cedural artifacts, and that vasectomy performed in the adult rat does not result in the extensive morphological changes reported for the immature rat.

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4. In this technique, a 1.0-cm midline incision is made 0.5 cm posterior to the penis, followed by a laterally adjacent 0.5-cm incision in the testicular sac. The testis is eased through the retracted incision and its length measured. The ductus deferens is then carefully dissected from its sheath and from the deferential blood vessels and ligated with silk at two points 0.6 cm apart, and 0.4 cm of the duct is removed from between the sutures. The testis is replaced and the incision closed with individual silk sutures, as is the skin incision after the procedure is repeated on the contralateral side. Intramuscular injections of penicillin and streptomycin are administered once a day for 3 days to reduce the probability of infection.
5. Testis length rather than weight was used as the primary measure of testis size because it allows for pre- and postsurgical comparisons. Pearson product moment correlations, calculated for the 20 animals from which both measures were obtained, were .71 for vasectomized rats ($P < .01$) and .54 for controls ($P < .05$). Since the ratio of length to width varies in these animals, these correlations, although significant, might be improved by use of size measurements that account for both length and width.
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Sperm Penetration of Housefly Eggs: Evidence for Involvement of a Female Accessory Secretion

Abstract. *Removal of the paired sex accessory glands associated with the posterior reproductive tract of female houseflies inhibited penetration of the eggs by sperm, but the insemination of females without these glands appeared to be unaffected. The results indicate that either the sperm are "activated" or the permeability of the egg membrane is altered by the secretion of the accessory glands before fertilization occurs.*

Some form of accessory gland is associated with the posterior region of the reproductive tracts of most species of female insects. These glands vary widely in structure, location, and product, but they generally produce secretions that aid in the attachment of the eggs to various substrates, or form protective coatings and egg cases. However, modifications of this usual function are known to occur. For example, venom is produced by an accessory gland in aculeate hymenopteran insects (*1*), and some viviparous dipterans provide nourishment for the larvae from glands associated with the uterine chamber (*2*). It has been further speculated that the secretion of the accessory glands of some insects may facilitate fertilization of the egg during oviposition (*3*).

Previous investigations of the internal genitalia of the female housefly, *Musca domestica*, established that paired accessory glands and the sperm storage organs (spermathecae) discharge their products into the vagina through ducts that have a common opening (*4*). This arrangement suggested that mixing of the sperm with secretion emitted from the glands would be inevitable, if the glands were functional during oviposition. The study reported here indicates that the accessory glands are functional during oviposition and that a mechanism involving the secretory product with sperm penetration does exist in the housefly.

Table 1. Effect of removal of accessory glands on the fertility of female houseflies. In each treatment 118 females were used. The figures in parentheses represent the total number of eggs deposited and examined.

Treatment	Eggs hatched (%)	Eggs fertilized (%)
Sham operation	74.03 (2569)	77.40 (600)
Glands removed	0.68 (2106)	4.20 (208)

Table 2. Effect of removal of accessory glands on insemination of female houseflies. The females were considered inseminated only if sperm were observed in the spermathecae.

Treatment	Females tested (No.)	Females inseminated (%)
Sham operation	147	64.6
Glands removed	112	68.8

Light microscopic examination of histological preparations of the reproductive tracts of houseflies taken at various stages of egg deposition established that sperm penetration of the egg occurs within an anterior chamber located just posterior to the junction of the vagina and common oviduct (*5*). Just before an egg leaves the common oviduct and enters the vaginal canal, several sperm traverse the short distance from the vaginal opening of the spermathecal ducts to the anterior chamber. As the egg enters the vagina, it is thrust into the anterior chamber and held there for several seconds before deposition while one to four sperm penetrate the micropyle and enter the ooplasm.

