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Location of Sperms in the Oviduct of the Domestic Fowl with Special Reference to Storage of Sperms in the Vaginal Gland

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(Text-figs. 1–22; Tables 1–5)

The domestic fowl maintains fertility for a long time after the last mating. Since a long time ago, many observations have been made on the distribution of sperms in the fertile oviduct, in connection with the mechanism of fertilization in the fowl. For instance, PAYNE (1914), ANDERSON (1922), WALTON *et al* (1933), VAN DRIMMELEN (1945, 1946), OLSEN *et al* (1948), and MIMURA (1939, 1941, 1958) examined the oviduct microscopically for the presence of sperms, by using such wet preparations as scraped mucosa, sucked fluids, and irrigation sediments collected from the inner surface of the oviduct. These workers generally agreed that sperms were held in the oviduct for a considerably long period after copulation. These temporary preparations, however, may not manifest the actual location and distribution of sperms in the fertile oviduct, though they serve for demonstration of the existence and motility of sperms.

VAN DRIMMELEN (1946) was the first to find in a section of the fertile oviduct that large numbers of sperms were stored in the mucosal crypts of the chalaziferous portion of the upper oviduct. He called such crypts the sperm nests. Judging from the structure of the oviduct, sperms discovered in the sperm nests may be a part of those actually distributed in the oviduct.

The present study was performed to obtain more detailed information on the actual distribution and location of sperms in the oviduct, by using histological preparations. In addition to the chalaziferous portion pointed out by VAN DRIMMELEN, the author found another reservoir of sperms in the vaginal gland at the beginning of the vagina. The present paper deals mainly with the storage of sperms in the vaginal gland.

MATERIALS AND METHODS

A total of 30 adult White Leghorn hens were used in this study. Histological preparations were made from the oviduct in the following way. The hens examined were killed at varying intervals of time after the last mating or artificial insemination, and the whole oviduct was removed. Small segments, about 1 *cm* long, were excised from several portions of the oviduct, including funnel, chalaziferous por-

tion, magnum (middle), isthmus (middle), uterus, uterovaginal junction (on either side of the vaginal orifice), and vagina (at the middle and caudal portions). Each segment was ligated with silk threads at both ends to prevent sperms from outflowing during the preparation of sections. It was fixed in Bouin's or Zenker's solution, embedded in paraffin or celloidin, and cut in sections thin enough to make the distribution of sperms clear. The sections were stained with Heidenhain's iron hematoxylin, his azan stain, and hematoxylin and eosin. Twenty sections were selected at random from each segment and examined for the presence of sperms.

The present studies were composed of two series of trials. The first series was carried out with the oviducts of naturally mated hens and the second with those of artificially inseminated hens.

RESULTS

1. Distribution and location of sperms in naturally mated hens.

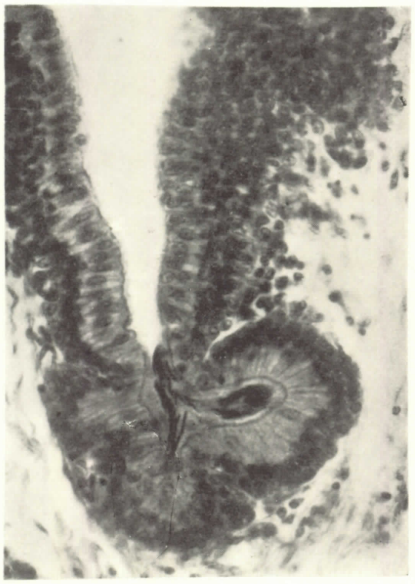
Seven naturally mated hens were used to determine in what portion of the oviduct sperms were located and how long they stayed there after the hens had been separated from the cock. They had been raised in flocks where the male to female ratio was one to ten. At the beginning of experiment, they were laying fertilized eggs, although the time of last mating was unknown. Table 1 shows the distribution of sperms at different levels of the oviduct in the naturally mated hens.

Table 1. Distribution of sperms at different levels of the oviduct in naturally mated hens.

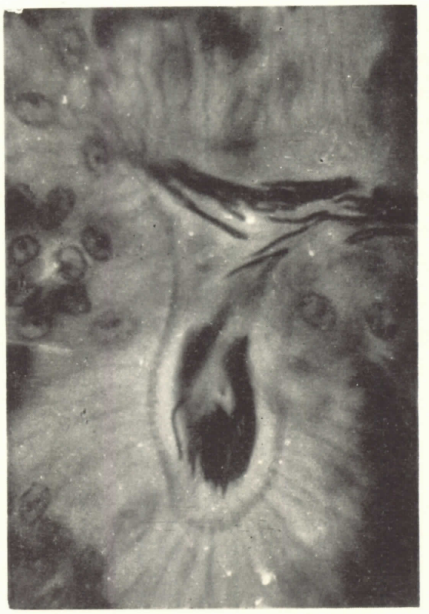
Hen No.	Days without cock	Distribution of sperms in each portion of the oviduct						
		Funnel	Chalaziferous portion	Magnum	Isthmus	Uterus	Uterovaginal junction	Vagina
11	1	+	|||||	—	—	—	|||||	|||
12	2	—	|||||	—	—	—	|||||	++
13	7	—	|||||	—	—	—	|||||	—
14	12	—	|||	—	—	—	|||	—
15	20	—	—	—	—	—	|||	—
22	27	—	—	—	—	—	++	—
23	27	—	—	—	—	—	++	—

Key to signs. — : none, + : very few, ++ : few, ||| : many, ||||| : numerous, ||||| : very numerous.

