THE PROTEINS OF BOVINE SPERMATOZOA FROM THE CAPUT AND CAUDA EPIDIDYIMIDIS*

U. LAVON,† R. VOLCANI AND D. DANON‡

Department of Animal Science, Volcani Institute of Agricultural Research, Rehovot, Israel, and ‡The Weizmann Institute of Science, Rehovot, Israel

(Received 31st March 1970)

Summary. Changes occurring in the proteins of bovine spermatozoa during their migration from the caput to the cauda epididymidis were studied.

The percentage of total nitrogen in the dry matter was greater in the cauda than in the caput spermatozoa, but the nitrogen content/ 10^9 cells was less in the cauda spermatozoa. The amount of DNA for the same number of cells remained constant during this stage of maturation. The decrease in the nitrogen content indicated a loss of protein during maturation of bovine spermatozoa in the epididymis. Amino acid analysis of the dry matter of the sperm cells showed an increase in the percentage of arginine and cystine and a decrease in the percentage of glutamic and aspartic acids, alanine, methionine, isoleucine and leucine during passage of spermatozoa through the epididymis. Lipoprotein content decreased during the maturation of the sperm cells, and a slight increase occurred in several of the other proteins. Amino acid analysis of the extracted proteins showed differences in the percentage of several amino acids between caput and cauda spermatozoa in the F_1 and F_2 fractions. Gel disc electrophoresis of the various caput and cauda sperm proteins indicated a different number of bands and a different colour intensity for those fractions in which a different amino acid composition was found.

The results suggest that the lipoproteins of the cell membrane may be the site of alterations in the quantity, composition and electrophoretic behaviour of the proteins of bovine spermatozoa during their maturation.

INTRODUCTION

Several publications report on the protein content and amino acid composition of the spermatozoa of different species; details are given by Mann (1964) and in the studies of Bril-Petersen & Westenbrink (1963), Hunter & Hafs (1964),

* Contribution from the Volcani Institute of Agricultural Research, Rehovot, Israel. 1969 Series, No. 1638-E.

† Present address: The Animal Research Station, 307 Huntingdon Road, Cambridge.

Henricks & Mayer (1965a, b), Gledhill, Gledhill, Rigler & Ringertz (1966) and Ingles, Trevithick, Smith & Dixon (1966). A few of them are concerned with the changes associated with the alteration in the basic proteins of the nucleus from second spermatocyte or round spermatid to the spermatozoon (Alfert, 1956; Vendrely, Knobloch & Vendrely, 1957; Gledhill *et al.*, 1966; Ingles *et al.*, 1966). Two reports deal with the changes in the proteins during sperm passage through the epididymis. Berry & Mayer (1960) reported an increase in the percentage of the basic amino acids and a decrease in that of the acidic acids of the basic proteins. Gledhill *et al.* (1966) assumed that there is only a small increase in the percentage of basic amino acids in the basic proteins of the nucleus following the sperm passage through the genital tract.

Several methods of sperm protein extraction are reported in the literature. Those adopted for fish spermatozoa generally use water or salt solutions and are described by Felix (1960), while, for mammalian spermatozoa, a stronger treatment is needed for the disruption of the cell membrane. Hence, the use of glass beads, enzymes or reducing agents was adopted (Green, 1940; Zittle & O'Dell, 1941; Berry & Mayer, 1959; Bhargava, Bishop & Work, 1959a; Bril-Petersen & Westenbrink, 1963; Hunter & Hafs, 1964; Henricks & Mayer, 1965a, b). As most of the studies have dealt with the basic proteins, it was necessary to find appropriate treatments which would facilitate the extraction of both the lipoproteins and the basic proteins in sequence.

The purpose of the present investigation was to determine the amount and composition of total proteins in the dry matter, to extract various proteins by chemical methods, to determine their quantity and composition, and to characterize them by gel disc electrophoresis, in order to compare the findings for spermatozoa derived from the caput and cauda epididymidis. This study included the following determinations: (a) total nitrogen, (b) desoxyribonucleic acid (DNA), (c) amino acid composition of the dry matter, (d) extraction of proteins from the dry matter, (e) the protein content in each fraction, (f) amino acid composition of the extracted proteins, and (g) gel disc electrophoresis of these proteins.

METHODS

Sperm collection

Spermatozoa from the caput and cauda epididymidis were obtained from seven 14- to 16-month-old bulls immediately after slaughter (Grogan, Mayer & Sikes, 1966). The cells were separated from fluids and remnants of tissue as described by Lavon, Volcani & Danon (1968). The cells were resuspended in a known volume of 0.9% NaCl solution in order to facilitate the estimation of their number.

