

proteolytic enzymes<sup>11</sup>. Thus it is possible that the *Glycine* agglutinin reacts with the ground structure of the MN blood groups<sup>12</sup>. The present communication is intended briefly to report that an agglutinin resembling anti-T is also present in the betel nut (*Areca catechu* Linn).

The *Areca* agglutinin, like that of *Glycine*, strongly agglutinates human erythrocytes treated with neuramidi-

nase or with papain. It differs from the *Glycine* agglutinin in that it does not agglutinate rabbit erythrocytes or untreated human red cells, and is not inhibited by *d*-galactose and related sugars.

Absorption studies show that a single agglutinin acts on both papainized and neuraminidase-treated erythrocytes. It is hoped that the *Areca* agglutinin will also find application in charting the agglutinin receptors of the erythrocyte membrane<sup>13</sup>.

Comparison of *Glycine soja* and *Areca catechu* agglutinins

Agglutination of red cells	Agglutinins from <i>Glycine soja</i>	<i>Areca catechu</i>
Rabbit	+++	–
Human, untreated (tested at 4°C)	++	–
Human, papainized	+++	+++
Human, neuraminidase treated	+++	+++
<i>Inhibition by sugars</i>		
<i>d</i> -Galactose	inhibited	not inhibited
Lactose		
Raffinose		
<i>l</i> -Arabinose		
<i>d</i> -Glucose	not inhibited	not inhibited
Sucrose		
Maltose		
Mannose		
Mannitol		
Dulcitol		

*Zusammenfassung.* Agglutinine aus Samen der *Areca catechu* Linn reagieren mit (1) dem T-Antigen und (2) einem Rezeptor der Erythrocytenoberfläche, der nach Behandlung mit Papain in Erscheinung tritt.

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Electron Microscopic Observations on Phagocytosis of Rabbit Spermatozoa in the Female Genital Tract

Uterine leukocytic infiltration occurs during estrus and following mating<sup>1,2</sup>. A similar response can be noted after estrogen stimulation<sup>3,4</sup>. Mobilization of leukocytes with phagocytosis of foreign bodies such as bacteria<sup>5</sup> and starch granules<sup>6</sup> has also been demonstrated in the uterine cavity. CHANG<sup>7</sup> and AUSTIN<sup>8</sup> have shown that many of the unfertilized spermatozoa are eliminated from the female genital tract by the phagocytic action of leukocytes. This report concerns light and electron microscopic observations on the mechanism of leukocytic phagocytosis of spermatozoa in rabbit uteri.

New Zealand female white rabbits, weighing between 4 and 5 kg, were housed separately from males for three weeks prior to the experiment. Half of the female rabbits were ovulated 12 h before operation with an intravenous injection of 500 IU of chorionic gonadotropin (Ayerst Laboratories, New York, N.Y., USA).

Ejaculates of sperm were collected from mature male rabbits weighing in excess of 5 kg. The volume of the ejaculates ranged between 0.8 and 2.0 ml, and the sperm counts were between 95 and 850 million per ml. Between 80 and 85% of the sperm showed good motility. Samples

were studied with light and electron microscopy and no morphological abnormality was observed. Several specimens of spermatozoa were gently washed three times with calcium-free Krebs-Ringer solution at room temperature, and aliquots of the washed sperm were diluted to 2 ml in calcium-free Krebs-Ringer solution before injection.

In all surgical procedures, animals were anesthetized with 2.3 to 3.0 ml of Diabutol (Diamond Laboratories, Des Moines, Iowa). At operation the sperm were injected slowly with an 18 gauge needle into the lumen of the uterus at a point 1 cm distal to the bifurcation of the cervix. Approximately 45 to 220 million sperm were injected into each uterine tube. After varying periods of time, the uterine tubes were surgically removed and the lumina gently flushed with cold isotonic osmic acid. The

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<sup>3</sup> W. G. SPECTOR and E. STORY, *J. Path. Bact.* 75, 387 (1958).  
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<sup>6</sup> J. KILLINGBECK and G. E. LAMMING, *Nature* 198, 111 (1963).  
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<sup>8</sup> C. R. AUSTIN, *J. Endocrinol.* 14, 335 (1957).

flushings were fixed in 1% osmium tetroxide in an isotonic pH 7.3 phosphate buffer and centrifuged at 180 rpm for 10 min at 2°C. The sediment was embedded in Vestopal W and cut at 250–400 Å. The sections for electron microscopy studies were contrasted with saturated uranyl acetate and lead monoxide. Electron microscopy of the sediment was carried out at 3500 to 16,000 magnification.

Flushings from all of the uterine tubes showed large accumulations of polymorphonuclear neutrophilic granulocytes, many fragmented sperm, occasional epithelial cells and platelet thrombi.

The majority of sperm underwent degradation and displayed fragmentation outside of the polymorphonuclear neutrophilic cell membrane prior to phagocytosis. Dislocation of the sperm head from the midpiece was the most frequent site of fragmentation (Figure 1). During phagocytosis of the midpiece, the cell membrane, mitochondrial sheath and axial fibers remained intact (Figure 2), although the tails were fragmented into varying lengths. All sperm portions (head, midpiece, tail fragments) were observed to undergo phagocytosis by polymorphonuclear leukocytes.

Sperm phagocytosis was observed when the uterus was securely ligated at both the uterocervical and the uterotubal junction. Phagocytosis occurred earlier in rabbits where the uterus was securely ligated at both ends when compared to the animals with untied uteri. Injection of either crude ejaculate or washed sperm into the uterine lumen evoked a definite leukocytic response and subsequent phagocytosis. Our experiments demonstrated significant leukocytic response to injection of spermatozoa without supplementation of estrogen or artificial ovula-

tion. No apparent hormonal stimulation need be present for this phenomenon to occur since we have observed sperm phagocytosis in ovulated and non-ovulated rabbits. From these observations we would conclude that intrauterine phagocytosis is a non-specific foreign body reaction responding to a variety of particulate matter, including spermatozoa. It is also apparent that a small number of motile spermatozoa successfully evade phagocytosis preceding and following the process of fertilization<sup>9,10</sup>.



Fig. 1 (upper left). Fragmentation of sperm head from midpiece prior to phagocytosis.  $\times 12,750$ .



Fig. 2 (upper right). Phagocyte ingesting a segment of sperm midpiece (arrow). Note the segments of sperm tail already engulfed in the cytoplasm, and sections of sperm head, midpiece and tail in the extracellular fluid.  $\times 13,125$ .

**Résumé.** La phagocytose intrautérine est un phénomène physiologique et non spécifique qui se produit par la présence de divers corps étrangers y-compris les spermatozoïdes.

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