Technical Papers

Electron Microscopic Study of Spermatozoa

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The problems of interest in sperm morphology are chiefly the head components, the filaments and fibrils that constitute the axial fiber, and the "brush effect" at the end of the sperm tail. No attempt will be made here to review the work of previous investigators (*1–15*). This report presents electron micrographs that may, it is thought, bring about a clearer conception of the fibrils near the head-neck junction of ram spermatozoa than those previously published, and it introduces NaOH as an effective reagent for denuding the spermatozoa for morphological investigations.

The semen specimen was clarified by washing with 33% alcohol, and centrifuging; it was washed and centrifuged again, and then filtered. The spermatozoa left on the filter paper were suspended in distilled water (1 to 2 million spermatozoa/ml). To reveal the fibrils of the spermatozoa, the membranes that surround the fibrils were removed by adding various amounts of NaOH solution to tubes containing the sperm suspension and were kept at 5° C for 12 to 72 hr. At the end of the treatment the NaOH was removed by centrifugation and the spermatozoa were again suspended in distilled water. They were then shadowed with chromium at an angle of 20° to the horizontal and observed with an RCA Model EMU-2D electron microscope.

The results obtained in the electron micrographs show that spermatozoa can be denuded by suitable concentrations of NaOH. The degree of disintegration of the sperm cells can be adjusted by varying the concentration of the reagent and the length of the reaction time. The electron micrographs provide evidence to substantiate the observations of Randall and Fried-

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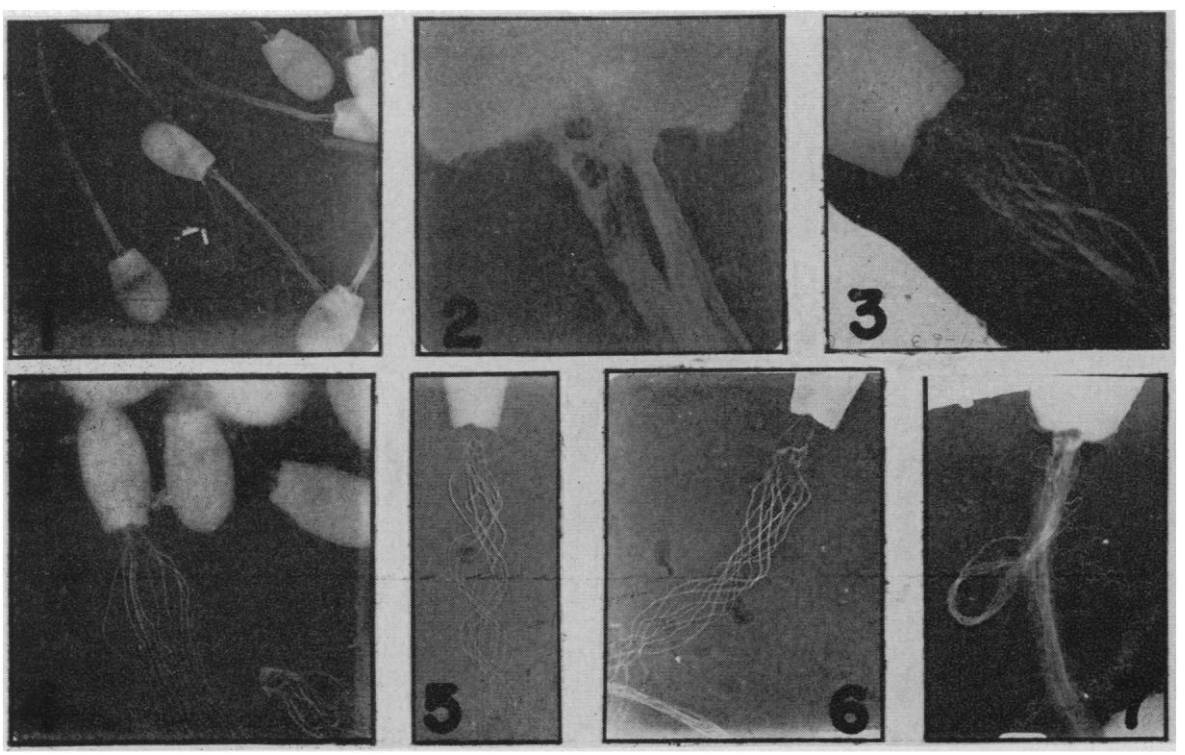

FIGS. 1–6. Ram spermatozoa treated with various concentrations of NaOH for 68 hr at 5° C. *1*, in 0.025 *N* NaOH, showing disintegrated membrane and loose fibrils in the neck, midpiece, and tail region (× 1980); *2*, in 0.025 *N* NaOH, showing partially denuded neck region, fibrils within the surrounding helix are clearly visible (× 16,800); *3*, in 0.15 *N* NaOH, showing denuded spermatozoa with 9 fibrils clearly visible up to the neck region; and a group of the fibrils near the neck region still remaining in the surrounding helix (× 7800); *4*, in 0.15 *N* NaOH, showing further denuded spermatozoa with 9 fibrils clearly visible up to the neck region (× 3600); *5* and *6*, in 0.15 *N* NaOH, showing denuded spermatozoa with 9 fibrils clearly visible (× 3600).