Sperm counting

The number of spermatozoa in a given collection was counted in duplicate samples using an EEL colorimeter and a haemocytometer (four counts on each sample).

Dry matter determination

This procedure was carried out as described by Lavon *et al.* (1968). Calculation of dry matter content was made in terms of percentage of the fresh weight and of mg/10⁹ cells.

Nitrogen determination

Total nitrogen was determined on 10-mg samples of the lyophilized dry matter by the micro-Kjeldahl method; the nitrogen was determined with a Technicon AutoAnalyzer according to the method of Clare & Stevenson (1964).

FLOW SHEET. A fractionation scheme for the isolation of sperm proteins from the caput and cauda epididymidis.

Determination of DNA

This was carried out on the same samples for which the total nitrogen was determined. The DNA was extracted from the cells according to Summerhill & Olds (1961) and was determined by its phosphorous content (9.89%) according to the method of Fiske & Subbarow (1925) in a Technicon AutoAnalyzer.

Amino acid analysis

This was carried out on 3-mg samples of the lyophilized dry matter of spermatozoa from the caput and cauda epididymidis of each of six bulls, using a Beckman 120B amino acid analyser. The analyses of the extracted proteins were performed on samples of 500 to 1000 μg of protein from the various fractions. The samples were hydrolysed in 6 N-HCl for 24 hr in evacuated sealed glass ampoules at 110° C before analysis.

Protein extraction

Sperm dry weight samples were treated with 0.1 N-NaOH, 1% NaCl or 0.1 N-HCl. The protein content of each extract was determined in an Elmer Perkins Hitachi Spectrophotometer at 280 m μ . Different sequences of treatments were tried and the scheme shown in the Flow Sheet was adopted. This scheme is based on the work of Berry & Mayer (1959), Henricks & Mayer (1965b) and Summerhill & Olds (1961).

The treatment with mercaptoethanol was incorporated as proposed by Green (1940) and by Henricks & Mayer (1965b) in order to disrupt the S—S linkages of the cell membrane, and in order to facilitate the extraction of the keratin-like basic proteins by 0.1 N-HCl solution. The extractable material in each fraction was calculated by subtraction of the residual material remaining after treatment, from that present before treatment.

Determination of total protein in the extracts

The protein content in each fraction obtained by using the above-mentioned sequence was determined according to Lowry, Rosebrough, Farr & Randall (1951) using a standard of bovine serum albumin as reference.

Electrophoresis

Gel disc electrophoresis was performed at both acidic and basic pH by the methods of Reisfeld, Lewis & Williams (1962) and Davis (1964), respectively. The quantity of protein in each gel was about 200 to 300 μg . The length of the acrylamide gels and the time of electrophoresis were changed according to the extract that was separated and to the pH used for the run. A modification was also adopted in which the staining of the gels with Coomassie Brilliant Blue (ICI R250) was carried out with diluted dye solution (0.01%) in TCA 12.5% trichloroacetic acid (TCA) for 18 hr. The removal of the surplus dye was achieved by repeated washings with 12.5% TCA.

Statistical analysis

The results for each of the parameters were analysed according to Snedecor (1948) by a two-way analysis of variance (different locations in the epididymis

and different bulls). The standard error (S.E.) of the means for the caput and the cauda and the least significant difference (L.S.D.) at the 5% level were given when significant differences were found.

RESULTS

Total nitrogen

Seven determinations showed an increase in the % total nitrogen in the dry matter (10 mg) from 11.08 ± 0.11 in the caput to 12.30 ± 0.11 in the cauda epididymidis (L.S.D. 5% = 0.39). However, when the results were related to the number of sperm cells from which the 10-mg samples were taken, a decrease from 3.41 ± 0.08 mg/ 10^9 cells in the caput to 3.11 ± 0.08 mg/ 10^9 cells in the cauda epididymidis was found (L.S.D. 5% = 0.27).

DNA content

The values obtained from five determinations, based on the phosphorous content, were 2.90 mg/ 10^9 cells for the caput and 3.03 mg/ 10^9 cells for the cauda epididymidis; the difference was not significant. These values are in agreement with those reported by other investigators (Mann, 1964, p. 147). Using a value of 15% nitrogen in the DNA (an average value of results quoted by Mann, 1964, Chapter VI), it can be calculated that the net protein nitrogen is 2.98 mg/ 10^9 cells in the caput and 2.66 mg/ 10^9 cells in the cauda epididymidis. This difference is equal to a decrease of 2.0 mg of protein in the dry weight of 10^9 spermatozoa during their migration from the caput to cauda epididymidis.

