THE CHEMISTRY AND CYTOLOGY OF THE SPERM MEMBRANE OF SHEEP [1]

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ONE PLATE (FIVE FIGURES)

The quality of spermatozoa may be measured by cytological or biochemical methods. Each method furnishes desirable but incomplete information pertinent to any given sample. The rate of glycolysis of sperm has been shown to be rather highly correlated with certain measures of quality, such as duration of motility (Comstock, '39). In addition, it has been found to be negatively correlated with the number of sperm possessing abnormal heads (Green and Comstock, '39). However, types of micropathology were not indicated by the rate of carbohydrate consumption. The abnormal heads and tails could be classified only by cytological methods. Up to the present time, with the exception of the correlation provided by micropathology, general cytological methods have failed to give much information as to the physiological potencies of sperm. Accordingly it has become desirable to find a morphological feature of the sperm which would provide an index of the quality of sperm as measured by biochemical methods.

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This paper has been read and criticized by Dr. L. M. Winters. The author is also indebted to Dr. R. E. Comstock for the glycolysis determinations and statistical treatment of the material.

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The structure and properties of the permanent limiting membrane of ram's sperm were studied in an attempt to find a variable portion or structure which could be correlated with the physiological status of the sperm and with some biochemical assay of seminal quality. Some authors (Cody, '23; Braus and Redenz, '24; Belonoschkin, '34; Popa, '27, '30; Popa and Marza, '29) have reported the acquisition of various types of protective layers about the head of spermatozoa of various species. These layers were supposedly associated with the physiological well being of the sperm and were acquired during the sperm's existence in the extra testicular portion of the male tract. Some of the layers reported were transitional rather than permanent features of the cell. The membrane here discussed may be observed at any time during the sperm's existence, and it may be demonstrated by the use of hypotonic solutions and/or certain stains.

The chemical properties of sperm which have been reported indicate that they may be inert toward a variety of reagents. Lynch ('20) reported that whitefish sperm were not attacked by many strong chemicals. Van Herwerden ('16) stated that the portion of sperm not removed by 1% hydrochloric acid was not attacked by nuclease. Offergeld ('27) found sperm resistant to pepsin and trypsin. In addition to these chemical methods Marza ('30, '30 b, '31) has located the position of various types of protein in the sperm of many species by histochemical technics.

MATERIALS AND METHODS

Semen of the ram was used throughout the entire series of experiments. It was collected by the same method as described by Comstock ('39).

Routine stains and fixatives were made according to the directions given by Guyer ('30). The aceto-carmine stain was prepared by simmering certified carmine in 45% acetic acid for 1 hour, using a reflux condenser. Directions for the preparation of the stain were furnished by Dr. T. S. Painter

(personal communication). Slides were prepared by placing a small quantity of semen or washed sperm onto a slide flooded with saline. The formula for the saline was given by Winters et al. ('38). The density of the sperm was then adjusted so that only one or two cells would be visible in one oil immersion field. Following this, the slide was flooded with Bouin's fixative and, after 10 minutes, drained and placed into aceto-carmine stain overnight. Finally, the slides were washed in distilled water to remove excess stain and allowed to air dry. Observations were made without the use of cover slips; the oil was placed directly onto the slide. Unless otherwise stated, microscopic examinations were made with dark field illumination.

The material for chemical analysis was prepared from sperm obtained by the daily matings of a group of eight mature rams. Regular use of the males eliminated as much as possible changes which might have arisen due to infrequent or too numerous services. Immediately after gathering, the sperm were centrifuged, washed twice with saline and once with N/100 sodium hydroxide. The cells were then extracted in the dilute alkali for a period of 3 days. Frequent changes of the solution removed dissolved materials; chloroform was used as a preservative. The material was then washed with distilled water and extracted for 3 days with N/100 sulfuric acid. After this the residue was reextracted for 3 days in the N/100 alkali. Following final extraction, the residue was washed with water, alcohol, and ether. The ether was removed by vacuum. After drying the material in an oven for 5 hours at 100°C., it was stored over sulfuric acid until used. After this treatment, neither Harris' hematoxylin nor Heidenhain's iron-hematoxylin revealed any nuclear material. Most of the tails were lost and no bacteria or leucocytes could be found.

