Scanning electron microscopic observation of sperimophage cell within the lumen of the epididymal duct of the vasectomized Japanese monkey (Macacus fuscatus)

M. Murakami, T. Shimada and K. Suefuji

Department of Anatomy, Kurume University School of Medicine, Kurume 830 (Japan), 30 December 1976

Summary. The spermiophagic process by intraluminal macrophages in the epididymal ducts of the vasectomized Japanese monkey was well visualized with scanning electron microscope. The fragments of disintegrated spermatozoa were seen in the phase enveloped in bulk by flap-like cytoplasmic extensions or in the phase ingested within the cytoplasm.

Intraluminal phagocytosis of spermatozoa by macrophages has recently been reported in the epididymal ducts after surgical or pathological obstruction of the deferent duct in several mammals[1–5]. The similar spermiophagy in the epididymal lumen has also been demonstrated, even in normal animals including men[6,7]. However, the observation concerning the spermiophagy hitherto reported was mainly carried out on tissue sections following light or transmission electron microscopy, and the description using technique of scanning electron microscopy which is particularly useful in visualizing the 3dimensional detail of phagocytosis has not yet been widely reported. Therefore, in the present study the spermiophagy by macrophages in the epididymal duct of the vasectomized Japanese monkey is observed with scanning electron microscope.

Materials and methods. 2 healthy adult Japanese monkeys (Macaca fuscata) weighing 8–12 kg were anesthetized with nembutal and vasctomized bilaterally. As vasectomy the epididymal extremity of the deferent duct was doubly ligated with silk and divided 1 month before the experiments. The same type of ligation was also placed around the efferent duct to prevent the transfer of the newly produced spermatozoa from the seminiferous tubules into the epididymal duct. The animals were sacrificed by vascular perfusion with Karnovsky's aldehyde agent[8]. Blocks of the tissues obtained from the initial, middle and terminal segments of the epididymal duct thus treated were immersed in the same agent for 5 h and gently washed several times in physiological saline solution to

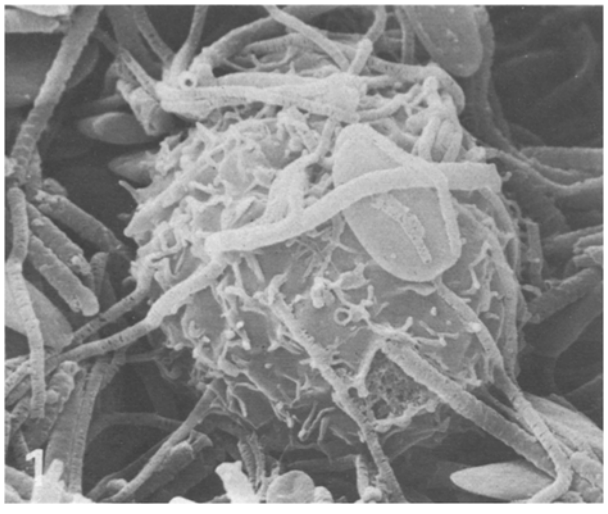

Fig. 1. Surface of an intraluminal macrophage viewed 30 days after vasectomy. ×4600.

1 A. M. Phadke, J. Reprod. Fertil. *7*, 1 (1964).
2 N. J. Alexander, J. Reprod. Fert. *31*, 399 (1972).
3 D. J. Flickinger, Biol. Reprod *13*, 50 (1975).
4 A. P. Hoffer, D. W. Hamilton and D. W. Fawcett, J. Reprod. Fert. *44*, 1 (1975).
5 L. H. Hoffman, N. Jahad and M. C. Orgebin-Crist, Cell Tiss. Res. *167*, 493 (1976).
6 J. D. Roussel, O. T. Stallcup and C. R. Austin, Fert. Steril. *18*, 508 (1967).
7 A. F. Holstein, Naturwiss. *54*, 89 (1967).
8 M. J. Karnovsky, J. Cell Biol. *27*, 137 (1965).

