CHEMICAL STUDIES ON MAMMALIAN SPERM*

by

R. DUNCAN DALLAM AND LLOYD E. THOMAS

Department of Biochemistry, University of Missouri, Columbia, Mo. (U.S.A.)

The first investigation on the chemical nature of the spermatozoa of mammals was carried out by MIESCHER[1]. He found that bull spermatozoa do not contain protamine or histone, but contain protein of an acid nature which he did not clearly characterize. Similar investigations were conducted by MATHEWS[2] under the direction of KOSSEL. He studied the spermatozoa of the bull and ram and came to the same conclusion as did MIESCHER, *i.e.*, that these cells do not contain protamines nor histones. In 1926 STEUDAL[3] again turned to the problem of the chemical nature of mammalian spermatozoa. He investigated stallion and human spermatozoa and was unable to demonstrate the presence of protamine or histone in either. BOSHIJAN[4] extracted lipid-free boar spermatozoa with 0.4% sodium hydroxide and obtained an acidic protein which precipitated near pH 4.6–4.7 with 2% acetic acid. ZITTLE AND O'DELL[5] reported some success in dissolving a part of the substance of bull sperm but made no attempt to characterize any of the soluble material. MAYER AND THOMAS[6,7] found that although water or salt solutions did not extract proteins or nucleic acid from boar or ram sperm, 1.0 *N* sodium hydroxide removed a considerable part of the substance of the sperm. Protein fractions and a nucleic acid fraction were precipitated from the alkaline extracts with acetic acid.

MATERIALS AND METHODS

Isolation of the sperm heads

Mammalian sperm can be obtained from epididymides or directly from ejaculated semen. The following methods were used in obtaining the sperm heads. Throughout this work homogenization was done with the POTTER-ELVEHJEM homogenizer.

Preparation of bull sperm heads. Bull epididymides were obtained from the abattoir and kept at about 3° to 5° C until used. To assure the use of uniformly aged sperm, only the caudal (tail) portion of the epidiymides were used. One to two pounds of caudal epididymides were cleaned of all external connective tissue and blood vessels and ground in a meat grinder. The mash was blended with physiological saline in the Waring blender for five minutes. The homogenate was then allowed to stand at 0°–4° C for about six hours, stirring occasionally. The mixture was strained through four layers of cheese cloth twice and eight layers of cheese cloth once and finally once through four layers of fine muslin. Microscopic examination of the solution showed no visible foreign matter, a few intact sperm but predominantly sperm broken into heads and combined tail-midpieces. The suspension was centrifuged for ten minutes at 4,800 r.p.m. The supernatant, pink in color due to a small amount of haemoglobin, was discarded. The residue was mixed with distilled water and blended in the Waring blender for three minutes at half the normal speed and centrifuged at 2,500 r.p.m. for seven minutes. This process of centrifugation and blending was repeated three times, gradually

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reducing the speed and time to 1,200 r.p.m. and three minutes. This procedure for differential centrifugation yielded a pure preparation of bull sperm heads (Fig. 1, a).

Preparation of dog sperm heads. Epididymides were excised from dog testicles immediately after death of the animals. The caudal epididymides were separated from the whole epididymides and dissected from the extravascular tissues. Due to the small number of epididymides used, usually ten to fifteen, grinding in the meat grinder was not necessary to break up the tissue. The cleaned caudal epididymides were blended with 0.14 *M* sodium chloride in the Waring blender for five minutes. The remainder of the procedure for the isolation of the dog sperm heads was carried out in the same manner as that outlined for the isolation of bull sperm heads.

Preparation of human sperm heads. Human semen was supplied by healthy donors. The semen was frozen within one hour after ejaculation, and within twenty-four hours after collection the frozen semen was warmed to room temperature, mixed with 0.14 *M* sodium chloride, strained through four layers of cheese cloth twice and centrifuged for ten minutes at 4,800 r.p.m. The firmly packed sperm cells were washed twice by homogenization with 0.14 *M* sodium chloride and centrifuged at 5000 r.p.m. for twenty minutes. The heads were isolated by the same procedure that was used for bull sperm heads.