Qualitative tests indicated the presence of protamine in the sheep sperm. Because protamines form coacervates rather easily with other proteins, some of the sperm residue was further extracted with 1.8% sulfuric acid in an attempt to

eliminate any protamine which would be removed by the more concentrated acid, if such protamine were present. Further studies will be required to ascertain whether or not a coacervate was formed. However, studies and observations up to this time indicate that the residue from the above extraction represented, to a great extent, the protein of the membrane itself. Until further studies are made, the material obtained after extraction by N/100 sodium hydroxide and N/100 sulfuric acid (1.8% acid in some cases) will be considered only as the residue resulting from such treatment.

Van Slyke protein analysis was made following slight modifications of the methods as suggested by Narayana and Sreenivasaya ('28) and Cavett ('32). Because of the apparent resistance of the sperm to hydrochloric acid, the material was hydrolyzed for 36 hours. Amino nitrogen was determined by the methods given by Van Slyke ('11, '12, '13–'14, '15) and the total nitrogen was found by the peroxide method as outlined by Peters and Van Slyke ('32). In all nitrogen determinations, N/100 sodium hydroxide and hydrochloric acid were used. Cystine was determined by the Tompsett ('31) modification of the Folin and Marenzi method. Arginine values were secured by hydrolysis with 30% potassium hydroxide; the Holm ('20) apparatus was used. Morrow and Sandstrom ('35) recommendations for the above technics were also observed. For the amino-nitrogen determinations during enzymatic trials, a macro-digestion chamber was equipped with a micro-burette. Glycolysis was determined by the same (Warburg) method as was used by Comstock ('39). All analyses were made in duplicate or triplicate. Although small quantities of materials were used, in the vast majority of cases, differences between check runs fell within the limits of burette readings. Blank determinations were made on all chemicals.

EXPERIMENTAL RESULTS

A. Chemical studies. The membrane about the sperm was quite resistant to dispersion or solution. Its solubility was

tested by placing a small drop of semen in 10 to 15 ml. of each solvent. Microscopic examination of the sperm membranes was made after varying intervals. The degrees of solution were as shown in table 1. Desiccated sperm treated with boiling 20% hydrochloric acid gave the same results as did the fresh cells. Residue from extracted sperm reacted the same as fresh sperm when treated with boiling water for 8 hours.

TABLE 1

The visible effect of various reagents on the membrane of ram's sperm

REAGENT	TEMPERATURE	EFFECT
Distilled water	Room	No injury after 2 weeks' exposure except some nuclear material was extruded from the tip of some cells
Physiol. saline or 0.8% NaCl	Room	No injury after 2 weeks' exposure
10% NaCl	Room	Little effect after 24 hours—changes probably due to dehydration
70% ethyl alcohol	Room	Results similar to 10% NaCl—13 days' exposure
N/1 NaOH	Room	Little injury at end of 1 hour. Complete fragmentation after 24 hours
N/2 NaOH	Room	Same as N/1 NaOH
N/10 NaOH	Room	Very little injury at end of 18 hours—most membranes normal
N/100 NaOH	Room	Less injury than N/10 NaOH—membranes thin at anterior end injured most
Conc. HCl	Room	No injury after exposure of 7 days
20% HCl	Boiling	Membranes more or less intact at the end of 15 hours (fig. 2)—a few almost intact at the end of 24 hours
Distilled water 100°C.		No visible change at end of 11 hours.

No action on the membrane was noted when fresh, washed sperm were treated with the reagents used by Young and Inman ('38): 7.2% sodium benzoate, 4.0% potassium cyanide, 20.9% potassium thiocyanide, 8.0% sodium salicylate, 1.0% sodium bicarbonate, and 16% barium sulfide. Ten cubic centimeters of solvent and one small drop of sperm were used in each case above and the tubes were kept at room temperature for 1 week. It should be recognized that material could have been removed from the membrane without changing the

microscopic outline of the structure. An attempt was made to check the above solubilities by total nitrogen determinations, but due to the nature of the residue from extraction, the results were not reliable enough to report.

The membrane was also quite resistant to the action of pepsin and trypsin when measured by visual or chemical methods. In all cases, a KCl-HCl buffer system was used for pepsin and a KH_2PO_4-NaOH system for trypsin. For microscopic tests the solutions were buffered to a pH of 0.9 and 8.0, respectively, and the temperature was held constant at 37°C. for 24 hours. Qualitative tests were made to be sure the enzymes were active in the concentrations used. Sperm, fresh when introduced into the enzyme, were not injured by pepsin. However, sperm which had been allowed to die while stored as semen, under oil, at 4°C. were slightly injured.