Amino acid composition of the dry matter

The amino acid composition of the proteins present in the dry matter of spermatozoa from the caput and cauda epididymidis is given in Table 1. It can be seen from this Table that a significant decrease in the acidic and neutral acids, and a parallel increase in the basic amino acids occur during sperm passage from caput to cauda epididymidis. The decrease in the acidic acids was manifested in both the glutamic and aspartic acids. In the neutral acids, there was a decrease in alanine, methionine, isoleucine and leucine, an increase in cystine, and no change in the others. The increase in arginine was 3.73%, while the percentage of lysine and histidine was not altered.

Protein extraction

The extractions with various agents showed a higher protein content in the dry matter of spermatozoa from the caput than the cauda epididymidis for all the chemical treatments. The results are presented in Table 2.

From these results, it was not clear whether the different agents extracted the same proteins, and if so, to what extent they achieved this. Therefore, the particular sequence of treatments, the results of which are given in Table 3, was employed.

The percentage dry matter extracted by 0.1 N-NaOH was 64 in the caput and 50 in the cauda epididymidis. The decrease of the extractable material from caput to cauda in this fraction occurred both in the proteins and in the

non-protein compounds (lipids, carbohydrates, etc.). This decrease was more significant when the values were expressed as mg/10⁹ cells rather than as percentage dry matter. Mayer, Madera-Orsini & Berry (1957), who employed the same initial treatment, identified the extracted proteins, by precipitation with acetic acid at pH 6.0 and by chemical analysis, to be mainly lipoproteins.

TABLE 1
AMINO ACID COMPOSITION OF THE PROTEINS OF SPERMATOOA
COLLECTED FROM THE CAPUT AND CAUDA EPIDIDYMDIS OF SIX BULLS

<i>Amino acid</i>	<i>Mean for caput*</i>	<i>Mean for cauda*</i>	<i>S.E. of means</i>	<i>L.S.D. 5%</i>
Lysine	6.65	6.37	NS	—
Histidine	2.46	2.61	NS	—
Arginine	15.79	19.52	±0.42	1.52
Aspartic acid	8.10	7.33	±0.18	0.64
Threonine	4.52	4.49	NS	—
Serine	4.93	4.85	NS	—
Glutamic acid	12.93	11.62	±0.19	0.69
Proline	5.11	4.92	NS	—
Glycine	4.40	4.22	NS	—
Alanine	4.10	3.71	±0.05	0.18
Half cystine	3.08	4.19	±0.17	0.62
Valine	5.13	4.94	NS	—
Methionine	2.29	2.05	±0.05	0.18
Isoleucine	3.90	3.64	±0.07	0.25
Leucine	8.01	7.49	±0.08	0.29
Tyrosine	4.32	4.08	NS	—
Phenylalanine	4.17	3.97	NS	—
Total basic acids	24.90	28.49	±0.57	2.06
Total acidic acids	21.03	19.24	±0.21	0.77
Total neutral acids	54.07	52.27	±0.42	1.52

* The amino acid content is expressed as a percentage of the protein.

NS = Not significant.

TABLE 2
PERCENTAGE OF PROTEINS IN THE DRY MATTER OF SPERMATOOA
FROM THE CAPUT AND CAUDA EPIDIDYMDIS OF FOUR BULLS
TREATED WITH VARIOUS AGENTS

<i>Treatment</i>	<i>Caput</i>	<i>Cauda</i>	<i>S.E. of means</i>	<i>L.S.D. 5%</i>
0.1 N-NaOH	35.0	26.2	±0.38	1.72
1% NaCl	28.9	20.7	±1.82	8.11
0.1 N-HCl	20.7	14.5	±1.23	5.50

Following the treatment with 0.1 N-HCl (Fraction F₂), the percentage of extractable material increased from caput to cauda. The mercaptoethanol facilitated the extraction of 4 to 6% of the dry matter (Fraction F₃). This finding is considerably lower than that of Henricks & Mayer (1965b) who obtained 12 to 15% basic proteins for ejaculated boar sperm cells. The remaining material was found to contain all the DNA (as no traces of it were identified in Fraction

F₁, F₂ and F₃) and residual proteins, most of them basic in character. The 3% NaOH extracted 20 to 30% of the dry matter (Fraction F₄), whereas the undissolved precipitate (Fraction F₅) comprises 5 to 8% of it. When the results were given as percentage dry matter, an increase in the extractable material and occasionally in the proteins or in the non-protein material from caput to cauda, was found in the F₂, F₃, F₄ and F₄+F₅ fractions. This increase was partly cancelled when the data were expressed as mg/10⁹ cells. In this case, the differences were significant only for the extractable material in Fraction F₂ and for the basic proteins of Fraction F₃.

