THE AMINO ACID COMPOSITION AND SOME PROPERTIES OF HISTONES

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In this paper the properties and the amino acid compositions of a group of histones and of a protamine-like material are described. Histones have been prepared from a variety of sources, and are now believed to occur in the nuclei of the somatic cells of most organisms. We have studied the amino acid compositions of histones prepared from calf liver, calf thymus, and fowl erythrocytes. The results have a bearing on the problem of whether histones prepared from different tissues of the same animal or from different animals vary in their amino acid compositions.

Protamines have been found in the nuclei of the sperm of many fish, but it is not known whether they occur in other tissues of these fish or in the sperm of other higher animals. We have prepared a protamine-like material from the sperm of the fowl. Its preparation, properties, and amino acid composition are described in detail. The preparation of the fowl erythrocyte histone and the protamine of fowl sperm makes it possible to compare the basic proteins of nuclei in the somatic and sperm cells of the same animal.

As histones are among the principal constituents of chromosomes, efforts have been made to determine their role in the structural organization of the chromosome. The action of proteolytic enzymes has sometimes been employed for this purpose (1, 2). The interpretation of such experiments depends on some knowledge of the action of the enzyme employed on the individual components of the chromosome. In order to investigate the validity of the conclusions drawn from such experiments, we have studied the action of pepsin on histones both when isolated and when in the chromosome. Studies of the specificity of proteolytic enzymes have emphasized the importance of the nature of the amino acids adjacent to the sensitive peptide bond (3). Therefore one of the important factors determining whether a protein will be digested by pepsin is the amino acid composition of the protein. We have found that pepsin does digest histones, and that its action is not surprising in view of the amino acid compositions of the histones.

Protamines were first isolated by Miescher from salmon sperm (4). They

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were found to be in salt-like combination with nucleic acid. Later work revealed the occurrence of protamines in the sperm of many species of fish. Histones were discovered somewhat later by Kossel who isolated the material from goose erythrocyte nuclei (5). Histones were also found in combination with nucleic acid and thus presented an analogy to the protamines. Compounds of nucleic acid with protamine or histone have been isolated from many tissues, but, as Kossel stated, "It is still unknown whether a compound of nucleic acid and histone is preformed in the thymus gland" (page 73). His statement applies equally well to the state of these compounds in other tissues. Kossel regarded the distribution of histones as essentially restricted, but recent work has shown that they can be isolated from a variety of sources, and it appears likely that either histones or protamines are constituents of the nuclei of most organisms (6).

Kossel observed that histones are precipitated by ammonia and are insoluble in an excess of the reagent (5). This indicates that they have basic isoelectric points; those which have been measured lie between pH 10 and 11 (6). In addition to their basic properties all the histones which have been prepared so far have several other properties in common by which they may be distinguished from other proteins: their nitrogen contents are about 18 per cent; histone molecules are large enough to be retained by a cellophane membrane; they contain only traces of tryptophane; they are soluble in a hot or cold solution of 0.34 M $HgSO_4$ in 1.88 M H_2SO_4 (6). This last property provides a convenient method for the release of histone and no other protein from nucleoproteins.

The protamines differ from the histones in the following respects: they are not precipitated by ammonia; they have a higher nitrogen content than the histones; they will pass through a cellophane membrane; finally it will now be shown that they are soluble in the hot $HgSO_4$–H_2SO_4 reagent and insoluble in the cold.

The question arises as to whether individual histones and protamines differ from each other with respect to their amino acid compositions. The amino acid composition of protamines isolated from the sperm of various species of fish was studied by Kossel and others, and their diversity of composition was soon recognized (5). They contain a smaller variety of amino acids than the typical proteins, and vary in composition with respect to the kinds of amino acids present as well as their distribution (7). The basic amino acids account for most of the nitrogen of the protamines. The histones differ from the protamines in that they contain most of the commonly occurring amino acids. Values for the basic amino acids have been reported for several histones; for thymus histone values for additional amino acids have been reported (5). With the possible exception of the basic amino acids many of these values are to be regarded as minimal on account of the limitations of the techniques employed. Among the basic amino acids there were significant variations in composition, some of which may have been the result of incomplete purification.