As shown in the Table, all the hens examined contained sperms in their oviducts, although the amount of stored sperms varied greatly according to the interval of time between the last day of cohabitation with a cock and the day of examination. The presence of sperms was restricted to the chalaziferous portion and the uterovaginal junction, except in hens Nos. 11 and 12 which were segregated a short time before. In both hens, small numbers of sperms were found in the funnel and the vagina, and relatively large numbers of them in the mucosal crypts of the vagina. Text-figs. 1 and 2 show the presence of sperms in the grooves among the



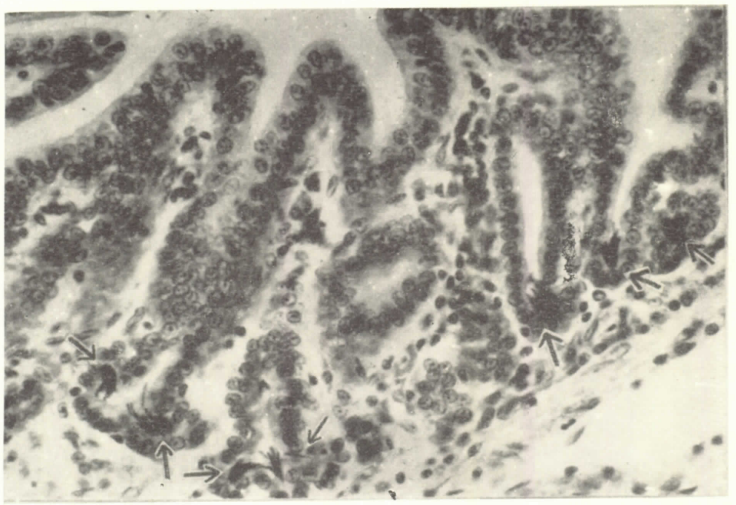
Text-fig. 1. Agglutination of sperms in the grooves between the secondary mucosal folds of the vagina. Hematoxylin and eosin (HE) stain. ×300.



Text-fig. 2. High-power magnification of Text-fig.1. HE stain. ×800.

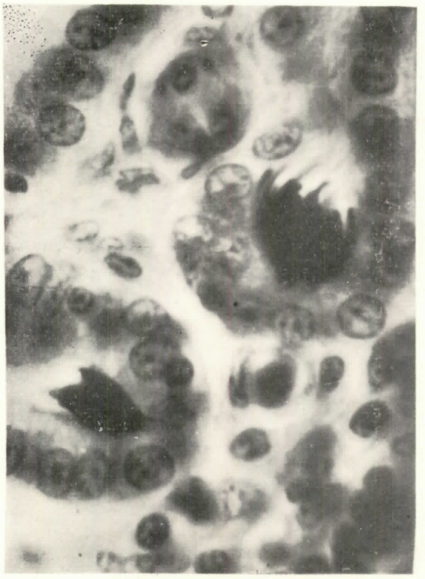
secondary ridges of the vagina. This finding, however, was rare.

In the chalaziferous portion, sperms were actually located in the crypts of the mucosal folds. These results agree with those given by VAN DRIMMELEN. Here some description is made to supplement his results. The histological picture in

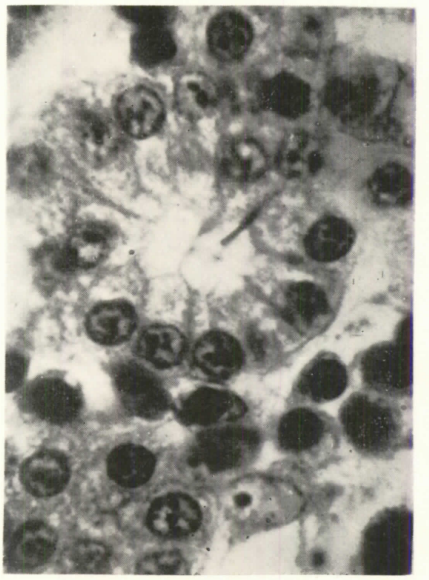


Text-fig. 3. Distribution and location of sperms in the chalaziferous portion. Sperms are located abundantly in the depths of the mucosal folds and chalaziferous glands. HE stain. ×300.

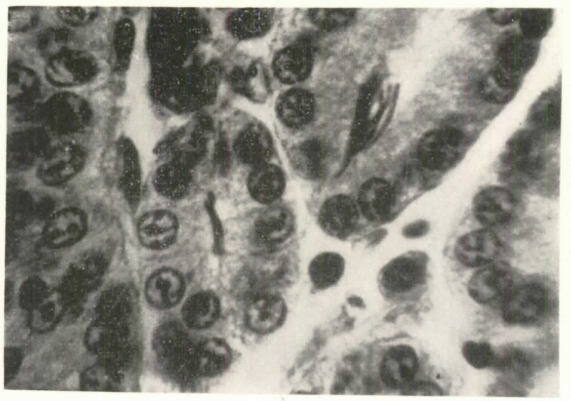
Text-fig. 3 shows the most typical localization of sperms in the chalaziferous portion. This specimen was taken from hen No. 11, which had been experimentally mated ten times, once a day, to induce a rich accumulation of sperms in the oviduct. As is clear in the figure, large numbers of sperms were located in the crevices of mucosal folds, called the *glandular grooves* by SURFACE (1912), and in the lumina of the tubular glands, extending from the bottom of these grooves and called the *chalaziferous glands* by RICHARDSON (1935). Some sperms were distributed sparsely around the openings of the grooves and the glands and among the cilia of the mucosal epithelium. At a higher magnification, sperms were situated in the depths of the grooves in such characteristic manner as forming bundle-like agglutinations,



Text-fig. 4. High-power magnification of sperms stored in the lumina of the chalaziferous glands. Note regularly arranged masses of sperms with their head attached to the free surface of glandular cells. HE stain. × 1,000.



Text-fig. 6. A sperm inserted into the intercellular cleft of the glandular cells. HE stain. × 1,000.