TABLE 3

THE TOTAL MATERIAL, PROTEINS AND NON-PROTEINS EXTRACTED FROM THE DRY MATTER OF SPERMATOZOA FROM THE CAPUT AND CAUDA EPIDIDYMDIS OF SIX BULLS, USING THE SEQUENCE OF TREATMENTS DESCRIBED IN THE FLOW SHEET

Fractions		% in dry matter				Mg/10 ⁹ cells			
		Caput	Cauda	S.E. of means	L.S.D. 5%	Caput	Cauda	S.E. of means	L.S.D. 5%
F ₁ 0.01 N-NaOH Extract	Total	64.02	49.68	± 0.99	3.60	19.14	12.29	± 0.45	2.22
	Proteins	29.64	22.46	± 1.58	5.73	8.81	5.54	± 0.40	1.50
	Non-proteins	34.38	27.22	± 0.92	3.34	10.33	6.76	± 0.31	1.13
F ₂ 0.1 N-HCl Extract	Total	4.16	8.04	± 0.51	1.85	1.28	2.03	± 0.10	0.36
	Proteins	1.96	3.43	NS	—	0.60	0.86	NS	—
	Non-proteins	2.20	4.61	± 0.49	1.90	0.68	1.17	NS	—
F ₃ 0.1 N-HCl Extract	Total	4.10	6.30	± 0.46	1.67	1.24	1.57	NS	—
	Proteins	1.80	2.78	± 0.24	0.87	0.54	0.69	± 0.04	0.14
	Non-proteins	2.30	3.52	± 0.19	0.69	0.70	0.88	NS	—
F ₄ +F ₅ Combined	Total	27.72	35.98	± 1.01	3.65	8.33	8.90	NS	—
	Proteins	16.78	20.78	NS	2.21	5.06	5.18	RS	—
	Non-proteins	10.94	15.20	NS	—	3.27	3.72	NS	—
F ₄ 3% NaOH Extract	Total	21.92	28.98	± 0.90	3.26	6.62	7.23	NS	—
	Proteins	10.98	13.78	NS	—	3.35	3.51	NS	—
	Non-proteins	10.94	15.20	± 0.65	2.36	3.27	3.72	NS	—
F ₅	Undissolved residue	5.80	7.00	NS	—	1.71	1.67	NS	—

The components are expressed as a percentage of the dry matter and as mg/10⁹ cells.

NS = Not significant.

Amino acid composition of the extracted proteins

The data for the various fractions are given in Table 4.

The data of the amino acid composition of both caput and cauda cells show a wide variation in the composition of the proteins of the various fractions. Several points have to be emphasized: (a) In general, significant differences were observed in the amino acid composition between caput and cauda cells for histidine and alanine in Fraction F₁ and arginine, serine and methionine in Fraction F₂. Differences were also noted in Fractions F₄ and F₅, although these were not significant. (b) Fraction F₁ was rich in aspartic and glutamic acids, lysine and leucine and very poor in arginine and cystine. (c) The content of

arginine and cystine was higher, and that of the acidic acids, lysine and some of the neutral acids, lower, in the F_2 than in the F_1 fraction. (d) In Fraction F_3 , arginine was the major amino acid and comprised almost 50% of the total acids. Cystine and tyrosine were also present in relatively high proportions, whereas the other acids contributed only a small percentage. (e) The combined residue $F_4 + F_5$ was less basic than the F_3 fraction, but contained a high percentage of arginine and cystine. (f) Fraction F_4 showed a percentage of basic amino acids similar to that in the $F_4 + F_5$ residue, though there appeared to be a decrease in the lysine and an increase in the arginine content from caput to

TABLE 4

THE AMINO ACID COMPOSITION OF THE EXTRACTED PROTEIN FRACTIONS FROM THE DRY MATTER OF SPERMATOOZOA FROM THE CAPUT AND CAUDA EPIDIDYIMIDIS