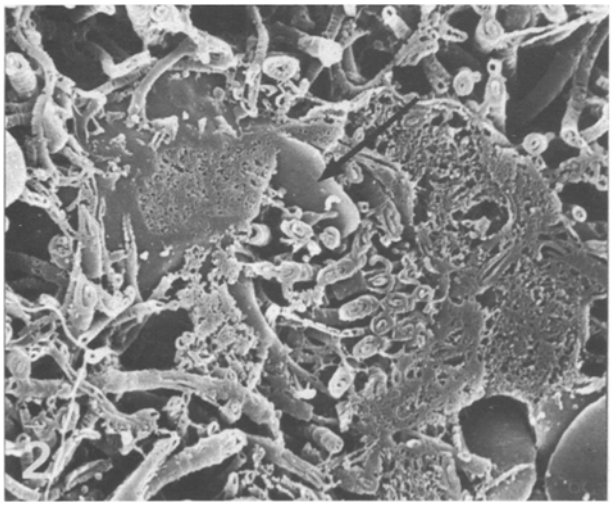

Fig. 2. Fractured surface of an intraluminal macrophage showing ingestion of fragments of spermatozoa by its flap-like cytoplasmic extensions. Arrow indicates an engulfed sperm head. ×4500.

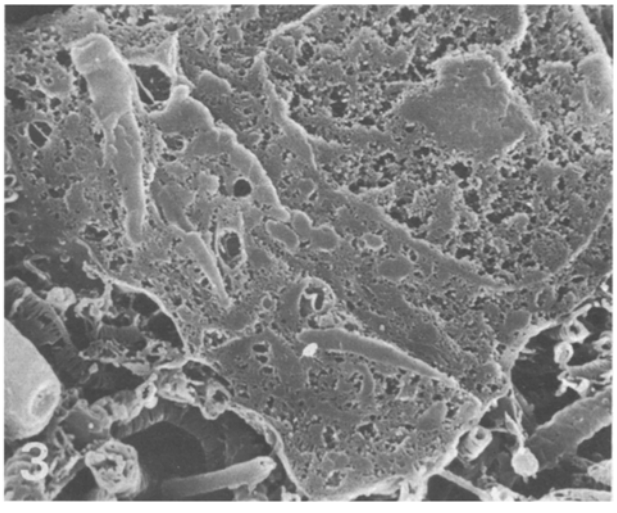

Fig. 3. Fractured surface of an intraluminal macrophage containing ingested sperm heads within its cytoplasm. ×8200.

rinse away mucus in the epididymal lumen. The specimens were postfixed in osmium tetroxide for 2 h, dehydrated in an ascending acetone series and dried by the critical point method using carbon dioxide. They were then coated with gold by ion sputter coating method and viewed in a Hitachi field emission scanning electron microscope (HFS-2). The remaining half of the specimens was fractured by frozen resin cracking method prior to the critical drying.

Results and discussion. In the epididymal ducts of the vasectomized animals, there are fragmented or disintegrated spermatozoa in a high concentration. The macrophages are present here and there among these disintegrated spermatozoa. The macrophages vary in shape from spherical to flattend, probably depending upon functional stage, and possess short ridge-like microvilli loosely distributed on their surface to which the fragmented portions of disintegrated spermatozoa adhere (figure 1). The fractured surfaces of the specimens reveal various stages of spermiophagy by the macrophage. Macrophage seen in figure 2 is relatively irregular in shape and engulfes in bulk such fragments of disintegrated spermatozoa as head and tail by flap-like cytoplasmic extensions. Figure 3 shows a surface of another macrophage fractured through the nucleus. This macrophage is more spherical than that in figure 2 and has no prominent cytoplasmic process. Its cytoplasm contains a number of fusiform inclusions suggestive of ingested sperm heads in addition to many spherical bodies presumably corresponding to lysosomes or residual bodies. The macrophage shown in figure 3 may be in more advanced stage of phagocytosis than that in figure 2. There is no morphological evidence for uptake of spermatozoa by epithelial cells in any region of the epididymal duct. The results indicate that disposal of disintegrated spermatozoa in the epididymal duct of the vasectomized Japanese monkey may be managed mainly, although not only, by macrophages appeared in the lumen as the results of the reports on this problem so far. The origin of the macrophage remains still obscure[1, 2, 4].