TABLE 2

Activity of enzymes used for digestion of extracted sheep sperm (Time, 2 hours; temperature, 36°C.)

ENZYME	pH	DIFFERENCE BETWEEN DIGESTION TUBE AND CONTROL IN MG. OF AMINO NITROGEN
Pepsin	1.9	0.12
Trypsin	7.7	0.18

No action was noted by trypsin on either the fresh or dead cells.

The chemical determination of the enzymatic action was made by measuring the amount of amino nitrogen liberated (Van Slyke analysis) under standard conditions. The buffer systems were adjusted to a pH of 1.9 for pepsin and 7.7 for trypsin. The activity of the enzymes was determined by using 5 ml. of a 2% gelatin sol plus 15 ml. of buffer and 1 ml. of a 1% enzyme solution. Blank tubes were inactivated by placing them into a boiling water bath. The activity of the enzymes was as shown in table 2. The action of the enzymes on the residue from the extracted sperm was determined by adding to 100 ml. of buffer, 5 ml of a 1% enzyme solution and 10 mg. of residue material. An addition of 5 ml. of enzyme solution

was made at the end of 48 hours. The amount of amino nitrogen present in the 10 mg. sample was calculated from the results of a previous Van Slyke protein analysis. Blank flasks were inactivated in a boiling water bath. The results, calculated on a corrected volume basis, were as shown in table 3.

Van Slyke protein analyses of the residue from the extracted sperm were made to secure information relative to the basic amino acid components of the material. In general the differences between duplicate analyses were small (table 4). Some discrepancy was noted in the humin nitrogen determinations. This was due to some factor other than the period of hydrolysis which was exactly 36 hours in each case. The cause of the apparent loss of nitrogen in one phosphotungstic acid liquor total nitrogen was not ascertained. In a

TABLE 3

Action of enzymes on sheep sperm extracted with N/100 NaOH, N/100 H_2SO_4, and 1.8% H_2SO_4 (temperature, 37°C.)

ENZYME	pH	MG. AMINO N IN SAMPLE	DIFFERENCE BETWEEN DIGESTION AND CONTROL FLASKS IN MG. OF AMINO NITROGEN	
			24 hours	72 hours
Pepsin	1.9	0.6	0.0	0.1
Trypsin	7.7	0.6	0.0	0.0

second analysis of N/100 reagent extracted material, duplicate readings of 7.1% and 7.35% cystine nitrogen were obtained. The relative high (19.3%) nitrogen content of the material resulting from N/100 reagent extraction was constant for that treatment. Four different samples, gathered at different seasons of the year, gave the same result.

B. Microscopic studies. The membrane was impossible or very difficult to observe if direct illumination were used in conjunction with Harris' hematoxylin, Heidenhain's iron-hematoxylin (fig. 1), neutral red, acid fuchsin stain, acid fast stain, carbol fuchsin, Gram's KI and I, or phenolphthalein (0.1% in alcohol). Although some of these stains colored and made the membrane distinguishable when used with dark field illumination, none of them were very satisfactory. After the completion of the chemical analysis, which gave informa-

tion concerning the composition of the protein portion of the membrane, aceto-carmine stain was selected as a possible dye for the membrane.

This aceto-carmine stain and dark field illumination revealed a small, hyaline vesicular structure contained in the membrane at the anterior border of some cells. The structure found will, throughout the rest of the paper, be termed a "vesicle" because of its appearance. The vesicle was not

TABLE 4

Van Slyke protein analysis of extracted sheep sperm. Results are given in per cent of total nitrogen

	METHOD OF EXTRACTION—N/100 SODIUM HYDROXIDE PLUS N/100 H_2SO_4		N/100 H_2SO_4 + 1.8% H_2SO_4	
	dupl.	*dupl.*	*dupl.*	*dupl.*
Weight of sample, grams	0.3115	0.2976	0.1927	0.24
Per cent nitrogen	19.3	19.3	17.0	17.0
Ammonia N	7.04	6.98	6.82	7.2
Humin N	6.95	8.7	9.14	7.99
Total N phosphotungstic acid precipitate	57.95	57.71	59.55	60.09
Total N phosphotungstic acid liquor	25.47	21.00	23.89	23.85
Amino N phosphotungstic acid precipitate	17.18	17.19	17.30	17.30
Amino N phosphotungstic acid liquor	15.42	15.68	18.19	17.80
Cystine N	6.60	7.01	2.81	3.15
Arginine N	38.42	37.01	41.76	42.00
Histidine N	17.86	19.13	16.39	16.87
Lysine N	0.00	0.00	0.00	0.00
Per cent recovery	97.41	94.39	99.40	99.13

observed on sperm recovered directly from the testis and its time of formation or cytological derivation was not studied. Therefore, it would be unwise to use any existing nomenclature when referring to the structure.