Amino acid	F_1		F_2		F_3		$F_4 + F_5$		F_4		F_5	
	Caput	Cauda	Caput	Cauda	Caput	Cauda	Caput	Cauda	Caput	Cauda	Caput	Cauda
Lysine	10.34	9.28	8.51	8.51	2.83	3.17	5.16	5.09	5.70	4.79	3.75	3.88
Histidine	2.22	2.54*	3.00	3.14	2.75	2.89	2.31	2.38	2.35	2.28	1.81	1.89
Arginine	4.59	5.22	9.45	7.82†	49.80	49.17	26.96	27.48	28.50	32.08	41.69	38.11
Aspartic acid	10.20	10.34	9.29	9.25	2.51	2.73	6.22	5.26	6.06	5.30	4.60	5.06
Threonine	5.07	5.24	4.71	4.82	4.16	4.20	4.06	4.05	3.55	3.54	3.63	3.69
Serine	5.00	5.42	5.10	5.42¶	2.81	3.51	4.48	4.38	3.84	3.70	3.37	3.48
Glutamic acid	15.66	15.89	14.60	14.18	6.01	6.17	8.56	8.27	9.91	8.51	6.86	7.17
Proline	5.66	5.72	5.87	6.46	1.64	2.06	4.47	4.88	4.05	4.67	3.22	4.04
Glycine	4.44	4.28	4.09	3.79	2.44	2.45	4.86	5.05	6.92	7.47	2.94	3.22
Alanine	5.15	4.78§	4.49	4.59	1.96	2.09	2.95	3.11	2.98	2.92	2.81	2.97
Half cystine	1.29	1.65	1.94	2.10	3.78	3.15	4.27	4.07	1.02	1.06	1.09	0.92
Valine	6.33	6.18	5.57	5.56	3.65	3.46	4.63	4.60	4.53	4.37	3.43	3.94
Methionine	2.46	2.30	1.75	2.46‡	0.97	1.14	1.93	1.82	1.54	1.42	1.44	1.57
Isoleucine	4.80	4.70	4.64	4.92	2.29	2.24	3.40	3.31	2.98	2.81	2.74	2.86
Leucine	9.21	9.14	8.28	8.47	4.29	4.56	7.21	7.10	7.17	6.35	7.27	7.26
Tyrosine	3.34	3.00	4.22	4.27	5.33	4.37	4.79	4.85	5.59	5.73	3.95	4.41
Phenylalanine	4.24	4.06	4.50	4.27	2.78	2.65	3.77	3.63	3.34	3.05	5.44	5.54

*S.E. = ± 0.02 , L.S.D. 5% = 0.12, †S.E. = ± 0.24 , L.S.D. 5% = 1.46, ‡S.E. = ± 0.11 , L.S.D. 5% = 0.67, §S.E. = ± 0.04 , L.S.D. 5% = 0.24, ¶S.E. = ± 0.05 , L.S.D. 5% = 0.30

The quantity of each amino acid is expressed as a percentage of the protein. The values in Fractions F_1 , F_2 and F_3 are derived from three bulls and in Fractions $F_4 + F_5$, F_4 and F_5 from two bulls.

cauda. The slight decrease in the acidic acids from caput to cauda, which was greater in Fraction F_4 than in the $F_4 + F_5$ residue, was not significant. The same was noted also for leucine. Minor changes from the $F_4 + F_5$ fraction were found for most of the acids, of which the high value for glycine and the very low one for cystine should be emphasized. (g) Fraction F_5 included basic proteins which contained about 40% arginine. The percentage of cystine, however, was very low (1%) contrary to what would have been expected from the results of the $F_4 + F_5$ and the F_4 fractions.

Gel disc electrophoresis

The results of the electrophoretic separations are shown in Text-figs. 1 to 4 for the four fractions (F_1 to F_4) which were obtained from the spermatozoa of the caput and the cauda epididymidis.

It can be seen that following the separation of the proteins of Fractions F_1 , F_2 and F_4 by gel disc electrophoresis, differences in the number of bands between sperm cells from the caput and cauda were found. Furthermore, the proportion of the individual bands in the total proteins of the fraction was altered during the maturation process. This phenomenon was very marked when 0.01 N-NaOH-extracted proteins were separated at pH 8.6. In this case,

TEXT-FIG. 1. Electrophoretic separation (a) at pH 4.5, and (b) at pH 8.6 of the proteins in the F_1 fraction extracted by 0.01 N-NaOH from spermatozoa of the caput and cauda epididymidis.

about twenty bands were identified and some of them stained to different intensity in the fractions from the caput and the cauda. At pH 4.5, however, smaller differences were observed between caput and cauda cell extracts. In the 0.1 N-HCl extract (F_2), typical differences between the caput and the cauda sperm cells were found at pH 4.5, whereas in the F_3 fraction no differences were observed. In the 3% NaOH extract (F_4), a small band was identified in the cauda which was absent in the sperm cells from the caput epididymidis.

TEXT-FIG. 2. Electrophoretic separation at pH 4.5 of the proteins in the F_2 fraction extracted by 0.1 N-HCl from spermatozoa of the caput and cauda epididymidis.

TEXT-FIG. 3. Electrophoretic separation at pH 4.5 of the basic proteins in the F_3 fraction extracted by 0.1 N-HCl from spermatozoa of the caput and cauda epididymidis following mercaptoethanol treatment.

TEXT-FIG. 4. Electrophoretic separation at pH 4.5 of the proteins in the F_4 fraction extracted by 3% NaOH from spermatozoa of the caput and cauda epididymidis.