By means of the recently developed chromatographic procedure of Moore and Stein (8–10), it is possible to carry out a complete analysis of the amino acids commonly occurring in protein hydrolysates. We have studied the composition of acid hydrolysates of histones prepared from different tissues of the same animal—calf thymus and calf liver, and from a different animal—fowl erythrocytes. We have also studied the composition of a protein obtained from fowl sperm which is analogous to the histone of the erythrocyte and is similar in many respects to the protamines isolated from fish sperm.

Preparation.—The histones were prepared by modifications of methods which have already been described (6). All the isolation procedures were carried out in a cold room at 2°C.

Thymus Histone I.—This preparation was made from isolated thymus nuclei (6). A suspension of nuclei was extracted with 0.2 N HCl. The suspension was centrifuged, and the supernatant was dialyzed overnight against distilled water. Histone was precipitated by the addition of NaOH until maximum precipitation was reached (pH approximately 10). After thorough washing with alcohol and ether, the histone was dried at room temperature *in vacuo*.

Thymus Histone II.—This preparation was made by a somewhat different procedure in which a weaker acid was used for the extraction. Isolated thymus chromosomes (11) were extracted with 1 M NaCl containing 1 per cent citric acid. Not all the histone is released from thymus chromosomes by this procedure. The suspension was centrifuged, and a clear supernatant was obtained. Its absorption spectrum indicated the absence of nucleic acid. The solution was dialyzed against distilled water overnight, and the histone was then precipitated by the addition of NaOH until maximum precipitation was reached (pH 9.2). It was washed with alcohol and ether and dried at room temperature *in vacuo*.

Calf Liver Histone.—This histone was prepared by a procedure similar to that employed for the preparation of thymus histone II. Isolated calf liver chromosomes (11) were treated with 1 M NaCl containing 1 per cent citric acid. All the histone is released from calf liver chromosomes by this procedure, but in subsequent steps much of it is lost. The suspension was stirred gently for 1 hour and then centrifuged. The supernatant was filtered. The absorption spectrum of this solution indicated the presence of a small amount of nucleic acid. The solution was dialyzed overnight against distilled water. After dialysis the solution was centrifuged, as it had become slightly turbid. The absorption spectrum of the clear supernatant indicated the absence of nucleic acid, and a Millon test produced a clear red solution indicating the absence of nonhistone protein. Histone was precipitated from the solution by the addition of 1 N NaOH until maximum precipitation was reached (pH approximately 9.5). After standing for 1 or 2 hours the suspension was centrifuged. The supernatant was tested for completeness of precipitation by the addition of NaOH; no precipitate was formed. The histone was washed thoroughly with alcohol and ether and then dried at room temperature *in vacuo*.

Fowl Erythrocyte Histone.—Chromatin threads obtained from fowl erythrocytes (12) were extracted with 0.1 N HCl. The extract was dialyzed overnight against dis-

tilled water. Histone was precipitated by the addition of NaOH until maximum precipitation was obtained, and then washed with alcohol and ether and dried at room temperature *in vacuo*.

The Basic Protein of Fowl Sperm.—Rooster semen was mixed with 0.14 M NaCl and centrifuged. The sperm cells were washed several times with saline and then with 0.2 per cent citric acid which removes tails and midpieces leaving a suspension of well formed nuclei. The nuclei were extracted with 0.25 N HCl.

To learn what kinds of proteins are extracted with acid, the acid extract was cautiously neutralized by the addition of NaOH. As the pH reached 7, a precipitate formed and was removed by centrifugation. The protein in this precipitate was but a small fraction of the protein extracted. After removal of the precipitate the addition of alkali was continued. When the pH reached 10 to 11, the region in which histones precipitate, no precipitate formed. The presence of a tyrosine-containing protein was demonstrated by adding to a portion of the solution an equal volume of $HgSO_4$–H_2SO_4, warming to 60°, and then adding one-twentieth the volume of 1 per cent $NaNO_2$. A clear red solution was obtained.

