The surfaces from which material, to be searched for the presence of spermatozoa, was obtained were the following [See figure (xii)]:—

(1) Amongst the coils of the intestines.

(2) Around the ovary—
- (*a*) in a fresh calyx,
- (*b*) on a large follicle,
- (*c*) on the small follicles.

(3) On the mucosa of the infundibulum—
- (*a*) on the fimbriae,
- (*b*) in the cranial part,
- (*c*) in the middle,
- (*d*) in the caudal part.

(4) On the albumen secreting mucosa—
- (*a*) cranial,
- (*b*) middle,
- (*c*) caudal.

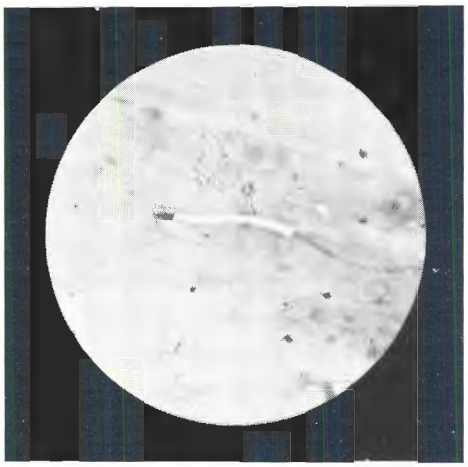
(5) On the mucosa of the isthmus—
- (*a*) cranial,
- (*b*) middle,
- (*c*) caudal.

(6) On the mucosa of the uterus.

(7) On the mucosa of the vagina.

(8) In the cloaca.

FIGURE (xiii) (*a*).—The Spermatozoon of the Fowl, stored *in Vivo* in the Oviduct.



Spermatozoon in a dry stained smear of mucus from the cranial part of the oviduct of hen No. 46, slaughtered eleven days after intraperitoneal insemination with 1 c.c. mixed semen from cocks Nos. 6, 47 and 55. Note the clear-cut shape and the acrosome, head, middlepiece and tail. (X 1400); (Stained by Williams' method after Lagerlöf).

(*Photo by Mr. Th. Meyer, for the Director of Veterinary Services, Onderstepoort.*)

The specimens were examined wet and fresh under thin glass coverslips or as hanging drop preparations placed immediately under the microscope. Smears of the material were made at the same time and dried immediately for later staining and microscopical examination. The stains used were Giemsa's azur-eosin, Nile blue sulphate, and the carbol-fuchsin-eosin-methylene blue method of Williams after Lagerlöf (1936) and also the carbol-acid-fuchsin-methyl green modification of the Altman-Bensley-Cowdry method (Jackson, 1944).

The time consumed by the making of wet preparations and their examination necessitated a modification of the dissection technique, because of the rapid chilling and drying out of the carcase. Consequently the birds killed after the fourth P.M. were fastened on their right side, and the specimens were collected through a small slit in the abdominal wall just ventral to the left acetabulum. Body heat was conserved by closing the opening with the semi-detached left leg and covering the carcase with a surgical towel and pad of cotton wool as described in the preliminary publication (v. Drimmelen 1945 b).

Results.—The findings in this experiment are set out in Table 61 to which the following remarks have to be added. The P.M. on hen No. 30 was also utilized for the location of coloured substance injected into the abdominal cavity [part (8) (*e*) (iii)] at the time of insemination.

The interpretation of microscopical appearances in the preparations was not without difficulty as to the question of defining " motility " and the use of the term " movement ":—

(i) Motility of the tail and middle-piece of a sperm-cell without head was often encountered in the examination of semen. However at no time was a sperm head only seen to progress definitely by its own motion. According to Phillips (1935) the motility of spermatozoa is apparently a function of the middle-piece. Whilst many authors have referred to spermatozoa as being " viable " or " alive " when they merely observed the cells to move, motile parts of a sperm-cell cannot be considered as a living entity. It is now generally accepted (Milovanov, 1940) that vigorous, progressive movement (when examined at body temperature) of a large percentage of cells is essential for fertilization.

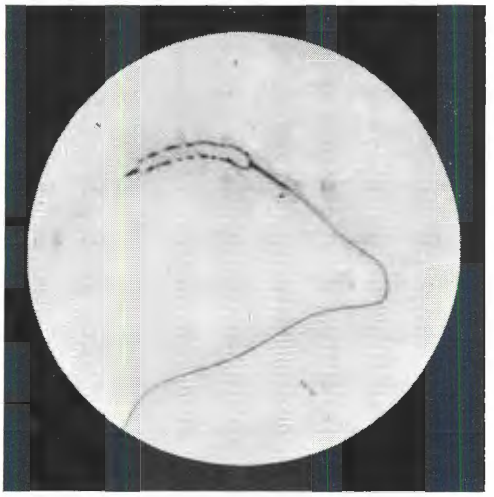
(ii) In small quantities of fluid containing mucus, yolk, cells or cilia there was so much Brownian movement and disturbance of the relative position of particles of substance, that no thin long body like the avian spermatozoon could remain stationary in a wet preparation made from body fluid slightly or not at all diluted in isotonic salines. Semen samples stored *in vitro* showed the same feature.