The number of vesicles present in fresh or stored samples varied between rams and among samples from a given ram. Tables 5 and 6 indicate these differences and also give the rate of vesicular loss when sperm were aged under different conditions.

Apparently a larger proportion of the sperm exhibited the vesicle after sperm ascent through the female reproductive tract. However, only two ewes were available for this work and therefore the results could serve only as an indication

TABLE 5

Individual differences and the effect of aging at 4°C. on the frequency of the vesicular structure expressed in per cent of the sperm possessing the vesicle. Per cent per 150 "normal" cells

RAM NO.:	54	36	67	32
12/29/38 (sample secured)	66.66	50.60	38.66	35.33
12/30/38	42.70	31.10	24.00	22.00
1/1/39	41.9	28.00	18.00	20.66
1/2/39	16.66	6.66	5.33	10.66
1/3/39	4.00	2.66	4.66	4.00
1/4/39	0.00	0.66	1.33	0.66
RAM NO.:	54	36	67	32
1/13/39 (sample secured)	42.0	38.0	16.66	18.66
1/16/39	16.0	3.33	7.33	4.00
1/17/39	0.0	1.33	0.0	2.0
RAM NO.:	117	107	112	
1/13/39 (sample secured)	26.00	6.66	6.66	
1/16/39	3.33	2.66	5.33	
1/17/39	1.33	1.33	1.33	

TABLE 6

Numbers of vesicular structures per 150 cells at the beginning and end of glycolysis determinations

RAM. NO.	NUMBER OF VESICLES/150 CELLS	
	Beginning	End
1/18/39		
32	18	0
36	19	1
37	15	2
54	15	2
61	13	1
67	22	5
1/21/39		
32	29	4
33	34	2
36	25	3
54	29	1
55	28	4
67	21	5

and may not be considered as conclusive. Nevertheless, the differences were great enough to warrant mention. Semen was obtained and introduced into an estral ewe by means of artificial insemination. The ewe was destroyed 12 hours after insemination and the sperm immediately washed from the infundibulum. The differences between the number of vesicles present in the original sample and the recovered sperm were as shown in table 7.

Many authors have found that the quality of sperm as measured by fertility studies progressively decreased if one male was allowed to mate a series of females during a relatively short period. Three trials were conducted to find whether or not frequent ejaculations affected the number of vesicles in a series of samples. Two series are reported; the

TABLE 7

Vesicular structures present before and after sperm ascent of the female reproductive tract

EWE NO.	PER CENT WITH VESICLES PER 150 SPERM	
	In original sample	At the infundibulum
57	8.66	30.00
58	6.00	32.66

third, although not entirely satisfactory because of technical reasons, gave results essentially the same as the reported trials. Only one ram was used per series and it was mated to a diestral ewe at the time periods given in table 8.

Contrary to the effect of head abnormalities the presence of tail pathology did not affect the rate of appearance of the vesicle. Sperm with normal heads were divided into two groups: one which had normal tails and the other, the cells which exhibited tail pathology. As table 9 indicates, the per cent of vesiculated heads was the same in both groups. Of a total of 72,500 abnormal heads counted not one has possessed a vesicular structure.

Within normal limits, the time from collection to the making of the slides did not affect the count of vesiculated cells. A sample was recovered and slides were made 2, 5, 8, 15, 25 and

35 minutes after ejaculation. The number of vesicles varied within 2% with no regular deviation in the count from one slide to another.