Preparation of ram sperm heads. Freshly ejaculated ram semen was shaken for one-half hour with 0.14 *M* sodium chloride and centrifuged. The sperm were then homogenized with distilled water until the majority of the sperm were broken into heads and combined tail-midpieces. Ram sperm heads were then isolated by the same differential centrifugation procedure outlined for the isolation of bull sperm heads.

Preparation of boar sperm heads. Freshly ejaculated boar semen was strained through four layers of cheese cloth twice and centrifuged. The sperm were then homogenized with distilled water and centrifuged. They were then passed through a WERKMAN[8] tissue grinder twice, which was the only mechanical means found that would separate the heads from the tail-midpieces. The suspension of broken sperm were centrifuged for 15 minutes at 400 r.p.m., the supernatant discarded and the residue homogenized with distilled water. The centrifugation was repeated at 400 r.p.m. for 20 minutes, the residue homogenized again with distilled water and the homogenate centrifuged at 100 r.p.m. for 10 minutes. The final residue consisted almost entirely of sperm heads.

Analytical methods

Nitrogen was determined by a micro-Kjeldahl method[9] and phosphorus by the method of ALLEN[10]. Pentose nucleic acid was determined by the method of MEJBAUM[11], using yeast nucleic acid for the standard (obtained from the Schwartz Laboratories and repurified). Desoxypentose nucleic acid was determined by the methods of STUMPF[12] and SEIBERT[13]. DNA from fish sperm (obtained from Nutritional Biochemical Laboratories and repurified) and DNA prepared from calf thymus were used for the standards. The two standards agreed very closely. Cholesterol was determined by a modified method of PIJOAN AND WALTER[14], arginine by the method of THOMAS, INGALLS AND LUCK[15] and tyrosine by the method of THOMAS[16]. Calcium was determined by means of a LUNDEGARDH flame spectrophotometer.

The lipid content of the lipoprotein was determined by direct weighing as follows: Aliquots of the lipoprotein in alkaline solution were transferred quantitatively into cellulose nitrate centrifuge tubes. The solutions were then acidified with 5% acetic acid (adding equal volumes to each tube) and centrifuged. The precipitate was washed twice with distilled water and the lipoprotein of each sample was then transferred quantitatively by the use of distilled water into an extraction thimble (each thimble had been previously extracted with boiling alcohol, placed in a designated weighing bottle and dried to constant weight in an oven at 90° C). Some of the samples were then dried to constant weight in an oven at 90° C.

The other thimbles were placed in a beaker of boiling alcohol for one hour. At 10 minute intervals during the extraction the thimble was raised and the boiling alcohol was allowed to drain through. The residue was then washed with boiling alcohol and the thimble, in the tared weighing bottle, dried to constant weight in an oven at 90° C. The lipid content was then calculated by difference. (This procedure was used because the lipid itself was hydroscopic and very difficult to weigh.)

The lipid content of the lipoprotein was also calculated from the nitrogen content (of the whole lipoprotein and that of the protein and lipid parts) and from the cholesterol content (of the whole lipoprotein and of the lipid part).

The quantities of the various constituents obtained from sperm heads were determined as follows: Whole sperm heads were homogenized with distilled water and transferred to a 250 ml volumetric flask. A 50 ml aliquot was removed from the flask with a pipette and placed in a cellulose nitrate centrifuge tube. The remaining 200 ml of the suspension was transferred to three cellulose nitrate centrifuge tubes. The 50 ml aliquot was centrifuged and the sperm heads washed into an extraction thimble. The sample was then dried to constant weight in an oven at 65° C. (Each extraction thimble was previously extracted with boiling alcohol, placed in a designated weighing bottle and dried to constant weight in an oven at 90° C.)

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The 200 ml aliquot was centrifuged and the sperm homogenized with 0.1 *N* sodium hydroxide and stirred for 30 minutes at room temperature. The residue was extracted a second time in the same manner. The combined extracts were acidified and the lipoprotein precipitate collected near pH 5.6. It was washed twice with distilled water, transferred to an extraction thimble and dried to constant weight in an oven at 76° C.