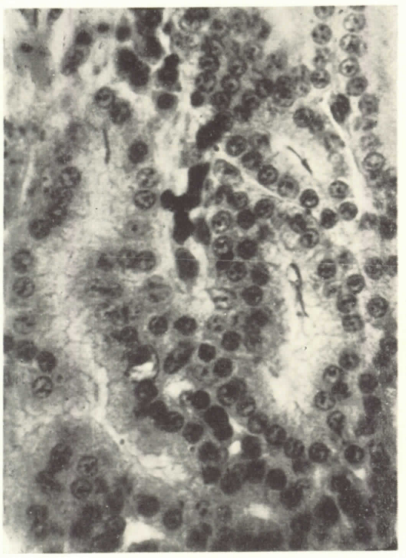
Text-fig. 5. Sperms with their head inserted into the cytoplasm of glandular cells of chalaziferous glands. The sperms have no tail. HE stain. × 1,000.

with their heads directed toward the fundus. At the bottom of the grooves, most sperms attached themselves closely to, or insert their heads directly into, the cytoplasm of the fundus cells, appearing to get nourishment from the cells (Text-figs. 4 to 6).

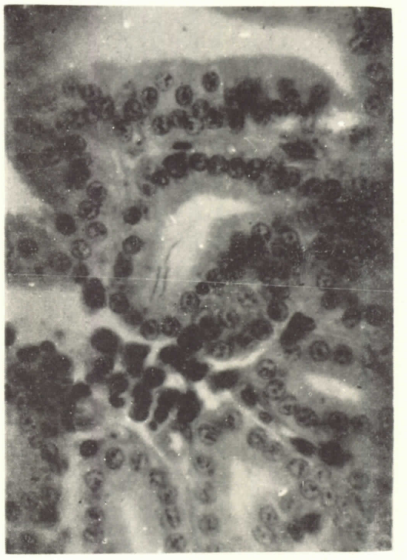
The sperms contained in the grooves and glands, however, decreased progressively in number toward the funnel portion and disappeared completely in the upper funnel. On the other hand, considerable amounts of sperms migrated into the lumina of primitive, undifferentiated albumen-secreting glands, which had been derived from the chalaziferous glands (BRADLEY, 1928) and situated at the beginning of the magnum (Text-fig. 7). As a whole, those sperms agglutinated in the grooves appeared normal in structure, with regular tails and heads. Therefore, they might be considered as living ones. Solitary sperms forming no agglutination had lost their acrosomes and tails. So they might be regarded as dead ones.

In hen No. 13 passing 7 days with no cock, the sperms stored in the chalaziferous portion decreased somewhat in number, but small clumps of sperms still remained in the grooves. On the 12th day after segregation from the cock (hen No. 14), sperms were retained in small numbers, scattered independently in the shallow position of the grooves. Their structure had been greatly disintegrated (Text-figs. 8 and 9). On the 27th day (Nos. 22 and 23), the oviduct had no sperms in the chalaziferous portion any longer.

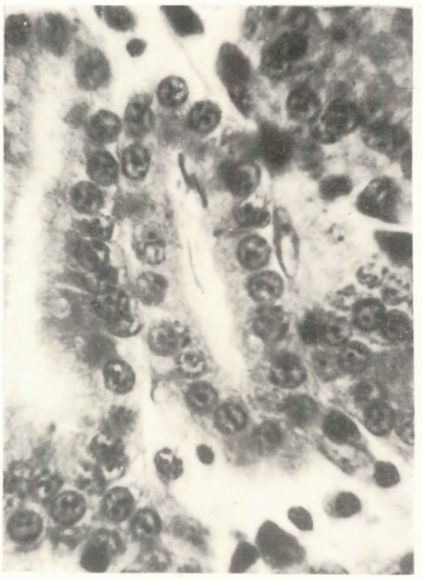
On the other hand, there was an accumulation of sperms in the uterovaginal junction. Here sperms were found in the lumina of the *vaginal glands*, so named by GIERSBERG (1922). In his previous paper, the author (1963) reported the struc-



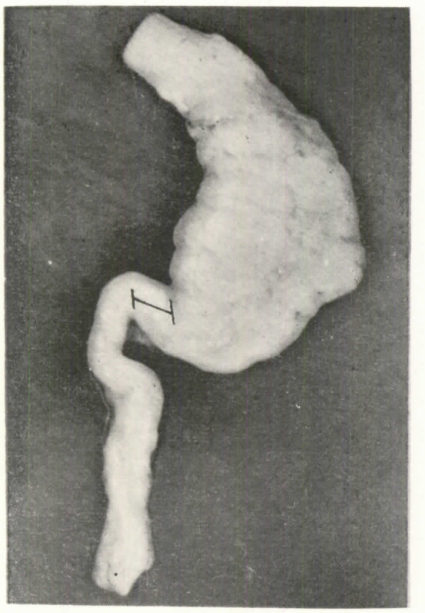
Text-fig. 7. Sperms distributed in the lumina of albumen-secreting glands. Azan stain. ×400.



Text-fig. 8. Small amounts of sperms located independently in chalaziferous glands. Such sperms increase in number with the lapse of time after insemination. ×400.

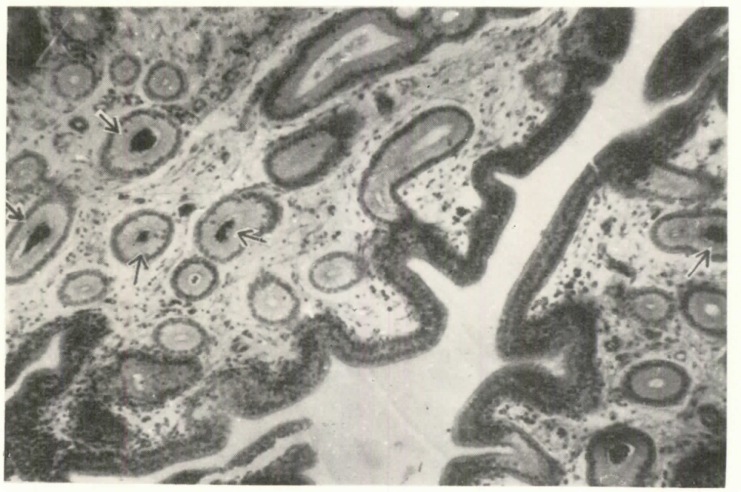


Text-fig. 9. Free sperms in chalaziferous glands. Explanation given in Text-fig. 8 is applicable. Sperms have been deformed in structure. HE stain. ×600.