The protein can be precipitated by adding 3 volumes of 95 per cent alcohol to the supernatant obtained after removal of the precipitate at pH 7. The precipitate was washed several times with 75 per cent alcohol to remove salt, dissolved in a small volume of water, filtered to remove a small amount of insoluble material, and dried while frozen. After being dried at 106° it was found to contain 24.4 per cent nitrogen. The ultraviolet absorption curve of the material shows a maximum at 2760 Å.u. and indicates clearly that no nucleic acid is present. The molecules of the protein are small enough to pass through a cellophane membrane. The high nitrogen content, non-precipitability by ammonia (or by NaOH at pH 10 to 11), and small molecular size are properties that distinguish this protein from a histone, and these properties together with its origin in a sperm nucleus are all characteristic of a protamine. We propose the name *gallin* for this protein consistent with the naming of other protamines such as salmin, clupein, sturin, etc.

The solubility of gallin differs in a curious way from the solubility of a histone, and, in this respect also, gallin resembles a protamine. Histones, unlike most other proteins, are soluble in $HgSO_4$–H_2SO_4; and when, after heating to 60°, one-twentieth volume of $NaNO_2$ is added to produce the Millon reaction, a red solution is obtained, whereas a red precipitate is obtained for proteins in general. Gallin also gives a red solution, but when the solution is cooled to 0°, the red gallin derivative precipitates, while the histone derivative remains in solution. The insolubility of gallin is made more apparent by formation of the red pigment, but even if no $NaNO_2$ is added, gallin is soluble in the $HgSO_4$–

H_2SO_4 reagent at 60° and insoluble at 0°. When the suspension is warmed to 60° the precipitate redissolves; warming and cooling with attendant dissolving and precipitation can be repeated any number of times.

The quantity of gallin present in the rooster sperm can be estimated by the Millon reaction. The red derivative has an absorption band at 5000 Å.u. and also a band in the ultraviolet at 3540 Å.u. All measurements must be made at 60° to prevent precipitation. The extinction coefficient at 3540 Å.u. of a 1 mg. per ml. solution of gallin is 2.3. For fowl histone it is 1.66, indicating that the tyrosine content of gallin is probably higher than that of the histone. To 2 ml. of a suspension of rooster sperm nuclei containing 4.2 mg. of nuclei was added an equal volume of the $HgSO_4$–H_2SO_4 reagent. The mixture was heated to 60° for 15 minutes, 0.2 ml. of $NaNO_2$ solution was added, and the mixture was kept at 60° for another 10 minutes. It was quickly centrifuged while hot, and the clear red supernatant was decanted. The extinction coefficient at 3540 was 0.620. From this it can be calculated that gallin forms 26.9 per cent of the weight of the nucleus. The experiment also shows that the sperm nucleus contains no histone, for when the red gallin-containing solution is cooled to 0°, no pigment remains in solution. Any histone present would have remained in solution at 0°. In the same way it can be shown that the protein extracted from fowl erythrocyte chromatin with $HgSO_4$–H_2SO_4 contains histone and no gallin, for when the solution is cooled to 0° no precipitate appears.

The presence of a histone in somatic cells of an organism and of a protamine in the sperm cells has also been demonstrated in the salmon (13). Miescher isolated the protamine, salmin, from salmon sperm, but found no salmin in unripe testes (4). From chromatin threads prepared from salmon erythrocytes (12) a protein can be extracted with 0.25 N HCl. This protein has all the characteristics of a typical histone: it is precipitated by ammonia, but insoluble in an excess of this reagent; it will not pass through a cellophane membrane; it is soluble in both hot and cold $HgSO_4$–H_2SO_4 solution.

Amino Acid Composition.—The proteins were hydrolyzed by refluxing with 200 times the sample weight of doubly distilled 6 N HCl for 18 hours (8). The amino acid compositions of the hydrolysates were determined by the procedure of Moore and Stein which consists of the chromatographic separation of the amino acids on starch columns and their quantitative estimation in the effluent fractions by a photometric ninhydrin procedure (8–10). The relative positions of the peaks corresponded to those obtained with a known mixture of amino acids, and no peaks were observed that could not be ascribed to the common amino acids. To confirm the positions assigned to the amino acids, threonine, serine, and histidine were added to the protein hydrolysates, and the designated peaks rose without loss of symmetry (14).