DISCUSSION

The results of the present study confirm that the decrease in the dry matter content of spermatozoa, which occurs during maturation (Lavon, Volcani & Danon, 1970), is partly attributable to a loss of proteins. This conclusion is based on the findings that the total nitrogen content decreases, whereas the DNA nitrogen remains constant when the spermatozoa migrate from the caput to cauda epididymidis. The results on DNA are in agreement with those reported by Bouters, Esnault, Salisbury & Ortavant (1967) and Bouters & Vandeplasse (1968). Other nitrogen compounds do not contribute to the decrease in nitrogen as their quantity is very low (Bhargava *et al.*, 1959a). The above findings are supported also by the results of the dry matter extraction, which showed a decrease in protein content during sperm passage from the caput to cauda epididymidis. The marked decline in the protein content in the F_1 fraction is responsible for this decrease. This finding is further strengthened by the fact that the proteins of the other fractions increase slightly during this stage of maturation.

The data concerning the amino acid composition of the lyophilized dry matter of spermatozoa from the caput and cauda epididymidis, demonstrate clearly that changes in various protein groups did occur during maturation. From the dry matter extraction, it can be concluded that the differences in the amino acid percentage in relation to the dry matter are due principally to quantitative

changes in the proteins of the various fractions. However, qualitative differences were also found. Significant changes in several amino acids were found in Fractions F_1 and F_2 and were indicated in other fractions. The marked decrease in the protein content from caput to cauda spermatozoa in the F_1 (0.01 N-NaOH extract) fraction is responsible for the decline in the percentage of the acidic and some of the neutral amino acids (such as leucine), all of which are present in high proportion in this fraction. The increase in the percentage of arginine in the total proteins is explained by the increase in the basic F_3 and $F_4 + F_5$ fractions, although the latter was not significant. This was attributed to the high arginine content in the proteins of those fractions. A slight increase of their quantity is sufficient to produce a marked elevation in the arginine percentage. This conclusion is supported by the findings of Berry & Mayer (1960) on the increase in the ratio between basic and acidic acids in the basic proteins during maturation. A similar reasoning seems to be acceptable for cystine, as its content in those fractions was much higher than in Fractions F_1 and F_2 .

From the results of Fractions F_4 and $F_4 + F_5$, it was obvious that the F_5 undissolved precipitate was rich in cystine and seemed to be a keratin-like protein. The presence of such a protein was postulated by Green (1940) and was indicated also by Zittle & O'Dell (1941), Berry & Mayer (1959) and Henricks & Mayer (1965b). Contrary to expectations, however, the percentage of cystine in this fraction was very low (1%). The fate of the 'residual' cystine, which was present in the $F_4 + F_5$ fractions, is not yet clear.

The electrophoretic separations of the extracted proteins support the findings on the amino acid composition of the same proteins, as both qualitative and quantitative differences in the individual protein bands between caput and cauda sperm cells were observed. These differences were found in those fractions which showed a different amino acid pattern for caput and cauda cells (F_1 , F_2 and F_4 , although the last is not significant), and might reflect the slight changes in the percentage of several amino acids in those proteins.

The decrease in the colour intensity of several bands in the electrophoretic separation of caput and cauda sperm proteins can be explained by the degradation of those proteins during maturation. A decrease in the amount of total lipids and of the main lipid fractions, particularly those connected in the lipo-protein complexes, occurs during sperm maturation in the epididymis (Scott, Dawson & Rowlands, 1963; Dawson & Scott, 1964; Grogan *et al.*, 1966; Pickett & Komarek, 1967; Pickett, Komarek, Gebauer, Benson & Gibson, 1967; Quinn & White, 1967; Scott, Voglmayr & Setchell, 1967; Scott & Dawson, 1968; Lavon *et al.*, 1970) and was assumed to be the result of their utilization as a source of energy in the absence of carbohydrates (Quinn & White, 1967; Scott *et al.*, 1967). It seems likely that a parallel degradation occurs in the related proteins during the maturation process.

The increase in the colour intensity of some bands and the appearance of new ones can be explained by two postulates: (a) the bands which seem to increase in proportion remain, in fact, constant in quantity, whereas the others decrease. This assumption does not explain the appearance of new bands during maturation. It is, however, possible that the new bands were present in very

small amounts and therefore could not be detected and identified in the caput, but later increased in proportion and appeared in the cauda sperm cells. It is also possible that they appeared as a result of protein degradation. (b) The synthesis of proteins continues during cell maturation. There is some evidence in the literature on the possibility of RNA and protein synthesis by ejaculated spermatozoa (Bhargava, 1957; Bhargava, Bishop & Work, 1959b; Abraham & Bhargava, 1963; Iype, Abraham & Bhargava, 1963). However, this synthesis was attributed by other investigators to bacteria contaminating the ejaculated cells and not to the spermatozoa themselves (Martin & Brachet, 1959; Setchell, Hinks, Voglmayr & Scott, 1967). The present study does not exclude the possibility of protein synthesis occurring during the passage of spermatozoa through the epididymis while they still possess cytoplasmic droplets.