The 0.1 *N* sodium hydroxide residue was homogenized and stirred with a one-to-one mixture of 2.0 *N* sodium hydroxide and 2.0 *M* sodium chloride for 20 minutes. The supernatant fluid was adjusted to pH 11.0 with acetic acid and the histone-like protein collected and transferred to an extraction thimble. The thimble was placed in a weighing bottle and dried to constant weight at 90° C. The residue from the extraction with the alkali-salt solution was washed three times by stirring with a large volume of distilled water, transferred to a weighing bottle and dried to constant weight at 90° C. This residue was nucleic acid.

Electrophoresis was carried out with a KLETT apparatus. The buffers used were those suggested by MILLER AND GOLDER[17], saturated with ether. FEVOLD AND LAUSTEN[18] have suggested the use of ether to decrease opalescence in lipoprotein solutions.

EXPERIMENTAL AND RESULTS

Solubility studies

Whole sperm heads. Toward the end of these investigations it was found that a 1 *M* solution of sodium chloride would remove a very small amount of nucleic acid and protein from bull sperm heads. The various other reagents tried probably removed this nucleic acid also but it had gone undetected in the work with those reagents.

Bull and boar sperm heads were used in the investigations reported below. A 0.1 *N* sodium hydroxide solution was used after each of the reagents, in order to determine what part of the lipoprotein had been removed by the reagent, since it was already known that 0.1 *N* sodium hydroxide would remove all of that fraction[19]. The Potter-Elvehjem homogenizer was used in preparing all homogenates.

In order to investigate further the possibility that mammalian sperm contain a keratin-like protein[20,5], bull and boar sperm heads were stirred for long periods with thioglycolic acid at about pH 3 and with a 4% solution of trimethylbenzylammonium hydroxide. The former extracted no detectable substance and the latter extracted all of the lipoprotein but left a large residue. These results are different from those of ZITTLE AND O'DELL[5].

"Nacconol", a mixture of sodium alkyl benzenesulphonates, extracted all of the lipoprotein from boar sperm heads, but it did not extract any other detectable protein or nucleic acid and it left a large residue. It has previously been found that "Nacconol" would dissolve a portion of whole boar sperm[21].

Boar sperm heads were stirred repeatedly for long periods with cysteine hydrochloride solutions at 36° C, and then stirred with a 1 *M* sodium chloride solution, to determine whether or not a reducing agent would make soluble a "keratin-like" protein. Neither the cysteine hydrochloride solutions nor the sodium chloride solution extracted any detectable protein.

A 0.5 *N* ammonium hydroxide solution extracted all of the lipoprotein from boar sperm heads and did not extract any other detectable substance. The lipoprotein extracted in this way would only partially redissolve in 0.5 *N* ammonium hydroxide after it has been precipitated with acid.

Bull sperm heads were homogenized with 0.1 *N* hydrochloric acid, and then stirred for one hour at 37° C. This failed to dissolve any substance precipitable by the addition of four volumes of alcohol or by increasing the pH of the extract to 10 with alkali.

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Treatment in the same way with 0.5 *N* sulfuric acid and with 0.1 *N* nitric acid gave similar results.

A 0.1 *N* sodium hydroxide solution removes all of the lipoprotein from the sperm, as previously described[22] and it also removes a small quantity of nucleic acid and protein, which is probably the same as that removed with a 1 *M* sodium chloride solution.

It was found during the latter part of this work that the lipoprotein could be extracted from bull sperm heads with a solution containing sodium chloride in 1 *M* concentration and sodium hydroxide in 0.005 *N* concentration. The final pH of this extraction homogenate was about 12.6.

After removing the small fraction of nucleic acid and protein with 1 *M* sodium chloride and then removing the lipoprotein, the residue consists entirely of heads somewhat smaller than the intact sperm heads (Fig. 1, a and b). They appear to be solid structures, rather than empty envelopes. This residue will be referred to as Residue I.