No cystine was observed on the chromatogram of any of these hydrolysates. As sulfur determinations indicated that methionine did not account for all of

the sulfur of the proteins, and nitroprusside-cyanide tests indicated the presence of cystine in the histones, it was thought that cystine might have been destroyed in the course of the hydrolysis. Therefore another hydrolytic procedure was employed for the estimation of this amino acid. The proteins were hydrolyzed with HCl in the presence of urea as recommended by Brand and Kassell (15) and the amounts of cysteine and cystine were determined in the hydrolysate by their photometric procedure (16). These estimations were carried out on the thymus histone and gallin only, as sufficient amounts of the other histones were not available. No appreciable amounts of cysteine were found, but any cysteine in the original protein might have been oxidized to cystine in the course of isolation. The amount of cystine found in gallin was so small that it could not be determined with any degree of accuracy; therefore the results are not reported. The methionine and cystine sulfur estimated by this procedure did not quite add up to the total organic sulfur of the proteins. No further attempts were made to account for all of the sulfur, as this was not considered essential for our comparisons.

In this way fairly complete amino acid compositions of the proteins were obtained. In the case of gallin reliable values could not be obtained for the amino acids leucine, isoleucine, and phenylalanine, as they were present in such small amounts. An approximate value for the percentage of the total nitrogen attributable to these combined amino acids was obtained from a chromatogram in which they emerge as a single peak.

The results are summarized in Table I. The data are subject to a certain amount of uncertainty, as the recoveries do not account for all of the nitrogen in the hydrolysates. The total nitrogen recoveries were: calf thymus histone I—87.2, calf thymus histone II—98.7, calf liver histone—92.7, fowl erythrocyte histone—96.2, and gallin—92.0 per cent. This is doubtless the result of decomposition of various amono acids in the course of the hydrolysis. Tryptophane is known to be almost completely destroyed in acid hydrolysates of proteins, but the tryptophane content of these proteins is extremely small (6). Appreciable amounts of threonine and serine are also decomposed in the course of acid hydrolysis, with the formation of ammonia. The values for these amino acids and for ammonia have been corrected in accordance with the estimates of Rees for the extent of decomposition (17). His estimates are probably applicable here (14). There is evidently some destruction of other amino acids for which we are unable to make corrections. The fact that the nitrogen recoveries are higher than the weight recoveries can probably be accounted for by decomposition of amino acids other than threonine and serine with the formation of ammonia. The appearance of the hydrolysates indicated that some destruction had taken place. The hydrolysate of thymus histone II was nearly colorless, while the other hydrolysates ranged in color from pale yellow to light brown (thymus histone I). The recoveries were nearly quantitative for thymus histone

II. It is well recognized that a single hydrolytic procedure is not satisfactory for the quantitative estimation of all the amino acids in a protein (18). However data of the sort presented here are of considerable value for comparative purposes.

TABLE I

Amino acid	Moles amino acid per 100 moles N					Gm. amino acid residue per 100 gm. protein				
	Calf thymus histone I	Calf thymus histone II	Calf liver histone	Fowl erythrocyte histone	Gallin	Calf thymus histone I	Calf thymus histone II	Calf liver histone	Fowl erythrocyte histone	Gallin
Leucine	5.38	5.11	5.38	5.81		7.77	7.93	7.82	8.68	
Isoleucine	2.80	2.16	2.74	3.68	0.35	4.05	3.35	3.98	5.50	
Phenylalanine	1.53	1.42	1.63	1.63		2.88	2.86	3.08	3.16	
Valine	3.72	2.72	3.64	3.94	0.68	4.72	3.70	4.65	5.16	1.18
Methionine	0.49	0.67	0.47	0.0	0.0	0.82	1.21	0.79	0.0	0.0
Tyrosine	1.31	1.60	1.66	1.62	1.58	2.73	3.58	3.48	3.49	4.49
Proline	2.86	2.51	2.31	2.68	1.94	3.55	3.34	2.89	3.44	3.28
Glutamic acid	5.87	6.18	5.44	5.75	0.45	9.68	10.9	9.02	9.81	1.01
Alanine	7.10	7.60	6.65	7.30	1.22	6.46	7.41	6.09	6.86	1.51
Threonine	3.78	3.85	4.20	3.39	0.72	4.79	5.33	5.45	4.52	1.28
Aspartic acid	3.87	3.82	3.20	3.49	0.28	5.70	6.02	4.73	5.30	0.56
Serine	3.24	3.17	3.05	4.54	3.89	3.61	3.79	3.42	5.23	5.91
Glycine	5.71	6.13	6.02	5.79	2.80	4.17	4.80	4.43	4.37	2.79
Ammonia	4.08	4.97	4.01	5.13	1.17					
Arginine	5.40	7.07	6.60	6.35	19.1	10.8	15.1	13.2	13.1	51.9
Lysine	5.00	7.22	6.23	6.29	0.0	8.19	12.7	10.2	10.6	0.0
Histidine	1.10	1.18	1.16	1.17	0.51	1.90	2.22	2.04	2.06	1.23
Cystine	0.26	0.28				0.68	0.74			
Total recovery	87.2	98.7	92.7	96.2	92.0	82.5	95.0	85.3	91.3	
Nitrogen content, *per cent*	17.9	19.2	18.0	18.5	24.4					