The participation of the secretion of the female accessory glands in the process of fertilization was investigated in part by surgically removing the glands from 3- to 7-day-old adult females that had mated previously. The glands were excised through a ventral midline incision made in the membrane located between the fifth and sixth abdominal segments. Other females were similarly treated, except that the glands were merely grasped with a forceps and not detached. Only females that appeared to have full control over their ovipositors after either procedure were retained for testing. The next day the females were allowed to oviposit, and most of the eggs deposited were used for determination of hatch; however, a small portion was collected immediately after oviposition, stained by the method of Leopold and Palmquist (*6*), and microscopically examined for the presence of sperm.

As Table 1 shows, fertility was reduced to less than 1 percent by removing the glands, but fecundity was only slightly affected. Further, the microscopic examination revealed that sperm were rarely present within eggs deposited by females lacking accessory glands. Thus, the secretion of these glands is apparently involved in the actual penetration of eggs by sperm, or aids the sperm in reaching the site of penetration.

To rule out the possibility that dam-

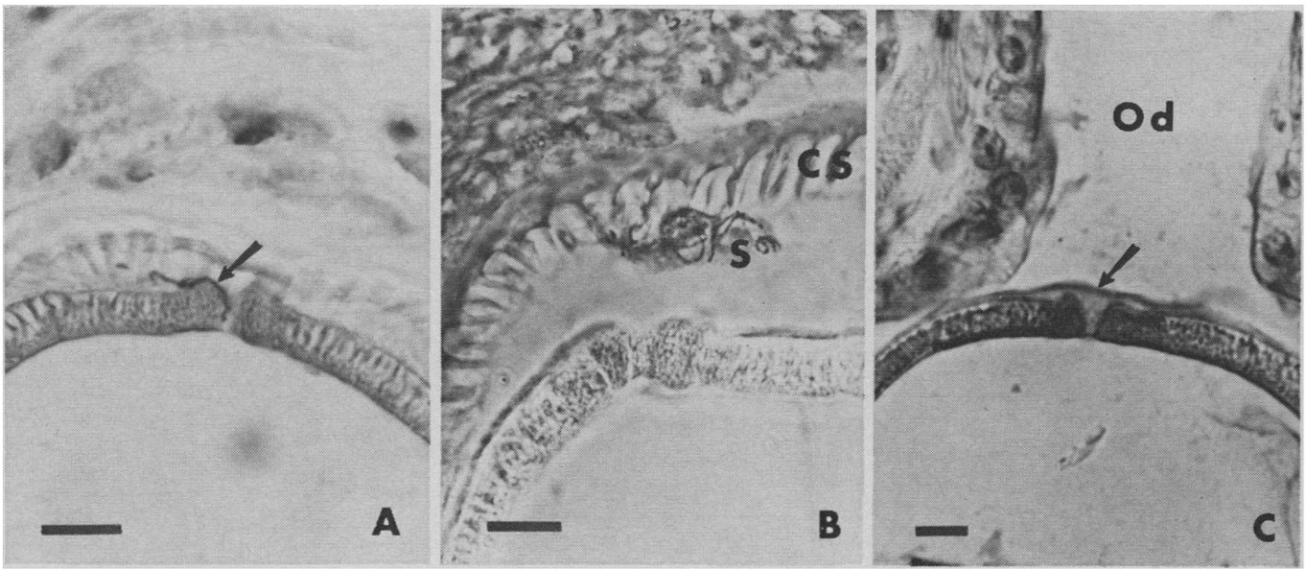

Fig. 1. Sagittal sections showing the micropyle region of eggs within reproductive tracts of female houseflies taken in the process of oviposition. (A) Sperm (arrow) are penetrating the micropyle of an egg held within the anterior chamber of a sham-operated female. (B) Numerous sperm (*S*) have coalesced into a single mass in the anterior chamber of a female without sex glands. Flexible cuticular spines (*CS*) project into the lumen of the anterior chamber (phase contrast illumination). (C) The arrow indicates the presence of the micropyle cap on an egg that has been stopped within the common oviduct (*Od*) of an ovipositing female. The tissues in (A), (B), and (C) were stained with the Feulgen–fast green technique. (Scale bars, 10 μm.)

age to the spermathecal ducts incurred during the excision of the glands prevented sperm from reaching the anterior chamber, a number of posterior reproductive tracts were examined by histological means (*5*). Although while most observations were made on reproductive tracts of ovipositing females that had been interrupted during the period when the eggs were being held in the anterior chamber, a few tracts were included that contained eggs in the oviducts. All females were allowed to deposit ten or more eggs before oviposition was terminated by dissection to establish the rhythmic sequence typical of housefly oviposition (*7*).