Residue I. Attempts were made to extract proteins or nucleic acids from Residue I with thioglycolic acid, cysteine hydrochloride, "Nacconol" and trimethylbenzylammonium hydroxide. In order to further test the possibility that a reducing agent would solubilize a keratin-like protein, hydrogen sulfide was bubbled through a homogenate of Residue I in 0.14 *M* sodium for six hours. In each case the residue was extracted with 0.1 *N* sodium hydroxide. None of these procedures removed any detectable protein or nucleic acid.

Residue I yielded a histone-like protein when it was homogenized with a solution containing sodium hydroxide and sodium chloride, each in 1 *M* concentration, and then stirred for 20 minutes. In the absence of the sodium chloride a higher concentration of sodium hydroxide was required to dissolve this protein. It precipitated when the supernatant was adjusted to pH 11 with acetic acid or hydrochloric acid and stirred vigorously for a few minutes or allowed to stand in a refrigerator at 2° C for one hour.

The residue from this extraction is gelatinous. Microscopic examination shows it to be composed entirely of structures similar in shape to intact sperm heads and to the head of Residue I, but smaller than the latter (Fig. 1, c). These also appear to be solid structures. This residue will be referred to as Residue II.

Residue II. When the extraction with the 1 *N* sodium hydroxide — 1 *M* sodium chloride solution is continued too long or when Residue II is mixed with an equal volume of distilled water the small remnants of the heads swell. After one minute standing in distilled water they are about as large as the original heads (Fig. 1, d) and have a "pitted" appearance. Blending in a Waring blender causes a stringing out of the substance of the residue (Fig. 1, e) and finally, after about five minutes blending, it goes into solution.

Residue II can be washed without going into solution if a large volume of distilled water is added, reducing the alkalinity. The thrice washed residue was found to be desoxyribose nucleic acid.

Isolation and characterization of the protein and nucleic acid of sperm

Nucleic acids and protein soluble in 1 M sodium chloride. Very small quantities of protein and nucleic acid were found to be extractable from the sperm heads with 1 *M* sodium chloride. During the process of isolation, the sperm heads were washed exhaustively with physiological saline. After extraction of the heads for 18 hours at 2° C

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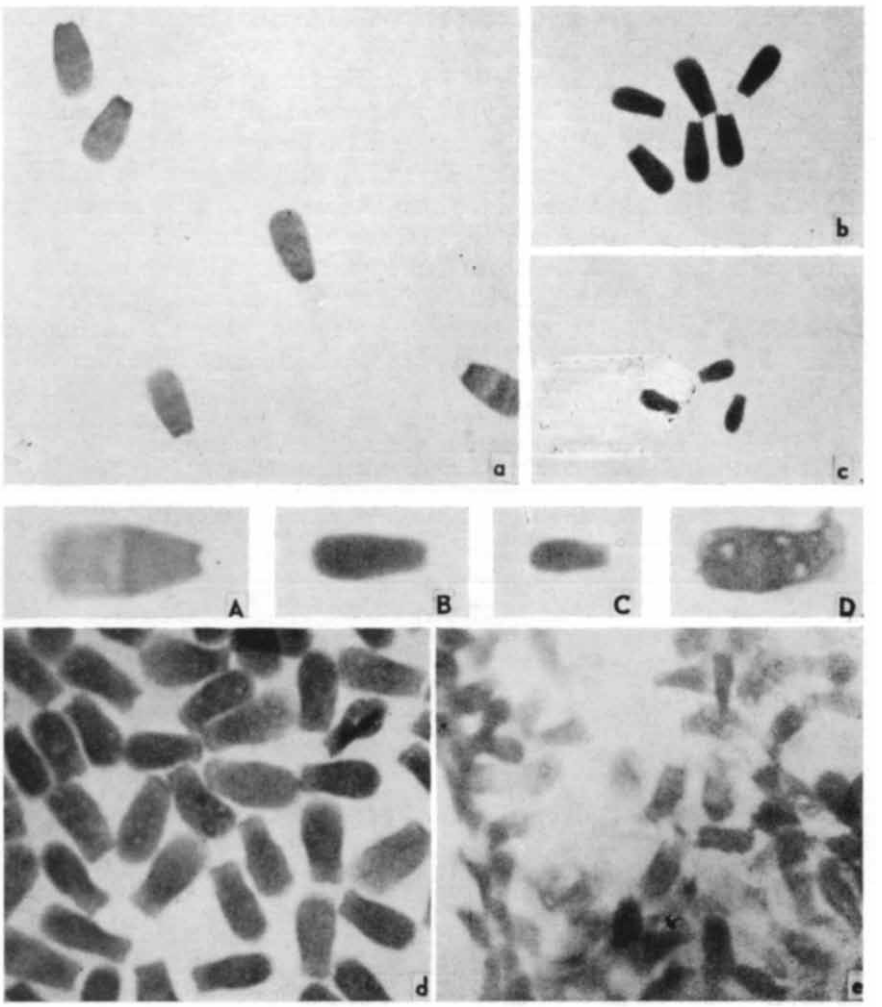