Observations regarding the vesicle were: (1) it was not removed by the action of ether, chloroform, or 70% alcohol after an exposure of 48 hours at room temperature, (2) the

TABLE 8

Effect of frequency of ejaculation on the numbers of vesicular structures

SERIES	TIME OF EJACULATION	QUANTITY OF SEMEN	STRUCTURES/150 CELLS
1	9:43 A.M.	1.1 ml.	32
	10:45	1.5	37
	11:45	0.5	22
	12:33 P.M.	0.2	22
	2:00	0.3	14
	2:50	0.15	20
2	9:23 A.M.	1.0 ml.	30
	10:17	1.2	22
	11:18	1.0	18
	12:12 P.M.	1.0	9[1]
	1:15	0.8	17
	2:20	0.9	11
	3:20	0.8	12
	4:14	0.8	5

[1] This slide stained poorly, which may account for the low figure.

TABLE 9

Comparison of per cents of normal sperm and those exhibiting tail pathology which had the vesicle present. All sperm exhibited normal heads

	NUMBER OF SAMPLES	NUMBER OF SPERM	PER CENT OF HEADS HAVING VESICLE	
			Normal sperm	Sperm with tail *abnormalities only*
Series 1	47	23,500	19.94	19.66
Series 2	25	12,500	16.51	16.23

same type of vesicular structure has been observed on bull sperm and a modified structure on boar cells.

The evidence obtained in the above experiments indicated a possible relationship between the presence of the vesicular structure and certain phases of physiological activity of the sperm. Therefore, trials were conducted to ascertain whether

or not a correlation existed between the rate of glycolysis and the presence of the structure. The trials were conducted in three series; each contained 25, 48, and 24 samples, respectively. Because only six samples could be run at one time, each series had to extend over a sufficient number of consecutive days to include the total number of samples. Therefore, correlations are reported for both the total correlation for each series and for the groupings of runs within each series. In the first two series, 500 cells per sample were counted while 150 cells were tabulated for the last group. The reason for counting the larger number in the first groups was the presence of a larger per cent of all types of cellular

TABLE 10

Correlations between the per cent of vesicular structures × count of sperm in billions and glycolysis for three series of determinations

r	GROUP 1	2	3	WEIGHTED AVERAGE
All samples	0.54	0.69	0.699	0.659 ± 0.06
Within runs	0.594	0.655	0.894	0.698 ± 0.064
Necessary for P 0.01				
All samples	0.505	0.372	0.515	0.270
Within runs	0.59	0.418	0.684	0.316

pathology. For correlation studies, the per cent of normal sperm exhibiting the vesicle was multiplied by the sperm count in billions per ml. and this figure correlated to the rate of glycolysis as reported in cubic millimeters of CO_2 produced in 40 minutes. The correlations for all samples, for samples within runs, and weighted means were calculated according to methods given by Fisher ('36). Glycolysis determinations and sperm counts were made by the same methods as described by Comstock ('39). The correlations were as reported in table 10.

DISCUSSION

The membrane surrounding the sperm of rams has furnished, through its structure, a new cytological measure of sperm quality and it has also been found to possess a protein

type somewhat unusual when compared to other proteins of mesodermal origin.

The insolubility of the structure in a wide variety of protein solvents, the absence of a change to a water soluble substance when treated with hot water for a long period of time, and its resistance to enzymatic action would classify the protein of the sperm membrane as an albuminoid. Young and Inman ('38) arrived at a similar classification for the protein casings of salmon eggs. Because the sperm membrane protein contained no lysine, it could not be characterized as a keratin or pseudo-keratin as Young and Inman classified the salmon casing substance. The protein here reported was of interest for two reasons: (1) its dibasic amino acid composition, the absence of lysine, and (2) the fact that it was one of a few albuminoid proteins reported which had its origin from mesodermal tissue in contrast to the more common ectodermal derivation of similarly classified proteins.

The sperm here studied apparently differs from the cells of some other species in the arrangement of the membrane about the sperm. Frequently, the nucleus of the cell is said to be covered with a membrane which, although thin, is thickened at the base so that the posterior portion of the nucleus sits in a somewhat thicker sheath similar to an "acorn in its cup." The posterior portion of the membrane of ram's sperm is thicker but the upper portion is continuous and equal with the border of the cup region as is shown in figures 3, 4 and 5. The latter is a drawing of a sperm head in which the anterior portion of the membrane has been pulled from its original position adjacent to the nuclear membrane. On an enlarged scale, it shows features similar to the ones exhibited in figure 4: the basal cup, the continuity of the external surfaces of the basal and anterior membrane regions, the thinning of the membrane anterior to the cup, and the position of the vesicle located in the membrane at the extreme anterior border of the cell.