In females with intact accessory glands, sperm were usually found either penetrating the micropyle (Fig. 1A) or within the ooplasm, when the oviposition process was terminated while the egg was held in the anterior chamber. Only 5 of 35 females examined had any residual sperm present in the anterior chamber, and of these, 3 had 3 sperm each and the other 2 had 5. In the anterior chambers of females lacking accessory sex glands, numerous sperm had usually aggregated into a single mass (Fig. 1B). Of the 12 females lacking accessory sex glands, 7 had more than 50 sperm in the anterior chamber, 4 had 3 to 15 sperm, and 1 had no sperm.

While the secretion of the accessory glands must be present for fertilization, it apparently does not aid the sperm to reach the spermathecae during the insemination of the female. Examination of the spermathecae for the presence of sperm in females without sex glands and sham-operated females that had mated 1 day after treatment revealed essentially no difference in the incidence of insemination (Table 2). Further, the secretion of the glands did not appear to participate in the removal of the substance that covers the anterior end of the egg and occludes the micropyle (Fig. 1C). This material is formed into a caplike structure and is present on oviducal eggs. No evidence of its existence was found on eggs held in the anterior chambers of females without sex glands or sham-operated females (Fig. 1, A and B). Apparently, it is either dissolved away by a vaginal secretion or possibly stripped from the egg by the prominent cuticular spines lining the anterior chamber (Fig. 1B).

If we thus discount the removal of the micropyle cap as a function of the secretion of the accessory glands, there remain at least two ways in which the secretion might facilitate fertilization. The vitelline membrane, like the chorion, is a barrier to the entry of the ooplasm by sperm. Conceivably, this membrane could be altered by the secretion in some manner that would allow penetration by sperm, or the secretion could act directly on the sperm to enable them to enter the egg. In mammals, an interaction of the sperm with the female reproductive tract is necessary before fertilization can be accomplished (*8*); this change in the potentiality of the sperm has been termed capacitation, and it is apparently manifested in the acrosomal region of the sperm head (*9*). Phenomena similar to capacitation have also been observed in an amphibian (*10*) and in a hydrozoan (*11*). While capacitation has not been demonstrated in an insect, modification of sperm within the spermathecae of certain insects has been suggested by Baccetti (*12*) to be a form of capacitation. Nevertheless, the occurrence of these morphological changes has not yet been proved necessary for penetration of the egg. Furthermore, until the process of sperm activation in lower animals is more clearly understood, the term capacitation should probably be reserved for mammalian species. Further elucidation of the mechanism controlling the activation of sperm in the housefly may be accomplished by procedures similar to the in vitro and ultrastructural methods that were successfully used with mammalian species.

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Phase Transitions and Heterogeneity in Lipid Bilayers

Abstract. *The optical reflectivity of several well-characterized lipid bilayer systems has been correlated with calorimetric studies of the membrane components. There is a large increase in mean membrane thickness when a bilayer is cooled below the transition temperature of the membrane lipid. Similar studies on membranes generated from a mixture of two lipids possessing different degrees of unsaturation suggest that between the characteristic transition temperatures of the two lipids, the bilayer contains clusters of gel and liquid crystalline lipid which coexist within the plane of the membrane.*

One explanation for the heterogeneity in the lipid composition of natural membranes may be that the different lipid species are required for the generation of a mosaic lipid membrane in which particular regions of the membrane are enriched with certain lipids. Indeed, recent physical studies suggest that some natural membranes contain heterogeneous lipid domains (*1*). According to this view of membrane structure, specific membrane functions would be gained by local interactions of the lipid mosaic with other membrane components. Thus it would be of considerable interest to examine the intrinsic physicochemical properties of lipid bilayer membranes in which heterogeneity within the plane of the membrane was established and subject to control. We now present evidence for the construction of a planar lipid bilayer membrane containing heterogeneous lipid (gel and liquid crystalline) domains. Evidence in support of this structure comes from measurements of the reflectivity of the membrane and from a correlation of the membrane composition with differential scanning calorimetric (DSC) studies of the membrane components.