Fig. 1. a. Normal bull sperm heads; b. Residue I; c. Residue II; d. Residue II dispersed in distilled water; e. Residue II after blending 1 minute with the Waring blender. a, b, c, d, and e, 1120 X. A, B, C, and D are uniform enlargements of a, b, c, and d, respectively.

with 1 *M* sodium chloride, a very small precipitate was produced in the extract by adjusting the pH to about 6.3. The precipitated substance gave positive biuret and MILLON tests and redissolved in 1 *M* sodium chloride.

After this protein material has been removed, the solution was diluted with 4 volumes of alcohol. After 6 hours standing at 2° C a very small quantity of white precipitate was collected. This material gave a maximum absorption at 2600 A and was found to be DNA and PNA in 1:4 ratio.

Lipoprotein. This complex was prepared from bull, boar, ram, dog and human sperm. For the characterization studies it was extracted with 0.1 *N* sodium hydroxide. It was precipitated by adding 5% acetic acid and then redissolved and reprecipitated in the same way three times. We found the lipoprotein to be soluble below pH 4 when

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an excess of acid was added very quickly to its alkaline solution, although it could not be dissolved directly in dilute acids.

Electrophoresis of the lipoprotein of bull or human sperm showed only one component if the nucleic acid soluble at pH 7 was removed before the extraction of the lipoprotein. Lipid was demonstrated to be present in the lipoprotein precipitated from solution after an electrophoretic run had shown only one component to be present.

The results of chemical analysis of the lipoprotein are shown in Table I. Lipoprotein that had been reprecipitated and redissolved eight times did not differ in lipid content significantly from that which had been reprecipitated and redissolved three times. Table II shows the amount of lipid removed from the complex by extraction with boiling alcohol, with alcohol at approximately 25° C, and with ether. Chloroform and methyl alcohol each removed a small part of the lipid at room temperature.

TABLE I

CHEMICAL ANALYSIS OF THE LIPOPROTEIN, HISTONE-LIKE PROTEIN AND NUCLEIC ACID OF SPERM HEADS*

	Lipoprotein			*Total lipid of the lipoprotein*	*Total protein of the lipoprotein*	*Histone-like protein*		*Nucleic acid*
	Ram	*Bull*	*Dog*	*Bull*	*Bull*	*Bull*	*Ram*	*Bull*
Nitrogen %		11.5		1.13	15.1	20.4	17.1	14.3
Phosphorus %				0.25				8.5
Cholesterol %		1.84		6.00				
Calcium %		0.45						
Arginine %					6.35	27.8	26.7	
Tyrosine %					7.22			
Total Lipid								
Direct weighing	25.5	31.1	23.3					
Calculated from nitrogen content		28.0						
Calculated from cholesterol content		31.0						
DNA %								100.6

* All determinations except those on ram sperm were done on two or more samples. Those on ram sperm were done on one sample.

TABLE II

THE AMOUNT OF LIPID EXTRACTED FROM THE LIPOPROTEIN BY ALCOHOL AND ETHER

	Extracted by boiling alcohol %	*Extracted by alcohol at ca. 25° C* %	*Extracted by ether* %
Lipoprotein from bull sperm heads	31.1	30.4	7.4

In preliminary experiments intact sperm heads were agglutinated by rabbit immune serum specific for the sperm lipoprotein.