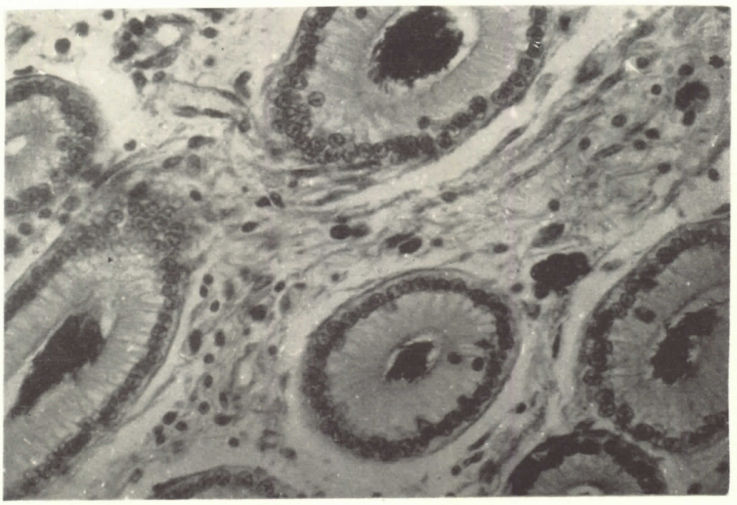


Text-fig. 10. Outside appearance of the uterus and the vaginal portion. The mark indicates an area where vaginal glands are distributed.

ture of the vaginal glands in detail. These glands were large simple tubular ones, about 70 μ in outside diameter. They were present abundantly in the lamina propria of the mucosal folds at the beginning of the vagina, about 1 *cm* from the vaginal orifice (Text-fig. 10). The histological pictures in Text-figs. 11 and 12 show sperms stored in the vaginal glands. They had been taken from a specimen collect-

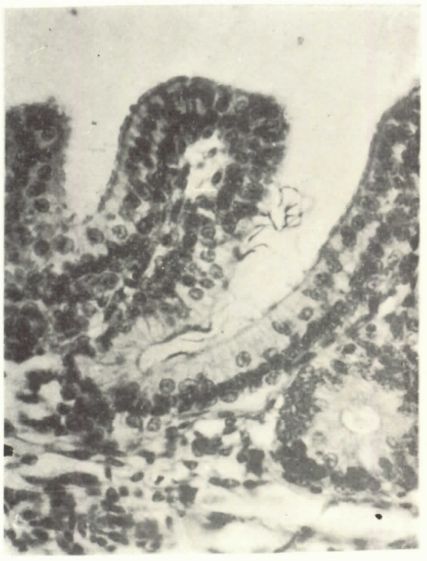


Text-fig. 11. Distribution of sperms in the lumina of vaginal glands. The lumen is filled with sperm masses varying in size. HE stain. ×150.

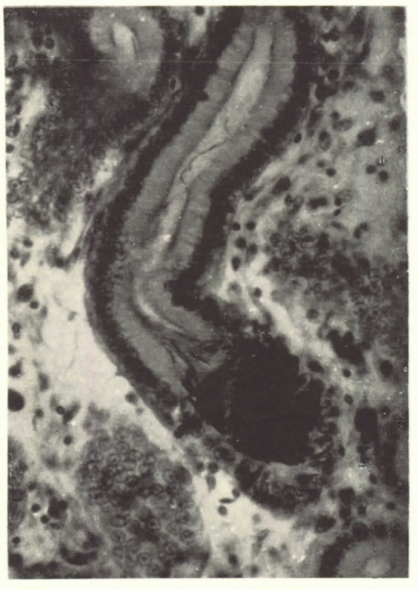


Text-fig. 12. High-power magnification of Text-fig. 11. Sperms are located having no contact with glandular cells. HE stain. ×300.

ed from the same hen No. 11. As shown in the pictures, a great many sperms occupied the wide lumina of the glands, forming dense masses. These clumps of sperms were located at various levels of the extending ducts. Occasionally, free sperms were seen in small numbers near the openings of the ducts (Text-fig. 13). The storage of sperms in these glands was very characteristic, as shown in Text-

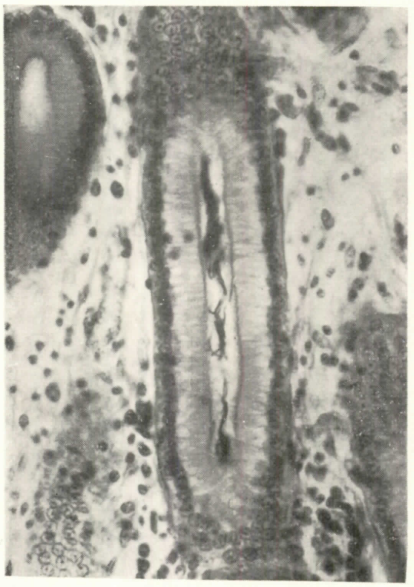


Text-fig. 13. Sperms distributed in the openings of vaginal glands. They are scattered without forming clumps. HE stain. ×300.



Text-fig. 14. Sperm clumps in a longitudinal section of vaginal glands. A large mass of sperms is seen at the bottom of a gland. Sperms have been agglutinated, preserving their regular form with a complete tail. A few free sperms are also seen. HE stain. ×300.

figs. 14 to 16. In general, the arrangement of sperms was somewhat regular like that in the crypts of the chalaziferous portion, but there were no signs for them to attach themselves to, or insert their heads into, the glandular epithelium.

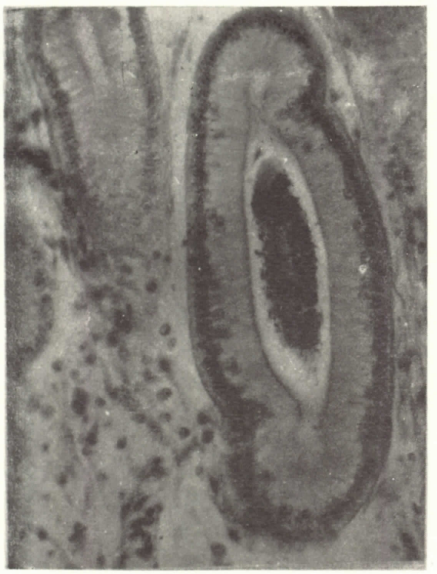


Text-fig. 15. Small masses of sperms in the lumina of vaginal glands. They are distributed at different levels of the lumen. HE stain. ×300.