The vesicle underwent certain changes somewhat correlated with other differences of the cells. As the sperm aged and

the vesicles diminished in frequency, the thinner anterior portion of the membranes thickened to a size comparable to that of the basal region. After the membrane had changed in this way, no vesicle was observed on the cell and the anterior end of the sperm was symmetrical and smooth in contrast to the contour when the vesicle was present. The vesicle always projected beyond the arc described by the anterior portion of the nucleus and the portion of the membrane base which was adjacent to it. In addition, the sperm tended to stain with less intensity and the basal cup was less pronounced either in samples which were reduced in the per cent of vesicles as a result of aging or in fresh samples which contained a lower initial number of vesicles. If the composition of the membrane changes as the physiological potencies change, then the differences in the reaction of the structure toward the stain may indicate chemical difference which may accompany the physical changes that do take place.

The exact role of the vesicle in the physiological activity of the cell has not been completely determined. This membrane about the cell was quite tough but also elastic. Some cells placed in hypotonic solutions or distilled water exhibited a swelling and when replaced into isotonic solutions, the membrane, if swelled beyond its limits of elasticity, would remain away from the nucleus in a circumjacent position. On the other hand, cells which were thin at the anterior border did not exhibit this result but nuclear material would extrude from the end and only the end of the cell, indicating a rupture of the membrane at that point. In addition, some reagents attacked the cell first at the anterior border if the membrane was thinner at that point. Because of physical differences, the anterior, vesiculated region may play a more or less dynamic role in phenomena such as osmotic changes, permeability, or possible mechanical or enzymatic action associated with fertilization. Although the vesicle's exact activity during fertilization is not known at the present time, it may be said that samples having a larger per cent of vesicles are more desirable than other samples because of the correlations now

found with certain physiological conditions of the sperm and the results of breeding tests now being conducted.

The correlation between the presence of the vesicles and the rate of glycolysis presents a technic for cytological evaluation of at least one if not two (glycolysis and respiration) biochemical measures of sperm quality. In addition to the ninety-seven samples reported here, other series of comparisons to be reported later have essentially the same correlation (0.7) between the rate of glycolysis and the number of vesicles. In addition to the relationship between the cytological and biochemical measures, the aceto-carmine technic further furnishes a method by which cellular pathology may be evaluated at the same time as counts are made for the vesicles. In this way a more complete picture of the sample may be secured.

The aceto-carmine technic is not offered as a substitute for glycolysis determinations. It should be used to supplement such readings. For the proper evaluation of sperm, both microscopic and biochemical methods are necessary and closer comparisons between the two methods will be desirable for future study and for a rational development of methods for sperm evaluation.

SUMMARY AND CONCLUSIONS

1. The membrane about the ram's sperm has been described. It stained poorly with some of the more common stains and was very difficult to observe if direct illumination was used.

2. It was visibly insoluble in many protein solvents. Trypsin did not attack it and only a slight action was noted when pepsin was used. Because of its properties, the protein of the membrane may be characterized as an albuminoid.

3. A Van Slyke protein analysis on the material remaining after the described extraction process indicated a high nitrogen content, high arginine, histidine, and cystine content, and an absence of lysine.

4. The use of aceto-carmine stain and dark field illumination made visible a small, hyaline vesicle located in the mem-

brane at the anterior portion of the sperm's head. The per cent of vesicles present varied between samples secured from different rams and among samples from any given ram. As sperm quality was apparently lowered due to aging of the cells or frequent ejaculations, the number of vesicles was lowered. Some evidence was found which favored a larger number of vesiculated cells reaching the infundibulum in a given time. Tail pathology did not alter the per cent of cells exhibiting the vesicle but no cell with an abnormal head has borne a vesicle.

5. A correlation of 0.7 was found between the number of vesicles present in a sample and the rate of glycolysis as measured in CO_2 production.

6. The sheep sperm membrane does not fit the "acorn in the cup" description.

7. The use of aceto-carmine stain and dark field illumination is suggested as a new technic for the evaluation of (sheep) sperm quality.