The distribution of amino acids in the different histones is rather similar. The absence of methionine in the fowl erythrocyte histone is the only striking difference. As the recovery of protein nitrogen was not complete it may be argued that methionine was destroyed in the course of the hydrolysis. In any case it would appear that the amount of methionine in the fowl erythrocyte histone is probably much less than in the other histones. Sulfur and cysteine analyses would have aided in providing an answer to the problem; but, as mentioned above, they were not carried out on this histone because of the limited quantities of material.

A comparison of the compositions of the histones of calf thymus and calf liver indicates that there are no great differences in this respect among histones prepared from different tissues of the same animal. Many of the differences may be attributable to losses incurred in the course of the hydrolysis. The absence of methionine in the fowl erythrocyte histone indicates that a greater difference may exist among histones prepared from different organisms. Nevertheless the general distribution of the other amino acids is rather similar to that of the calf histones. In evaluating these comparisons it should be kept in mind that these histone preparations are not single substances but histone fractions.

The basic character of the histones reflects their amino acid compositions. The presence of high concentrations of the basic amino acids, particularly arginine and lysine, was demonstrated by Kossel and other investigators (5). Our values for arginine in the thymus histones and the fowl erythrocyte histone are not very different from theirs. The values which we obtained for lysine are somewhat different from earlier values, but some of the latter were obtained by difference. We find that arginine and lysine are present in nearly equimolecular proportions. In view of the basic nature of histones it may be surprising that they contain considerable amounts of dicarboxylic amino acids. Furthermore the amount of amide nitrogen calculated on a molar basis is much less than the total amount of dicarboxylic amino acids; therefore there must be a number of free carboxyl groups in the histones. The basic character of the histones, therefore, depends primarily on a high concentration of basic amino acids and not on a scarcity of acidic amino acids.

The results have indicated that there may be no great differences in composition among the basic proteins in the somatic nuclei of an organism, but it is apparent that a great difference exists between the basic proteins of sperm and somatic nuclei of the fowl. The amino acid analyses have confirmed what was evident from an examination of other properties—that gallin is an entirely different protein from the erythrocyte histone. Gallin contains all the amino acids found in the histone with the exception of lysine, but in entirely different proportions. The amounts of tyrosine, proline, and serine are not very different from the amounts found in the histone, the amount of arginine is greatly increased, and the amounts of the other amino acids greatly decreased. The decrease in the amounts of these amino acids is not uniform. A uniform decrease would have been expected if gallin had been formed from the histone simply by the removal of a large portion of the peptide chains containing these amino acids leaving an arginine-rich residue. It was suggested by Kossel that protamines might be formed in this manner (5). It should be emphasized, however, that there is no reason to believe that gallin is a single chemical substance; it may consist of a mixture of protamine-like substances.

Histones and protamines are components of chromosomes. The composition of chromosomes in different tissues of the same organism is the same in at least

one respect. It has been shown that the amount of desoxypentose nucleic acid per nucleus is a constant in the somatic cells of an organism, and the amount in the sperm cells is one-half that value (19–21). In the fowl and in the salmon histones are present in the somatic chromosomes, and protamines are found in the sperm chromosomes. This shows that great variations in the composition of chromosomes can exist in a single organism.