Planar bilayer membranes were formed by standard techniques (*2*) in an all-quartz water-jacketed cell from *n*-hexadecane (*n*-C_{16}) solutions of (i) glyceryl monooleate (GMO), (ii) glyceryl monostearate (GMS), or (iii) a mixture of GMO and GMS (1 : 1) at concentrations of 10 mg of monoglyceride per milliliter of solvent. These systems were chosen for study because the bilayers that form spontaneously from them are chemically well defined, the number of molecules of monoglyceride and neutral hydrocarbon per unit area of membrane having recently been determined with the use of radioactively labeled components and a special sampling technique (*3*). The GMO and GMS were obtained from Sigma Chemical Co., and *n*-C_{16} was obtained from Koch Light Ltd. Membranes formed from solutions (ii) or (iii) were generated above 65° to 70°C and were subsequently cooled to the desired temperature.

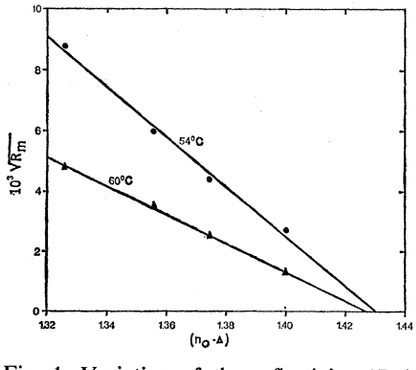

Fig. 1. Variation of the reflectivity (R_m) of bilayers formed from glyceryl monostearate (GMS) and *n*-hexadecane with refractive index ($n_o - \Delta$) at 60° and 54°C.

The thicknesses of the lipid membranes were determined from reflectivity measurements by a modification of the technique developed by Cherry and Chapman (*4*), in which the membranes were illuminated at near normal incidence by light from a helium-neon laser, and the membrane reflectance was obtained by comparing the reflected intensity with that of a quartz plate placed in the same aqueous solution. The reflectivity of the membrane, R_m, is related to its thickness by the previously derived equations (*4*)

$$\frac{\lambda R_m^{1/2}}{2\pi d} = \bar{n} - n_o + \Delta \quad (1)$$

$$\Delta = (\bar{n} - n_o)/(\bar{n} + n_o) \quad (2)$$

where λ is the wavelength of the incident light, d is the total membrane thickness, $\bar{n}$ is the mean refractive index in the plane of the membrane, and n_o is the refractive index of the aqueous phase, which is varied by forming the membranes in different solutions of sucrose or NaCl. By plotting $R_m^{1/2}$ against n_o, an approximate value of n is obtained. This value was used to calculate the correction term Δ; the final values of d and $\bar{n}$ were obtained by plotting $R_m^{1/2}$ against $(n_o - \Delta)$. For each of the systems examined, R_m was determined at four values of n_o, each value of R_m being the average of five measurements.

We have observed a large increase in the membrane reflectance when lipid bilayers formed from GMS and *n*-C_{16} at 65°C are slowly cooled below 55°C. This increase in reflectivity occurs rapidly (< 1 second for a bilayer of area 0.03 cm^2) at ~54.5°C, and the resulting membrane is extremely fragile. In several instances it was possible to reheat such a membrane without causing membrane rupture, and the membrane reflectivity was observed to return to its lower initial value. This phenomenon has been studied quantitatively as outlined above, and the data are summarized in Fig. 1. For each membrane R_m was measured at 60° and 54°C. From Eqs. 1 and 2 and the data in Fig. 1, we calculated that, on cooling, the membrane thickness, d, increased from 45 ± 1 Å to 77 ± 4 Å, while the