From the results of this study, it can be concluded that the major changes in quantity, composition and electrophoretic behaviour of the sperm proteins are demonstrated in the best way in the F_1 (0.01 N-NaOH-extracted) fraction. The site of alterations is, therefore, most probably located in the lipoprotein complexes, e.g. in the cell membrane. In addition, minor changes appear to occur in the nucleoproteins.

ACKNOWLEDGMENTS

This study has been financed in part by a grant awarded by the United States Department of Agriculture under P.L. 480. The authors are indebted to Mr D. Zismann for his technical assistance.

REFERENCES

- ABRAHAM, K. A. & BHARGAVA, P. M. (1963) The uptake of radioactive amino acids by spermatozoa. *Biochem. J.* **86**, 308.
- ALFERT, M. (1956) Chemical differentiation of nuclear proteins during spermatogenesis in the salmon. *J. biophys. biochem. Cytol.* **2**, 109.
- BERRY, R. E. & MAYER, D. T. (1959) The isolation of some cellular constituents of bovine spermatozoa. *Exptl Cell Res.* **18**, 398.
- BERRY, R. E. & MAYER, D. T. (1960) The histone-like basic protein of bovine spermatozoa. *Exptl Cell Res.* **20**, 116.
- BHARGAVA, P. M. (1957) Incorporation of radioactive amino acids in the proteins of bull spermatozoa. *Nature, Lond.* **179**, 1120.
- BHARGAVA, P. M., BISHOP, M. W. H. & WORK, T. S. (1959a) The chemical composition of bull semen with special reference to nucleic acids, free nucleotides and free amino acids. *Biochem. J.* **73**, 242.
- BHARGAVA, P. M., BISHOP, M. W. H. & WORK, T. S. (1959b) Incorporation of [^{14}C] amino acids into the proteins of bull spermatozoa. *Biochem. J.* **73**, 247.
- BOUTERS, R., ESNAULT, C., SALISBURY, G. W. & ORTAVANT, R. (1967) Discrepancies in analyses of deoxyribonucleic acid in rabbit spermatozoa, involving Feulgen staining (Feulgen-DNA) and ultraviolet light absorption (UV-DNA) measurements. *J. Reprod. Fert.* **17**, 355.
- BOUTERS, R. & VANDEPLASSCHE, M. (1968) Vergleichende mikrospektrophotometrische Bestimmungen von Feulgen-DNS an epididymären, ampullären, ejakulierten und durch Massage gewonnenen Spermien von Bullen. *Zuchthygiene*, **3**, 1.
- BRIL-PETERSEN, E. & WESTENBRINK, H. G. K. (1963) A structural basic protein as a counterpart of deoxyribonucleic acid in mammalian spermatozoa. *Biochim. biophys. Acta*, **76**, 152.
- CLARE, N. T. & STEVENSON, A. E. (1964) Measurement of feed intake by grazing cattle and sheep. X. Determination of nitrogen in faeces and feeds using an auto-analyzer. *N.Z. J. agric. Res.* **7**, 198.