Recombination of integral and peripheral protein fractions from human red cell membrane with homologous lipids

M. Barzilay, A. de Vries and Eleonora Condrea

Rogoff-Wellcome Medical Research Institute, Tel-Aviv University Medical School, Beilinson Medical Center, Petah Tikva (Israel), 24 December 1976

Summary. Integral and peripheral protein fractions from human red cell membranes were recombined with a total red cell lipid extract and with homologous lipids in varying mixtures, by dialysis from 2-chlorethanol solutions. The 2 protein fractions were compared for lipid binding capacity and for selectivity towards individual lipids.

Red blood cell membrane proteins and lipids can be dissociated and recombined, in a variety of experimental conditions, to form membrane-like structures which lack enzymatic activities[1–5]. Membranes which are formed by dialysing 2-chlorethanol (2-CE) solutions of membrane proteins and lipids against neutral aqueous buffer, possess but few intramembranal particles[6]. It has been shown, however, that the membrane protein structure is preserved sufficiently to retain lipid-binding specificity[7].

The membrane proteins have been classified as integral and peripheral[8, 9]. While the peripheral proteins interact weakly with lipids, the integral proteins exhibit strong hydrophobic interactions with the lipid bilayer whom they penetrate[8–11]. In this report we present a comparative study on the lipid-binding capacity and selectivity for lipid of the integral and peripheral protein fractions from human red cell membranes. We compared, for each protein fraction, the binding of charged and uncharged phospholipids and of neutral lipid, both from a total red cell lipid extract and from varying mixtures.

Materials and methods. Ghosts were prepared from human blood stored in acid-citrate-dextrose for 22–23 days according to the method of Fairbanks et al.[12], the 5 mM sodium phosphate buffer (pH 8) being supplemented with trasylol (19 kIU/ml). Peripheral proteins were obtained by successively extracting the ghosts with 1 mM EDTA[12] and H_2O at pH 11[13]. The proteins in the combined extracts were precipitated with ammonium sulphate at 50% saturation, freed from ammonium sulfate by dialysis, lyophilized and stored as dry powder at 4 °C. The peripheral proteins contained 0.0197 μmoles N-acetylneuraminic acid per mg protein. Acrylamide gel electrophoresis showed the predominance of protein bands I, II and V (figure).

The integral protein fraction was prepared from the residual ghost fragments by extraction with 8 M urea-1 mM EDTA (pH 8) – 1% β-mercaptoethanol[13]. Following dialysis, the pellet was delipidated[13]. Integral proteins were stored as a dry powder at 4 °C. This fraction contained 0.1222 μmoles N-acetylneuraminic acid per mg protein, and residual phosphatidylserine. Acrylamide gel electrophoresis shows the predominance of band III in this fraction (figure).

1 R. F. A. Zwaal and L. L. M. Van Deenen, Chem. phys. Lipids *4*, 311 (1970).
2 D. Schubert, J. Poensgen and G. Werner, Hoppe-Seylers Z. physiol. Chem. *353*, 1034 (1972).
3 D. Schubert, Hoppe-Seylers Z. Physiol. Chem. *354*, 781 (1973).
4 M. Barzilay, E. Condrea, E. Ben-David and A. de Vries, Biochim. biophys. Acta *311*, 576 (1973).
5 P. Zahler and E. R. Weibel, Biochim. biophys. Acta *219*, 320 (1970).
6 E. Wehrli, S. Moser and P. Zahler, Biochim. biophys. Acta *426*, 271 (1976).
7 R. Kramer, Ch. Schlatter and P. Zahler, Biochim. biophys. Acta *282*, 146 (1972).
8 S. J. Singer, A. Rev. Biochem. *43*, 805 (1974).
9 S. J. Singer and G. L. Nicolson, Science *175*, 720 (1972).
10 T. L. Steck, J. Cell Biol. *62*, 1 (1974).
11 R. F. A. Zwaal, B. Roelofsen and C. M. Colley, Biochim. biophys. Acta *300*, 159 (1973).
12 G. Fairbanks, T. L. Steck and D. F. H. Wallach, Biochemistry *10*, 2606 (1971).
13 R. J. Juliano and A. Rothstein, Biochim. biophys. Acta *249*, 227 (1971).