The Action of Pepsin on Histones.—The action of proteolytic enzymes on chromosomes has been employed in an attempt to determine the relation of the protein components of the chromosomes to their structure. It has been observed that the action of pepsin on chromosomes brings about a shrinkage but no breakage of their structure. Mazia's interpretation of this observation is based on the assumption that pepsin does not digest histone in the chromosome (1). From a study of the action of pepsin on a series of peptides Fruton and Bergmann concluded that the action of this enzyme on a peptide linkage requires the presence of an aromatic amino acid adjacent to the linkage and is promoted by the proximity of a carboxyl group and inhibited by the proximity of a basic group (3). Mazia concluded from this that pepsin would not be expected to digest many of the linkages in a basic protein such as histone. However an examination of the amino acid composition of histones suggests that pepsin would be expected to digest histones as there are high concentrations of both aromatic amino acids and free carboxyl groups. It is highly possible that these groups might be arranged in such a way as to promote extensive digestion by pepsin. It should also be mentioned that acidic amino acid residues may not be the only requisite for extensive digestion by pepsin. Harington and Pitt Rivers have shown that certain peptides containing tyrosine and cysteine or cystine are readily attacked by pepsin (22). Furthermore the extent of digestion of one of these peptides, which contains cysteine, is comparable to that of the most sensitive substrate employed by Fruton and Bergmann. Histones contain cystine, but the amounts are relatively small. The histones may also contain cysteine in their original state, but the procedures which we have employed for their isolation may permit the oxidation of cysteine to cystine.

Mazia reported that pepsin exerts no observable effect on artificial fibers prepared from histone and nucleohistone. This interesting observation may have no bearing on the problem of whether histones in the chromosome are digested by pepsin. To settle this problem it is necessary to demonstrate directly whether the amount of histone in chromosomes decreases after treatment with pepsin. We have been able to do this, and we find that pepsin does indeed digest a large proportion of the histone in chromosomes.

Kaufmann and his collaborators have accepted Mazia's conclusion that pepsin does not digest histones. Earlier investigators had reported that pepsin digests isolated histones. Kaufmann suggested that their results might be attributable to the use of crude enzyme preparations. We have found that

crystalline pepsin does digest isolated histones. The conclusions drawn by Mazia and Kaufmann *et al.* from their experiments with pepsin are therefore open to question, as they are based on an erroneous assumption. Mazia has claimed that histone is responsible for the continuous structure of chromosomes. We have found that histone may be removed quantitatively from isolated chromosomes without altering their microscopic appearance. These experiments will be described elsewhere.

The Action of Pepsin on Isolated Histone.—The histone used in this experiment was prepared from isolated turtle erythrocyte chromosomes (11). To 60 ml. of a suspension of chromosomes was added 10 ml. of 0.5 N HCl in the cold. The suspension was centrifuged, and the clear supernatant, which gave an intense soluble color with the Millon reagent, was dialyzed for 2 days in the cold against distilled water. The supernatant remained clear, and the histone was precipitated by adding 0.1 N NaOH until maximum precipitation was obtained (pH 9–10). After standing for 2 hours in the cold the suspension was centrifuged. The supernatant gave no precipitate on the addition of more alkali. The precipitate was suspended in water and immediately went into solution when a few drops of 0.5 N HCl were added.

The histone solution contained 0.80 mg. of nitrogen per ml. To 0.5 ml. of the solution were added 0.4 ml. of water and 0.1 ml. of 3.6 M trichloroacetic acid, and a heavy precipitate formed immediately. The material was centrifuged, and a nitrogen determination indicated that the clear supernatant contained practically no nitrogen. Another 0.5 ml. portion of the histone solution was treated with 0.1 mg. of crystalline pepsin (obtained from Dr. Northrop) in the presence of 1 per cent citric acid, pH 2.5, for 1 hour at 24°. The volume of the solution was 0.9 ml. Upon the addition of 0.1 ml. of 3.6 M trichloroacetic acid a slight opalescence appeared. The suspension was centrifuged, and a nitrogen determination on the clear supernatant showed that 93 per cent of the histone nitrogen was now in the trichloroacetic acid supernatant. This indicated that pepsin had digested the histone converting most of it into products no longer precipitable by trichloroacetic acid.