Text-fig. 16. High-power magnification of sperms stored in the vaginal gland. Arrangement of sperms is somewhat irregular. HE stain. ×600.

Quite similar findings were obtained from the vaginal glands of the other naturally mated hens. The sperms stored in these glands decreased in number, like those in the previous glands, with the lapse of time after segregation from the cock. On the 12th and 20th days after segregation, however, considerable amounts of sperms were found, forming clumps in the vaginal glands (Text-fig. 17). On the 27th day when sperms disappeared completely from the chalaziferous portion, the vaginal glands still contained small numbers of sperms which had undergone great structural changes (Text-fig. 18). It must be noted that no sperms entered the uterine glands, which are tubular ones situated near the vaginal glands.

The present results obtained from sections of the oviduct are essentially the same as those of some previous workers obtained from wet preparations, so far as the distribution of sperms in the oviduct is concerned. However, it is very interesting to note that the vaginal glands stored large numbers of sperms for a long time after segregation from the cock, and that they stored much larger numbers of sperms for a much longer time than the crypts of the chalaziferous portion. From these results, it is suggested that the vaginal glands may have some favorable factor for the survival of sperms.



Text-fig. 17. A large mass of sperms in the vaginal gland. Sperms have been agglutinated compactly, with their body greatly disintegrated. HE stain. ×300.

Text-fig. 18. High-power magnification of sperms with their structure greatly changed. Such sperms increase in number generally with the lapse of time after insemination. HE stain. ×600.

2. Distribution and location of sperms in artificially inseminated hens.

In the preceding section, it has been clarified that the sperms introduced naturally by copulation were stored both in the crypts of the chalaziferous portion and in the lumina of the vaginal glands. This series of experiments was undertaken to obtain more sufficient information concerning the site for the storage of sperms, by using artificially inseminated hens. It comprised the following experiments: *a*) regular intravaginal insemination, *b*) intravaginal insemination of hens at the resting stage, *c*) intravaginal insemination with dead sperms, and *d*) intra-uterine insemination.

The hens used in these experiments were adults which had never been mated. Semen samples for insemination were collected from adult White Leghorn cocks by the massage of the abdomen described by BURROWS & QUINN (1939). Before insemination, the character of a semen sample was inspected routinely, and only active one was used undiluted.

The density of sperms of the inseminated semen was about 2–3 millions per cubic millimeter, and much larger quantities of sperms than usual were inseminated to obtain abundant accumulation of sperms in the oviduct. At various intervals of time after insemination, the hens were subjected to the examination in the same manner as before.

a) *Observation after regular intravaginal insemination.*

This experiment was carried out to determine whether or not sperms introduced artificially into the vagina were stored at the same site of the oviduct as those in naturally inseminated hens. Eight laying hens were inseminated intravaginally with a varying dose of semen by the ordinary technique. The results are given in Table 2.

Table 2. Distribution of sperms at different levels of the oviduct in intravaginally inseminated hens.

Hen No.	Days after insemination	Distribution of sperms in each portion of the oviduct							Dose of insemination (*cc*)
		Funnel	Chalaziferous portion	Magnum	Isthmus	Uterus	Utero-vaginal junction	Vagina	
18	1	—	—	—	—	—	###	—	0.2
19	1	—	##	—	—	—	###	##	0.2
17	2	—	+	—	—	—	####	—	0.3
9	2	—	+	—	—	—	###	—	0.2
10	3	—	###	—	—	—	####	—	0.3
6	5	—	##	—	—	—	###	—	0.3
28	19	—	+	—	—	—	##	—	0.1
29	19	—	—	—	—	—	##	—	0.1

For the signs see Table 1.

As shown in the table, sperms were found in the oviduct up to the 19th day after insemination. The storage site of sperms in the oviduct was identical with that in naturally mated hens. However, the amount of stored sperms varied greatly in each case, and was much smaller than that in naturally mated hens. Less accumulation of sperms in artificially inseminated hens was due to the insemination not repeated. In this experiment, some noticeable findings were obtained. As is seen in hens Nos. 9, 17, 18, and 19 within 48 hours after insemination, large numbers of sperms were located in the uterovaginal junction (in the vaginal gland), while a few or none of them were in the chalaziferous portion. This indicates that it takes a considerable long time for many sperms to reach the infundibulum and form masses in the crypts. This result does not agree with those of MIMURA (1939) and ALLEN *et al.* (1957), who observed that sperms reached the upper part of the oviduct in a short time, or within half an hour, after intravaginal insemination. This might be due to the difference of the inspection method used. The present author employed histological sections, MIMURA the smear method, and ALLEN *et al.* the radioactive tracer technique. Another finding was the relationship between the amount of sperms stored in the oviduct and the volume of inseminated semen. In general, the amount of sperms stored in the vaginal glands accorded with the volume of inseminated semen, but that in the chalaziferous portion did not. According to MIMURA (1941), there was a great difference in time required for sperms to reach the upper end of the oviduct, according to the position of eggs within the oviduct.

b) Observation after intravaginal insemination of hens at the resting stage.