Histone-like protein. This protein was extracted as described above from Residue I of the sperm of bull, boar, ram, dog and human. It is important that the extraction

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not be continued longer than 20 minutes to avoid bringing some of the nucleic acid into solution. The solution is very viscous and it was necessary to centrifuge it at 12,000 r.p.m. for 30 minutes in order to obtain a residue-free supernatant.

The histone-like protein precipitated from the sodium chloride-sodium hydroxide solution by dilution with four volumes of water; acidification to pH 11, followed by vigorous stirring or standing in the refrigerator for one hour; or by dialysis against distilled water overnight at 4° C. (No protein dialysed through the membrane.)

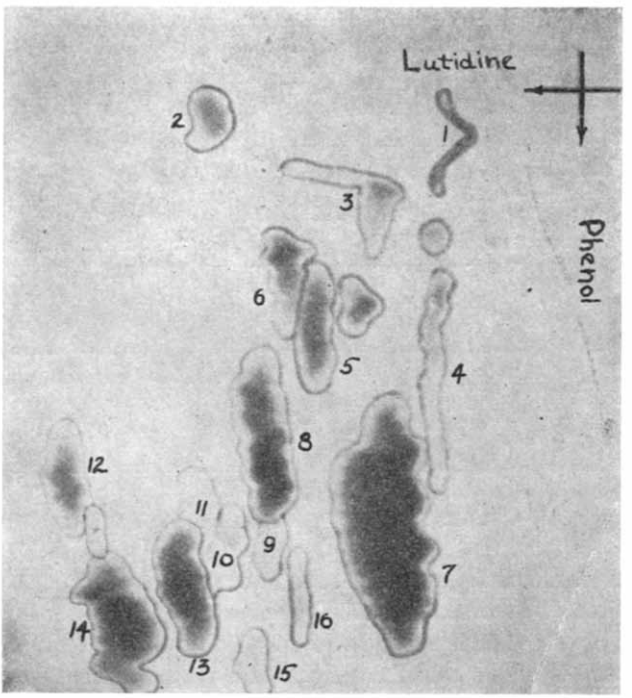

Fig. 2. Paper chromatogram of an acid hydrolysate of the histone-like protein from ram sperm heads.

1. Aspartic acid
2. Cystine (cystic acid)
3. Glutamic acid
4. Lysine
5. Glycine
6. Serine
7. Arginine
8. Threonine and Alanine
9. Hydroxyproline
10. Valine
11. Methionine sulfone
12. Tyrosine
13. Leucine and *Iso*leucine
14. Phenylalanine
15. Proline
16. Histidine

Once this histone-like protein has precipitated from solution, it will not redissolve in a 1 *N* sodium hydroxide solution. Therefore it cannot at present be purified by re-precipitation. The precipitated protein has been found not to contain nucleic acid, however. In Table I are shown the results of some chemical analyses of this protein fraction. A chromatogram of an acid hydrolysate of the histone-like protein of ram sperm is shown in Fig. 2.

Nucleic acid. Residue II was washed three times by stirring with a large volume of distilled water. It was then dissolved in 0.001 *N* sodium hydroxide and precipitated

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at about pH 2 by the addition of dilute hydrochloric acid. It was redissolved and reprecipitated in the same way three times.

The nucleic acid prepared in this way did not give the biuret, Millons or xanthroproteic tests. Its ultraviolet light absorption curve is typical of nucleic acid. The absence of protein was further indicated by the results of shaking a 0.001 *N* sodium hydroxide solution of the nucleic acid with a chloroform-octyl alcohol mixture according to the method of SEVAG. No protein was collected at the water-chloroform interface. The nucleic acid in this fraction was found to be entirely desoxyribose nucleic acid. The results of some chemical analyses are presented in Table I.

When the nucleic acid solution was dialysed against distilled water for 48 hours no purine nor pyrimidine bases passed through the membrane.

Quantitative composition of mammalian sperm. The amount of lipoprotein, histone-like protein and nucleic acid found in bull and dog sperm heads is shown in Table III. The total lipid of the bull sperm heads was found to be 6.5%.