The Action of Pepsin on Thymus Chromosomes.—A suspension of isolated thymus chromosomes (11) was centrifuged, and the precipitate of chromosomes was dried while frozen. The nitrogen content of this preparation was 13.0 per cent, and the phosphorus content was 3.63 per cent. As the nitrogen-phosphorus ratio of purified nucleic acid preparations is 1.7, the nucleic acid nitrogen was calculated to be 6.15 per cent, and the protein nitrogen was therefore 6.85 per cent.

Three hundred mg. of chromosomes was treated with 8 ml. of a solution containing 8.16 mg. of crystalline pepsin in 1 per cent citric acid for 2 hours at 25°. As a control an equal weight of chromosomes was treated with 8 ml. of 1 per cent citric acid only, under the same conditions. The suspensions were centrifuged, the residues were washed with 1 per cent citric acid, and the nitrogen contents of the combined supernatants and washings were measured. To a portion of the control solution pepsin was added in the same concentration as that used to digest the chromosomes, and the nitrogen content of the resulting solution was also determined. The control solution contained very little nitrogen, the solution from the pepsin-treated chromosomes contained 3.94 mg. of nitrogen, and the control plus pepsin 1.06 mg. Therefore 2.88 mg. of nitro-

gen was released from the chromosomes by the action of pepsin. This corresponds to 14 per cent of the protein nitrogen of the chromosomes. Considering the fact that in thymus chromosomes there is far more histone than non-histone protein, it is probable that much of this nitrogen is derived from histone, but this is not certain. A direct estimation of the histone content of chromosomes before and after digestion with pepsin is required.

Since histones and no other proteins in thymus chromosomes are soluble in a cold solution of 0.34 M $HgSO_4$ in 1.88 M H_2SO_4, this reagent can be used to extract histones alone from thymus chromosomes. The relative amounts of histone released can be estimated by the Millon reaction. Accordingly the residues of the chromosomes which had been treated with pepsin and of the control were resuspended and brought to a volume of 3 ml. with water. Three ml. of the $HgSO_4$–H_2SO_4 reagent was added at room temperature, and the suspensions were allowed to stand for 25 minutes at 0°. The suspensions were then centrifuged and the residues were reextraced with 1 ml. of water and 1 ml. of the $HgSO_4$–H_2SO_4 reagent. The total volume of the extracts was 7 ml. They were dialyzed against 0.94 M H_2SO_4 for 18 hours in the cold. In the course of dialysis substances of low molecular weight which might give a positive Millon test are removed while histones are retained. After dialysis the extracts were tested with the Millon reagent: To 1 ml. of extract were added 1 ml. of water and 2 ml. of the $HgSO_4$–H_2SO_4 reagent. The mixture was heated for 15 minutes at 60°, allowed to cool, and 0.2 ml. of 1 per cent $NaNO_2$ was added. The extinction measured at 5000 Å.u. was twice as high for the extract from the control as for the extract from the pepsin-digested chromosomes. This indicated that half of the histone in the chromosomes, as determined by the Millon reagent, had been digested by pepsin. In another experiment the Millon reaction was carried out on the extracts before dialysis, and a similar result was obtained: the pepsin-treated chromosomes contained a little more than half as much histone as the untreated chromosomes.

Another sample of chromosomes was digested with pepsin in the presence of 0.01 N HCl instead of citric acid. In this experiment the extracts were not dialyzed before the Millon reaction was carried out. As before pepsin was found to digest a great deal of the histone in the chromosomes. A control experiment showed that the action of 0.01 N HCl alone under the same conditions would not release histone from the chromosomes.

SUMMARY

Some of the properties and the amino acid compositions of the histones of calf thymus, calf liver, fowl erythrocytes, and of a protamine-like material isolated from rooster sperm were described.

The amino acid compositions of the histones were rather similar except that no methionine was found in the fowl erythrocyte histone.

In the fowl, histones are found in the somatic chromosomes and protamines are found in the sperm chromosomes. This shows that great variations in chromosome composition can exist in an organism.

Histone is digested by pepsin both when isolated and when in the chromosome.

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