The preceding data were obtained from laying hens with the oviduct actively

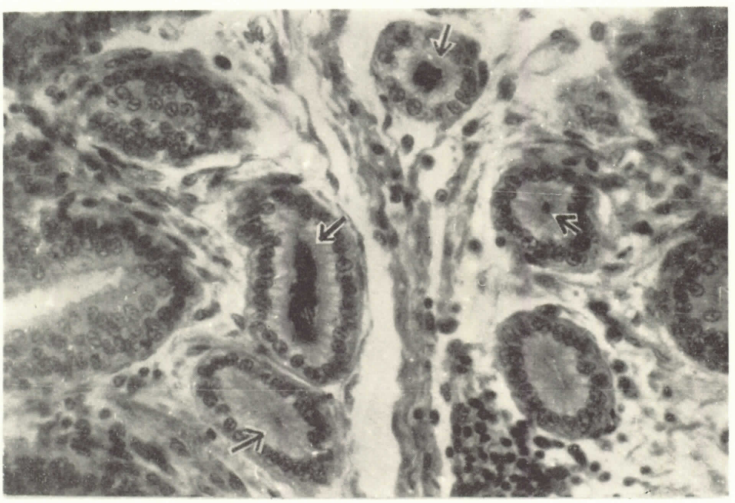
functioning. It is supposed, however, that the oviduct of nonlaying hens may differ from that of laying hens in the acceptability of sperms, since the former has undergone remarkable morphological and functional changes. This experiment was conducted to examine the transport of sperms through the oviduct and their location in the oviduct under unfavorable conditions of the oviduct. For this purpose, five moulting hens, in which the oviduct was inactive, were inseminated intravaginally with 0.3 *cc* of fresh semen. The results are presented in Table 3.

Table 3. Distribution of sperms at different levels of the oviduct in inseminated resting hens.

Hen No.	Days after insemination	Distribution of sperms in each portion of the oviduct						
		Funnel	Chalaziferous portion	Magnum	Isthmus	Uterus	Uterovaginal junction	Vagina
16	3	—	+	—	—	—	++	+++
24	3	—	+	—	—	—	+++	++++
21	4	—	—	—	—	—	++	+++
25	5	—	+	—	—	—	+++	+++
26	7	—	—	—	—	—	—	++
20	10	—	—	—	—	—	+++	+++

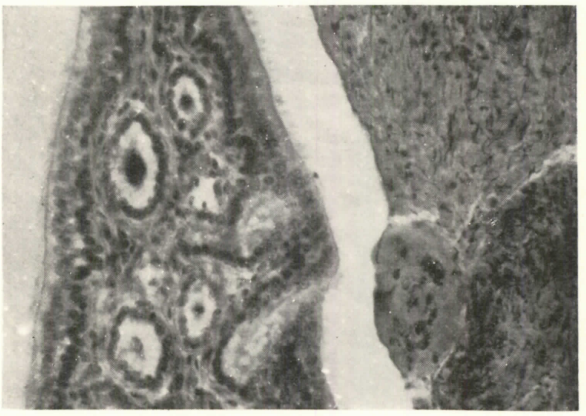
For the signs see Table 1.

In almost all cases in this experiment, small numbers of sperms were present in the chalaziferous portion and the uterovaginal junction (in the vaginal glands); sperms were rather abundant in the latter. This finding was quite similar to that obtained from the same experiment on laying hens, though the number of stored

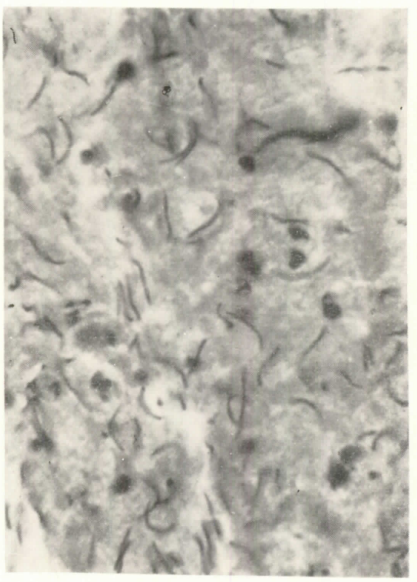


Text-fig. 19. Distribution of sperms in atrophied vaginal glands of a moulting hen after intravaginal insemination. Compare with Text-fig. 12 showing active vaginal glands. The finding is essentially the same as that of Text-fig. 12, except atrophy of the glands. HE stain. ×300.

sperms was smaller in nonlaying hens. In the chalaziferous portion, sperms were distributed scatteringly, without forming any clumps of sperms, in a shallow position of the grooves. Those sperms located in the vaginal glands were comparatively large in number and filled the atrophied glandular lumina, as shown in Text-fig. 19. On the other hand, large numbers of sperms were scattered in the vaginal cavity in all the birds examined (Text-figs. 20 and 21). They were embedded in a thick mucus and crooked largely with disintegrated structure. Presumably, they died soon after insemination owing to unfavorable conditions in the oviduct and remained there, as there was no expelling of eggs.



Text-fig. 20. Distribution of sperms in the vagina of a moulting hen. Some sperms have entered vaginal glands, but most of them are scattered in the cavity of the vagina, embedded in thick mucus. HE stain. ×200.



Text-fig. 21. High-power magnification of sperms distributed in the cavity of the vagina. Sperms are crooked, with no tailes. HE stain. ×800.

This result indicates that even when the genital tract is inactive, some sperms reach the infundibulum through the oviduct, though most of them remain in the vagina. Particularly, the fact that the vaginal glands allow a considerable amount of sperms to migrate suggests that they have some favorable action on the survival of sperms.

c) Observation after intra-uterine insemination.

In the preceding experiments *a*) and *b*), the semen was all deposited in the lumen of the caudal portion of the vagina. Therefore, it is possible that the introduced sperms may migrate involuntarily into the vaginal glands, since they are situated along the pathway of sperm transport through the vagina. If this is the case, no sperms will enter the vaginal glands when they are injected directly into the uterine cavity beyond the region of vaginal glands. Then five laying hens were injected with 0.2 *cc* of semen by the intra-uterine route, by using the syringe described by ALLEN *et al.* (1955). The results are shown in Table 4.

Table 4. Distribution of sperms at different levels of the oviduct in hens after intra-uterine insemination.

Hen No.	Days after insemination	Distribution of sperms in each portion of the oviduct						
		Funnel	Chalaziferous portion	Magnum	Isthmus	Uterus	Utero-vaginal junction	Vagina
41	3	−	−	−	−	−	−	−
36	5	−	++	−	−	−	++	−
50	7	−	+++	−	−	−	++	−
55	7	−	+++	−	−	−	++	−
37	9	−	+++	−	−	−	++	−

For the signs see Table 1.