TABLE III

THE AMOUNT OF LIPOPROTEIN, HISTONE-LIKE PROTEIN AND NUCLEIC ACID IN SPERM HEADS

	Lipoprotein %	*Histone-like protein* %	*Nucleic acid (other than that soluble at pH 7)* %	*Total* %
Bull sperm heads	19.6	28.7	48.0	96.3
Dog sperm heads	17.0	25.0	55.3	97.3

DISCUSSION AND CONCLUSIONS

The following characteristics of the substance extracted from the sperm heads by 0.1 *N* sodium hydroxide or weaker alkali leave no doubt that it is a lipoprotein complex and not a simple mixture of lipid and protein: The lipid content of the substance is constant regardless of the number of times that it is reprecipitated. Lipid can be demonstrated to be present in the precipitate recovered from a solution after an electrophoretic analysis of that solution has shown only one component to be present. WANG *et al* [23] have isolated a similar lipoprotein from cellular nuclei.

The solubility of the lipoprotein of sperm seems to depend on the alkalinity and ionic strength of the reagent and not on any other property of the reagent. The lipoprotein with which we are dealing must be classed with those which are present in the structural elements of various kinds of cells rather than with the more soluble lipoproteins, such as those found in blood, hens eggs, etc. The microscopic appearance of the sperm heads after removal of the lipoprotein (Residue) indicates that this substance forms a membrane or membrane-like structure around the head.

The presence of lipoprotein as a covering of the sperm head is not unexpected in view of the results of birefringence studies[24, 25], which indicate that cell membranes are composed of lipids and proteins and the finding that the mammalian erythrocyte membrane contains lipoprotein[26].

The total lipid of the bull sperm head (6.5%) can be accounted for, within experimental error, by the lipid content (31.1%) of the lipoprotein. The latter comprises 19.6% of the whole sperm head.

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Although the immunological tests reported here were preliminary, the finding that antiserum specific for the lipoprotein will agglutinate intact sperm heads seems to offer some additional support for the belief that the former is on the surface of the head.

The method of extraction and precipitation of the histone-like protein described above apparently denatures it so that it does not redissolve in the same reagent. It is, however, free of nucleic acid. We feel that the values obtained for the nitrogen and arginine content of this protein are therefore sufficiently accurate to place it in the general category of basic proteins along with histones and protamines. It does not seem necessary at present, however, to alter the classification of basic proteins by designating those found in mammalian sperm as a third group. We prefer to call these "histone-like proteins" until more information is obtained about them and about those found in the sperm of birds and other animals. The basic protein isolated by the MIRSKY group[27] from rooster sperm, designated "galin" by those authors, may eventually be placed in a group other than the protamines because of the large number of amino acids of which it is composed.

A useful system of nomenclature will obtain if the basic proteins found in the sperm of any animal species are named according to the method already used for the protamines of fish sperm and for "galin" of rooster sperm, *i.e.*, by using the genus name. The basic proteins of sperm will then be named by the same system whether they are protamines, histones, or others which have no group name at present, the only criterion for such naming to be the possession of characteristics which identify a protein of sperm as a basic protein. The names "bovin", "ovin", "canin", "hominin" and "suin" are therefore proposed for the histone-like proteins of the sperm of the bull, ram, dog, man and boar, respectively.

The microscopic appearance of Residue I and Residue II would seem to indicate that the histone-like protein forms a layer inside the layer of lipoprotein and outside the "core" of DNA. There may be another explanation for the appearance of these residues, however. (It should be pointed out that other sperm studied, such as fish sperm, have contained nucleic acid and basic protein which are easily soluble and therefore any such structure, *i.e.*, a morphological separation of nucleic acid and basic protein, would not be easily detected.) The failure of the DNA to dissolve until after the histone-like protein is removed may be due simply to a difference in the properties of the two components.

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SUMMARY

1. Methods are described for the preparation of the heads of the sperm of five mammalian species, free from the tails and midpieces.

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2. 1.0 *M* sodium chloride removes a very small amount of protein and nucleic acid (DNA and PNA) from bull sperm heads.