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PLATE 1

EXPLANATION OF FIGURES

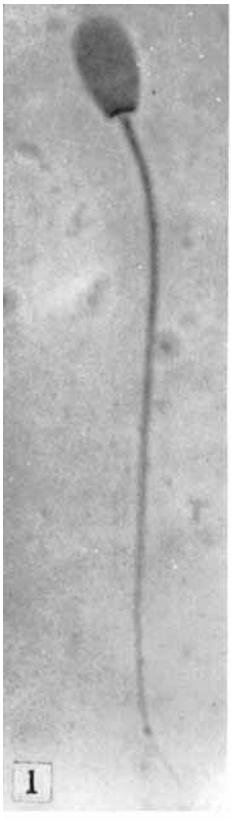
1 Sheep sperm; Bouin's fixative, iron-hematoxylin stain. × 1720.

2 Sheep sperm after treatment with boiling 20% hydrochloric acid for 15 hours. Unstained, dark field illumination. × 1825.

3 Sheep sperm; Bouin's fixative, aceto-carmine stain. The vesicular structure appears as a clear drop-like protrusion at the anterior border of the head. The tail is abnormally curved. Dark field illumination. × 2250.

4 Normal sheep sperm; Bouin's fixative, aceto-carmine stain. The vesicle and membrane continuity may be noted. Dark field illumination. × 900.

5 Drawing of sheep sperm head showing the relation of the various membrane structures.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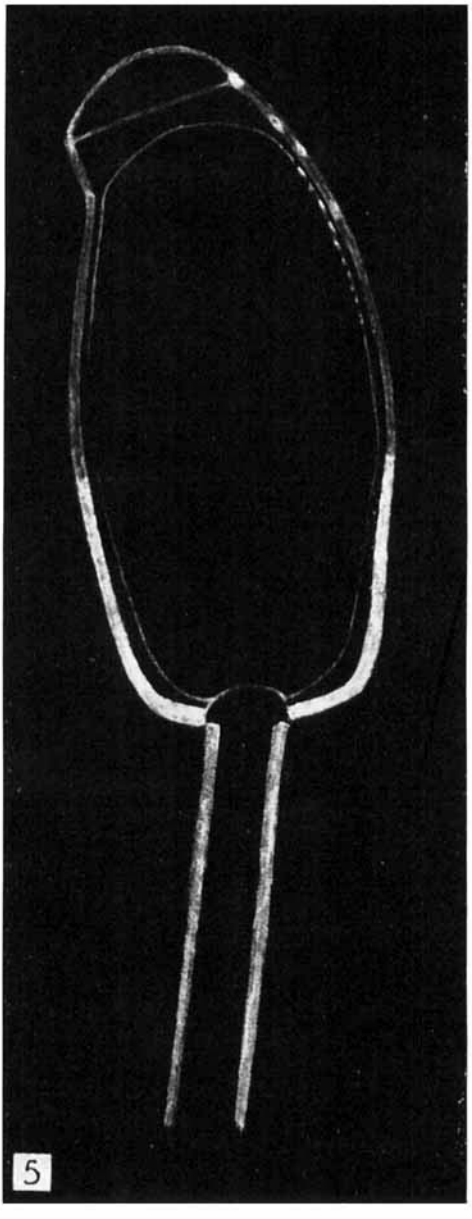
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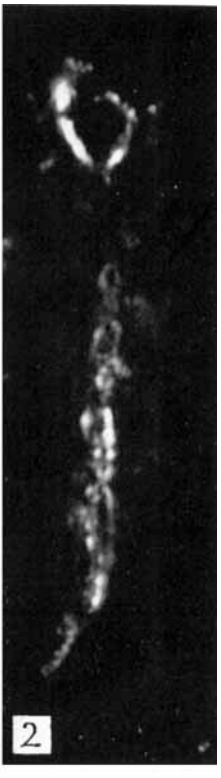
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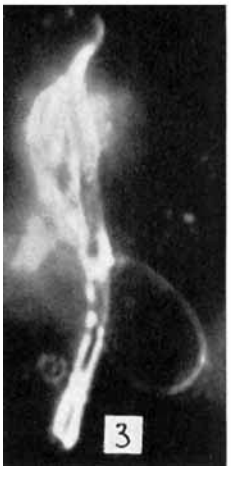
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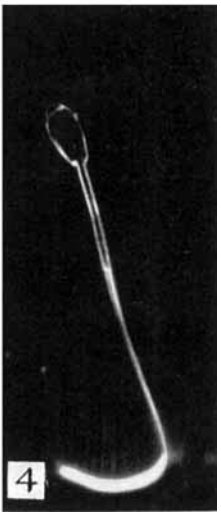
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