Contrary to expectation, the migration of sperms into the vaginal glands occurred in almost all cases examined. The mucosal crypts of the chalaziferous portion also contained considerable amounts of sperms. However, the number of stored sperms in the vaginal glands was much smaller than in the case of intravaginal insemination. From this result, it may be concluded that most of the sperms injected into the uterine cavity traversed the genital tract to reach the chalaziferous portion, while some of them went downward beyond the vaginal orifice to enter the vaginal glands. Previously, MIMURA (1939) demonstrated that the upward traversing of sperms through the oviduct was caused chiefly by the antiperistalsis of the oviduct. Based on this view, it is considered that sperms travelled against such movement. In other words, this result indicates that the vaginal gland has some factor to attract the sperm.

d) Observation after intravaginal insemination with dead sperms.

ALLEN & GRIGG (1957), who studied sperm transport in the genital tract, suggested a peculiar spasmodic movement which prevented sperms from passing the upper vagina. If sperms migrate into the vaginal gland on account of such movement, even

dead sperms introduced in the vagina must be carried into the vaginal gland. To affirm this view, two laying and two nonlaying hens were inseminated intravaginally with 0.2 *cc* each of dead sperms which had been heated at 60°C in a water-bath. The results are listed in Table 5.

Table 5. Distribution of sperms at different levels of the oviduct in hens intravaginally inseminated with dead sperms.

Hen No.	Days after insemination	Distribution of sperms in each portion of the oviduct						
		Funnel	Chalaziferous portion	Magnum	Isthmus	Uterus	Uterovaginal junction	Vagina
56 (laying)	3	—	—	—	—	—	—	++++
57 (nonlaying)	3	—	—	—	—	—	—	++++
41 (laying)	5	—	—	—	—	—	—	++
42 (nonlaying)	5	—	—	—	—	—	—	++++

For the signs see Table 1.

As shown in the table, sperms were present neither in the vaginal glands nor in the crypts of the chalaziferous portion of any bird. On the other hand, the vaginal cavity, where semen had been deposited, contained a large number of sperms. Sperms were particularly abundant in the posterior vagina of the nonlaying hen. The scarcity of sperms in the vagina of the laying hen was probably brought about by dejection due to repeated passage of eggs. The sperms distributed in the vaginal cavity were scattered widely in the duct and disintegrated greatly in structure, because they had been heated before use. It must be noted, however, that some sperms were distributed around the openings of the vaginal glands, but that no sperms entered the lumina of the glands. This indicates that dead sperms never migrated in the vaginal glands, and that they passed the uterovaginal junction. In fact, ALLEN *et al.* (1957) already reported that motile sperms traversed the uterovaginal junction freely, but that dead sperms did not. From this experiment, it may be concluded that the migration of sperms into the vaginal gland is caused not by such muscular movement of the vaginal wall as suggested by ALLEN, but by the motility of the sperms. In other words, only living sperms may be able to migrate into the vaginal glands.

DISCUSSION

Many investigators have examined microscopically the presence of sperms in the fertile oviduct of hens. PAYNE (1914) found sperms in the oviduct from 30 minutes to 56 days after insemination. ANDERSON (1922) stated that no sperms were seen in the oviduct on the 15th day after copulation. WALLEN *et al.* (1929), WALTON *et al.* (1933), and NOVIK (1958) demonstrated sperms in the oviduct 18,

15, and 65 days, respectively, after insemination. All these workers were interested mainly in the maximum duration of sperms in the oviduct and had no concern about the actual location of them.

Lately, MIMURA (1957, 1958) observed in detail the distribution of sperms in the oviduct for 3 weeks after intravaginal insemination, using smear preparations of the contents of the oviduct. He stated that 74 per cent of the hens observed contained sperms in the oviduct for the first ten days after insemination, and that 24 per cent of them did for the following ten days. He also reported that sperms were distributed in all portions of the oviduct at an early period after insemination, exclusively in the vagina and infundibulum at later periods, and restrictedly in the upper vagina at a much later period. From these results, he concluded that the anterior end of the vagina might be reservoir of sperms in the oviduct.

On the other hand, VAN DRIMMELEN (1946, 1949, 1951) examined the distribution of sperms in the oviduct scrupulously, using various new methods to collect sperms. He stated that an active and morphologically normal sperm had been found in the infundibulum even on the 14th day after insemination, although such sperm as this was not visible in the other portions after 72 hours. These results, obtained from the inspection of wet preparations, agree with the finding that sperms were distributed exclusively in the infundibulum and the vagina. As mentioned above, VAN DRIMMELEN was the first to confirm in histological preparations that the sperms distributed in the infundibulum portion were located in the mucosal crypts there.

In the present study, it was found that sperms were stored in two parts of the oviduct, the crypts of the chalaziferous portion and the lumina of the vaginal glands at the beginning of the vagina. The storage of sperms in the former part of the oviduct was already reported by VAN DRIMMELEN, as mentioned above, but that in the latter part was the most interesting finding in this study. Thus, the assumption of MIMURA made from his observation of wet preparations can be explained clearly by the presence of a reservoir of sperms in the vaginal gland. In addition, it must be noted that the migration of sperms into the vaginal glands is not spontaneous, but is caused by the attractive action of sperms. This finding may be supported by the fact that all the experimental hens naturally mated or artificially inseminated retained sperms constantly for 27 days after insemination. A more reliable evidence was obtained from the following experimental results: Living sperms were stored in the vaginal glands, but dead sperms were not. Even when sperms were introduced into the uterine cavity, they entered the vaginal glands later. The migration of sperms into the vaginal glands was not caused by the movement of the vaginal wall, but by the motility of the sperms themselves. The migration of sperms into the vaginal glands was also selective, and no sperms entered the uterine glands, which are situated near the vaginal glands.