- DAVIS, B. J. (1964) Disc electrophoresis. II. Method and application to human serum proteins. *Ann. N.Y. Acad. Sci.* **121**, 404.
- DAWSON, R. M. G. & SCOTT, T. W. (1964) Phospholipid composition of epididymal spermatozoa prepared by density gradient centrifugation. *Nature, Lond.* **202**, 292.
- FELIX, K. (1960) Protamines. *Adv. Protein Chem.* **15**, 1.
- FISKE, C. & SUBBAROW, Y. (1925) The colorimetric determination of phosphorus. *J. biol. Chem.* **66**, 275.
- GLEDHILL, B. L., GLEDHILL, M. P., RIGLER, R., JR & RINGERTZ, N. R. (1966) Changes in deoxyribonucleoprotein during spermiogenesis in the bull. *Expl Cell Res.* **41**, 652.
- GREEN, N. W. (1940) The chemistry and cytology of the sperm membrane of sheep. *Anat. Rec.* **76**, 467.
- GROGAN, D. E., MAYER, D. T. & SIKES, J. D. (1966) Quantitative differences in phospholipids of ejaculated spermatozoa and spermatozoa from three levels of the epididymis of the boar. *J. Reprod. Fert.* **12**, 431.
- HENRICKS, D. M. & MAYER, D. T. (1965a) Characterization of the basic proteins associated with DNA in mammalian spermatozoa. *Proc. Soc. exp. Biol. Med.* **119**, 769.
- HENRICKS, D. M. & MAYER, D. T. (1965b) Isolation and characterization of a basic keratin-like protein from mammalian spermatozoa. *Expl Cell Res.* **40**, 402.
- HUNTER, A. G. & HAFS, H. D. (1964) Some physicochemical properties of saline-soluble proteins in bovine spermatozoa. *J. Reprod. Fert.* **8**, 243.
- INGLES, C. J., TREVITHICK, J. R., SMITH, M. & DIXON, G. H. (1966) Biosynthesis of protamine during spermatogenesis in salmonoid fish. *Biochem. biophys. Res. Commun.* **22**, 627.
- IYPE, P. T., ABRAHAM, K. A. & BHARGAVA, P. M. (1963) Further evidence for a positive role of acrosome in the uptake of labelled amino acids by bovine and avian spermatozoa. *J. Reprod. Fert.* **5**, 151.
- LAVON, U., VOLCANI, R. & DANON, D. (1968) Determination of dry matter percentage of ejaculated and epididymal bull spermatozoa after differential flotation. *J. Reprod. Fert.* **17**, 151.
- LAVON, U., VOLCANI, R. & DANON, D. (1970) The lipid content of bovine spermatozoa during maturation and ageing. *J. Reprod. Fert.* **23**, 215.
- LOWRY, O. H., ROSEBROUGH, N. J., FARR, A. L. & RANDALL, R. J. (1951) Protein measurement with the Folin phenol reagent. *J. biol. Chem.* **193**, 265.
- MANN, T. (1964) *The biochemistry of semen and of the male reproductive tract*, Chap. V and VI. Methuen, London.
- MARTIN, F. & BRACHET, J. (1959) Autoradiographic studies on the incorporation of amino acids into spermatozoa. *Expl Cell Res.* **17**, 399.
- MAYER, D. T., MADERA-ORSINI, F. & BERRY, R. E. (1957) Lipoprotein complex, nucleic acids and other constituents of bovine spermatozoa. *Fedn Proc. Fedn Am. Soc. exp. Biol.* **16**, 219.
- PICKETT, B. W. & KOMAREK, R. J. (1967) Lipid and dry weight of bovine seminal plasma and spermatozoa from first and second ejaculates. *J. Dairy Sci.* **50**, 742.
- PICKETT, B. W., KOMAREK, R. J., GEBAUER, M. R., BENSON, R. W. & GIBSON, E. W. (1967) Lipid and dry weight of ejaculated, epididymal and post-castrate semen from boars. *J. Anim. Sci.* **26**, 792.
- QUINN, P. J. & WHITE, I. G. (1967) Phospholipid and cholesterol content of epididymal and ejaculated ram spermatozoa and seminal plasma in relation to cold shock. *Aust. J. biol. Sci.* **20**, 1205.
- REISFELD, R. A., LEWIS, U. J. & WILLIAMS, D. E. (1962) Disc electrophoresis of basic proteins and peptides on polyacrylamide gels. *Nature, Lond.* **195**, 281.
- SCOTT, T. W. & DAWSON, R. M. C. (1968) Metabolism of phospholipids by spermatozoa and seminal plasma. *Biochem. J.* **108**, 457.
- SCOTT, T. W., DAWSON, R. M. C. & ROWLANDS, I. W. (1963) Phospholipids interrelationships in rat epididymal tissue and spermatozoa. *Biochem. J.* **87**, 507.
- SCOTT, T. W., VOGLMAYR, J. K. & SETCHELL, B. P. (1967) Lipid composition and metabolism in testicular and ejaculated spermatozoa. *Biochem. J.* **102**, 456.
- SETCHELL, B. P., HINKS, N. T., VOGLMAYR, J. K. & SCOTT, T. W. (1967) Amino acids in ram testicular fluid and semen and their metabolism by spermatozoa. *Biochem. J.* **107**, 1061.
- SNEDECOR, G. W. (1948) *Statistical methods*. Iowa State College Press, Ames, Iowa.
- SUMMERHILL, W. R. & OLDS, D. (1961) Levels of deoxyribonucleic acid in bovine spermatozoa and their relationship to fertility. *J. Dairy Sci.* **44**, 548.
- VENDRELY, R., KNOBLOCH, A. & VENDRELY, C. (1957) An attempt of using biochemical methods for cytochemical problems; the deoxyribonucleoprotein of spermatogenic cells of bull testis. *Expl Cell Res. Suppl.* **4**, 279.
- ZITTE, C. A. & O'DELL, R. A. (1941) Chemical studies of bull spermatozoa. Lipid, sulfur, phosphorus and nucleic acids of whole spermatozoa and of the parts obtained by physical means. *J. biol. Chem.* **140**, 899.