FIG. 7. Rabbit spermatozoa treated with 0.045 *N* NaOH for 12 hr at 5° C showing partially denuded spermatozoa with fibrils visible through the remaining membrane (× 4800).

laender in that the axial fibrils of ram spermatozoa are continuous from the head through the neck to the midpiece. In addition, the micrographs show clearly that there are 9 fibrils up to the head-neck junction in ram spermatozoa. The axial filaments that constitute these fibrils and the microstructure of spermatozoa from other species are under investigation. Detailed results will be reported later.

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A Fungistatic Substance Extracted from Vitrain

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In 1951 W. D. Evans (*1, 2*) reported the presence of a bacteriostatic substance in vitrain in English coals. He named it vitricin because of its occurrence in the vitrainous parts of coal seams. He demonstrated that it inhibited *Bacillus subtilis* and attributed differences in prevalence of pneumoconiosis among mine workers to differences in the amount of vitrain coal in different mines: the higher the percentage of vitrain coal in a mine, the lower the incidence of pneumoconiosis among the miners.

Vitrains from southern and northern Illinois and Wyoming coals were examined by a chromatograph technic similar to that used by Evans. The coal from northern Illinois was No. 2 coal, C rank, from Kankakee County, that from southern Illinois was No. 6 coal, B rank, from Franklin County, and that from Wyoming was a Hanna Basin coal. The examination of these coals revealed that all of them contained soluble material fungistatic in nature (Table 1). Subsequently R. M. Kosanke (*3*) of the Illinois State Geological Survey modified Evans's extraction technic in several ways and extracts obtained by these methods were tested for their fungistatic capacities. The method

TABLE. 1. Inhibition of *Endoconidiophora fagacearum* by extracts of vitrains. Measurements represent the maximum widths and lengths of the areas of inhibition around the extract-treated filter paper strips.

Origin of coal	Clear area measurements	
	Width (mm)	Length (mm)
Illinois Coal—Franklin County	11	58
Illinois Coal—Kankakee County	6	20
Wyoming Coal—Hanna Basin	17	76

used in obtaining the extract reported on below is briefly as follows. Vitrain, after being ground into particles of small size, is washed several times with a mixture of equal parts of acetone and methanol. The liquid resulting from this elution is then poured into a Coors porcelain evaporating dish. A sheet of Whatman No. 1 filter paper $2\frac{1}{4} \times 5\frac{1}{2}$ in. is suspended so that about $\frac{1}{2}$ in. of the paper is submerged in the liquid. The liquid is absorbed by the filter paper and, as the solvents evaporate from the filter paper, a chromatograph is produced that has several distinct bands of light and dark brown.

Two such chromatographs developed from Hanna Basin vitrain coal of Wyoming were tested against eight fungi: *Aspergillus niger, A. terreus, Myrothecium verrucaria, Glomerella cingulata, Colletotrichum trifolii, Verticillium albo-atrum, Endoconidiophora fagacearum,* and *Ceratostomella ulmi. A. niger, A. terreus,* and *M. verrucaria* are saprophytes. *G. cingulata* causes bitter rot of apples; *C. trifolii,* anthracnose of clover. The remaining three are vascular parasites of plants; *E. fagacearum* and *C. ulmi* being highly virulent pathogens respectively of oak and elm, *V. albo-atrum* a destructive pathogen of various trees, shrubs, and herbs.

To test the potency of the material deposited in the chromatograph the following procedure was used. Eight milliliters of potato dextrose agar, pH 5.6, were poured into a sterile Petri dish. After the agar had solidified, the surface was thinly flooded with a suspension of spores obtained from young cultures grown on test tube slants of potato dextrose agar, pH 5.6, at 25° C. A strip $2\frac{1}{4}$ in. wide was cut from the top of the chromatograph, as it contained none of the fungistatic material, and the remainder, $3\frac{1}{4}$ in. long, was cut vertically into strips 3/16 in. wide. After allowing sufficient time for the water of the spore suspension to be absorbed by the agar, a strip chosen at random was placed on the surface of the agar. The Petri dish was then incubated at 25° C and observed at intervals. The test for each fungus was run in triplicate.

That the extract in the chromatograph inhibited spore germination was shown by the maintenance on either side of the chromatograph strip of clear areas into which the fungistatic material had diffused in sufficient amount to prevent germination of the spores. The diagrams, Fig. 1, show the patterns of inhibition