aggregated or the aggregated form is a natural constituent of the nucleus, is pointless as the natural conditions of the histones cease to exist as soon as they are dissociated from the deoxyribonucleic acid.

As we have already remarked, we did not believe that we would be able to prove by our experiments that the fractions, designated as component I and component II, are homogeneous. We wish to stress, however, that neither we nor UI[5] have obtained evidence to the contrary. Many different sedimentation constants of thymus histones have been reported in the literature (see Table IV). In our opinion all values above 2.0 correspond to aggregates of component I. In the experiments of MURRAY LUCK, COOK AND ELDREDGE[8] and of BAKAY, KOLB AND TOENNIES[9] components I and II do not appear to have been separated in the ultracentrifuge, so that they have missed the component with a sedimentation constant of about 1. All observations reported support the view that the histone extracted from the glands consists of two well-defined components, provided aggregation is prevented. MURRAY LUCK *et al.*[10] eluted

TABLE IV

SEDIMENTATION CONSTANTS

Authors	*Group 0.7–1.0*	*Group 1.6–2.0*	*Group 8.4–30 (aggregate)*	*% aggregate*
AHLSTRÖM	0.9	—	8.7	40
LUCK *et al.*	·	1.7	30	40
BUTLER *et al.*	1.0	2.0	10	0–10
BAKAY *et al.*	–	1.6	8.4	60
UI	0.7	2.0	·	·
Own expts.	0.9 ± 0.1	1.7 ± 0.1	·	·

References p. 512.

many fractions from histone on an Amberlite I.R.C. 50 column by increasing concentrations of guanidine hydrochloride. As long as no rechromatography of the fractions obtained has been carried out we are not convinced that they are not artefacts.

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SUMMARY

An analytical ethanol precipitation technique, similar to DERRIEN's salting-out procedure, was used for the characterisation of whole thymus histone and the products obtained by preparative ethanol fractionation. The analysis was carried out at $-5°$ C and pH 6.5. Whole histone prepared according to CRAMPTON *et al.*, and to BUTLER *et al.* (the product prepared according to the latter having been dialysed against phosphate, pH 6.5) appeared not to differ essentially; it contained among other substances a component precipitating at about 7% ethanol. If dialysis or storage at pH values approaching the neutral point were avoided in the BUTLER *et al.* method, this component was no longer observed. By means of ultracentrifugation it was proved to be an aggregate, the formation of which cannot be prevented in the CRAMPTON *et al.* method.

The BUTLER *et al.* method was simplified and the HCl concentration determined at which the histone is completely split off from the nucleohistone. Ethanol precipitation analysis revealed the presence of two components in the whole histone prepared in this way, if the above-mentioned measures are taken to prevent aggregate formation. Component I, representing 80% of the whole histone was precipitated between 13 and 17% ethanol; component II, representing 20% of the whole histone, never completely precipitated upon increasing the ethanol concentration. The latter component was dissociated from the nucleohistone at much lower acidity than the former.

Component I ($S_{20} = 1.7 \pm 0.1$) appeared to be arginine-rich, Component II ($S_{20} = 0.9 \pm 0.1$) lysine-rich. Ethanol fractionation is recommended for preparing the arginine-rich and lysine-rich histone fractions.

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