ALLEN & GRIGG (1957) examined the transport of sperms through the oviduct quantitatively, using sperms labelled with ^{32}P. They found that the number of sperms having reached the infundibulum was much smaller after intravaginal in-

semination than after intra-uterine one. Based on this fact, they considered that there was a barrier to check the transport of sperms, which, according to them, was a spasmodic movement of the vaginal wall at the junction of the vagina and the uterus. As mentioned above, the vaginal glands which stored large amounts of sperms were situated at the beginning of the vagina, or the uterovaginal junction. Judging from the number and size of vaginal glands, the amount of sperms stored in the glands must be enormous. Thus, the above-mentioned assumption of ALLEN *et al.* may also be explained clearly by the migration of sperms into the vaginal glands.

In connection with the presence of sperms in the oviduct, it should be taken into consideration whether stored sperms are living or dead. Many workers are not of the same opinion with regard to the activity and morphology of stored sperms. According to PAYNE (1914), most of the sperms lose their tails within 24–48 hours after insemination. WARREN & KILAPATRICK (1929) had a similar view. However, VAN DRIMMELEN (1945) found an active normal sperm in the oviduct on the 14th day after insemination. GRIGG (1957) reported that sperms stored in the crypts of the chalaziferous portion were quite intact, with an apical cap and a tail. He considered that the abnormal sperms observed by ALLEN *et al.* were probably those which had not been stored in the crypts of the infundibulum or which had undergone autolysis and phagocytosis.

In the present experiment, it was impossible to know whether the stored sperms were living or dead, since they were observed in section preparation. It may be generally said, however, that the sperms forming regular clumps in the crypts of the mucosal folds were apparently normal in structure. On the other hand, sperms scattering individually in the lumen of the duct were mostly tailless or had no acrosome.

A suggestion is made that the vaginal glands may have some histological and histochemical properties by which these glands can be differentiated from the other glands of the oviduct. GIERSBERG (1922) considered the vaginal glands as mucous ones. In his previous report, the author (1963) has proved that they are not mucous glands and are characterized by the presence of large amounts of cholesterin-ester-like lipids (Text-fig. 22). No other structural features were found in them.

At any rate, from the above-mentioned results, it is assumed that the vaginal glands may be a reservoir of sperms in the oviduct of the domestic fowl. GRIGG (1957) observed experimentally that sperms were squeezed out mechanically from the crypts of the chalaziferous portion into the duct when the infundibulum had been greatly enlarged to pass eggs in it. The mechanism of storage of sperms in the vaginal glands may be explained as follows: The sperms introduced in the caudal portion of the vagina reach the upper end of the vagina a short time after insemination, as reported by MIMURA (1939), and most of them migrate into the vaginal glands and are stored there. Then they move into the duct by the similar mechanism mentioned above, traveling upward through the genital tract. Finally, they reach the infundibulum to be stored there. In other words, the vaginal glands

Text-fig. 22. Deposition of sudan-positive granules in the glandular cells of vaginal glands. Sudan black B stain. ×300.

are considered as a primary reservoir of sperms in the oviduct.

SUMMARY

The distribution and location of sperms in the fertile oviduct of the domestic fowl was observed. For this purpose, section preparations were made from the oviduct at varying intervals of time after the last mating or artificial insemination. The results obtained are summarized as follows.

1. Sperms were distributed constantly in abundance and observed for a long time after insemination both in the chalaziferous portion and at the beginning of the vagina. The sperms were actually located in the grooves of the mucosal folds and the chalaziferous glands of the chalaziferous portion and in the lumina of the vaginal glands at the beginning of the vagina. The chalaziferous portion had been described by VAN DRIMMELEN as location of sperms, but the vaginal glands were found by the present author as such for the first time.

2. The vaginal glands were large simple tubular ones, about 1 *cm* long, situated in the lamina propria of the mucosal folds, far from the vaginal orifice. They seemed to be special glands characterized by the presence of cholesterin-ester-like lipids.

3. More sperms were stored in the vaginal glands for a longer time than in the chalaziferous portion. Sperms were found in the chalaziferous portion 2 or 3 weeks after insemination and in the vaginal gland on the 27th day after insemination, although it was not clear whether they were active or dead.

4. The sperms stored in the crypts of the chalaziferous portion were agglu-

tinated in the depths of the grooves, forming bundle-like clumps, shortly after insemination. They became scattered with the lapse of time after insemination. The sperms stored in the vaginal glands formed packed masses, which were not disintegrated for a long time.

5. The sperms forming masses had generally a normal structure, but those distributed independently had an abnormal one, lacking tail or acrosome.

6. The migration of sperms into the vaginal glands was voluntary. This was confirmed by using various experimental methods of insemination. In other words, the vaginal glands seemed to have some factor which favored the survival of sperms.

From these results, it is concluded that the vaginal glands may act as a preliminary reservoir for sperms until the sperms reach the infundibulum, where they are finally stored.

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鶏の卵管内の精子の分布と局在，特に膣腺における精子の貯溜について

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鳥類は哺乳類と異なり交尾後かなりの期間卵管内に精子を保持していることはよく知られている．この研究は鶏の交尾後の卵管内の精子の分布と局在ならびに滞溜期間を卵管の切片標本によって組織学的に調べた．観察は受精後一定間隔毎の自然交尾および各種の方法による人工受精鶏の卵管によって行われた．

調べた受精後約4週間以内の卵管30例はすべて精子を含んでいた．卵管内に多量の精子が長期間認められるところは卵管上部のカラザ部と卵管下部の膣起始部（膣開口より下方約 1 cm の間）の2部分であった．詳しい精子の位置はカラザ部では主に粘膜ヒダの間隙やカラザ分泌腺の腺腔であり，膣起始部ではこの部に特徴的に存在する大型の管状腺（膣腺）の腺腔内であった．この所見は従来の卵管の内容物からの塗抹標本による精子の分布の所見と一致するのみならず，さらに卵管の組織学的構造の面から精子の局在を明かにしたことになる．

一般に膣腺がカラザ部より，より多量に且つ長期に精子を保持していた．このことは従来の精子の貯溜部と考えられていたカラザ部に対し，膣腺が一次的貯溜所の役割を果しているように見える．