3. A lipoprotein can be extracted from the sperm heads of each of the five species by means of 0.1 *N* sodium hydroxide or a 1 *M* solution of sodium chloride at pH 12.6. More weakly alkaline reagents dissolve the lipoprotein less readily.

4. A histone-like protein can be extracted from the sperm heads with very strongly alkaline solutions. High salt concentrations aids in this extraction.

5. After the histone-like protein is removed from the heads, the nucleic acid dissolves in more weakly alkaline solutions.

6. The three major components were characterized by chemical analysis and ultraviolet light absorption studies. The lipoprotein was also studied by electrophoresis and the histone-like protein by paper chromatography.

7. The relation of the chemical composition of the sperm heads to their morphology is discussed.

8. The names "bovin", "ovin", "canin", "hominin" and "suin" are suggested for the histone-like proteins from the sperm of the bull, ram, dog, man and boar, respectively.

RÉSUMÉ

1. Des méthodes sont données pour la préparation de têtes de spermatozoïdes sans queues ni parties centrales, de cinq espèces de mammifères.

2. Le chlorure de sodium de concentration 1.0 *M* ne dissout qu'une très petite partie des protéines et des acides nucléique (ADN et APN) des têtes de spermatozoïdes de taureau.

3. On peut extraire une lipoprotéine des têtes de spermatozoïdes de taureau au moyen d'une solution 0.1 *N* de soude caustique, ou bien d'une solution 1.0 *M* de chlorure de sodium à pH 12.6. L'extraction de lipoprotéine devient plus difficile si la solution est moins basique.

4. Une protéine ayant certaines des propriétes d'une histone peut être extraite de têtes de spermatozoïdes au moyen d'une solution fortement basique. Une forte concentration de sel facilite cette extraction.

5. Après l'extraction de la protéine ayant des propriétés d'histone, l'extraction de l'acide nucléique peut se faire par des solutions moins basiques.

6. Les trois substances principales ont été caractérisées par analyse chimique, ainsi que par leur absorption optique dans l'ultraviolet. La lipoprotéine a aussi été étudiée par électrophorèse, et la protéine à propriétés d'histone par chromatographie sur papier.

7. Les rapports existant entre la composition chimique des têtes de spermatozoïdes et leurs morphologie sont considérés.

8. Nous proposons l'usage des noms (en anglais) de "bovin", "ovin", "canin", "hominin" et "suin" pour la protéine à propriétés d'histone provenant respectivement des spermatozoïdes de taureau, de bélier, de chien, d'homme et de verrat.

ZUSAMMENFASSUNG

1. Methoden zur Herstellung von Spermienköpfen von fünf Säugetierarten frei von Schwänzen und Mittelstücken werden beschrieben.

2. Eine 1.0 *M* Natriumchlorid-Lösung entzieht eine sehr geringe Menge Protein und Nucleinsäure (DNS und PNS) von den Spermienköpfen des Stiers.

3. Ein Lipoprotein kann den Spermienköpfen jeder der fünf Arten mittels 0.1 *N* Natriumhydroxyd oder einer 1.0 *M* Natriumchlorid-Lösung bei pH 12.6 entzogen werden. Schwächere alkalische Reagentien lösen die Lipoproteine nicht so leicht auf.

4. Ein histonähnliches Protein kann den Spermienköpfen mit sehr starken alkalischen Lösungen entzogen werden. Hohe Salzkonzentrationen erleichtern diese Extraktion.

5. Nachdem das histonähnliche Protein aus den Köpfen entfernt ist, löst sich die Nucleinsäure in schwächeren alkalischen Lösungen.

6. Die drei Hauptbestandteile wurden durch chemische Analyse und Absorptionstudien im ultravioletten Licht gekennzeichnet. Das Lipoprotein wurde ausserdem elektrophoretisch und das histonähnliche Protein chromatographisch studiert.

7. Die Beziehung der chemischen Zusammensetzung der Spermienköpfe zu ihrer Morphologie wird besprochen.

8. Die Namen "bovin", "ovin", "canin", "hominin" und "susin" werden (im Englischen) für die histonähnlichen Proteine der Spermien des Stiers, Widders, Hundes, Mannes beziehungsweise des Ebers vorgeschlagen.

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