(iii) Numerous cilia were seen in the material collected in the oviduct. Apparently their presence did not depend on the mechanical destruction of the mucosa and it would thus appear that the casting off of cilia (Richardson, 1935) from cells lining the oviduct might be a prolific source of error. On numerous occasions detached ciliated cells or portions of cells or free cilia resembled abnormal spermatozoa. Some bundles of closely interwoven cilia could only be distinguished from spermatozoan heads by staining the dried film with different dyes, to show that neither the acrosome, nor the proximal centrosome at the point of attachment of the middlepiece to the head, could be defined. These structures were retained in sperm heads when the middle-piece and tail were detached during storage *in vitro* [see figure (xiii)].

Discussion.

It would appear that the spermatozoa in the fowl hen, when awaiting ova for fertilization are only to be found in the infundibulum and not in any other locality, after periods of more than 72 hours following insemination. The wall of the infundibulum is, however, very thin (Surface 1912, Warren and Scott, 1935) and the mucous membrane very shallow compared with that of other parts of the oviduct, and besides these, the mucosa assists in secretion. McNally (1942) found the layer of mucin which strengthens the collagenous vitelline membrane of the ovum at the beginning of egg-formation, to be deposited in this region.

FIGURE (xiii) (*b*).—The Spermatozoon of the Fowl, stored *in Vivo* in the Oviduct.



Spermatozoon in a dry stained film of fluid from the cranial part of the oviduct of hen No. 46, slaughtered eleven days after intraperitoneal insemination with 1 c.c. mixed semen from cocks Nos. 6, 47 and 55. Note the intact appearance of the acrosome, middlepiece and tail, the " capsule " of the head has apparently been slightly broken by the process of staining. (X 1400) ; (Stain Williams' after Lagerlöf).

(*Photo by Mr. Th. Meyer, for the Director of Veterinary Services, Onderstepoort.*)

Although many of the spermatozoa seen in the case of hen No. 46 (11 days storage *in vivo*) appeared to be in close contact with the cells of the mucosa, it must be realised, that these cells could have adopted this position after death. The observation of Walton (1927) cited and confirmed by Quinlan *e.a.* (1932) that mammalian sperms appear to attempt entry into any cells present in their surroundings, may explain this finding.

Mimura (1939) found sperms alive or dead to have reached the ostium abdominale of the fowl's oviduct in 26 minutes after deposition at the caudal end of the uterus. Pullet No. 20 showed live sperms two hours after intraperitoneal injection of semen in all parts of the oviduct between the ostium abdominale and the isthmus. These facts suggest that in the upper divisions of the fowl hen's genital tract, material in suspension and sperm-cells alike, are moved up and down the lumen with great rapidity. The ventral and lateral mucosa of the oviduct bears cilia with an abovarian action in all parts of the duct; the dorsal

TABLE 61.—(See Appendix C, Table 61.)

The Results of Examinations of the Genitalia of 9 Fowl Hens by Various Methods at Different Times after Insemination in the Third Series of Insemination.

Line.	Method of Examination.	Method of Insemination.	Number of Hens Examined by the Method.	Interval between Insemination and Examination.	Localities in which indications of Live Sperm were found.
	Column (1)	Column (2)	Column (3)	Column (4)	Column (5)
a	Dry stained films.....	Intraperitoneal...	6	Two hours.....	Infundibulum once. Albumen region doubtful.
b				Eleven days.....	Infundibulum once.
c		*Per Vaginam*.....	4	Three and Fourteen days	Not found.
d	Platinum loop and Ringer's solution	*Per Vaginam*.....	1	Three days.....	Infundibulum once. Albumen region once. Uterus once.
e	Glass pipette and Ringer's solution	Intraperitoneal...	2	Four days.....	Infundibulum doubtful.
f				Eight days..... Eight days.....	Infundibulum doubtful. Albumen region doubtful.
g	Glass capillary.....	Intraperitoneal...	5	Two hours.....	Infundibulum once. Albumen region once.
h				Eleven days.....	Infundibulum once.
i		*Per Vaginam*.....	3	Fourteen days....	Infundibulum once.

mucosa, however, bears pro-ovarian cilia from the uterus to the ostium abdominale and abovarian cilia from the uterus to the genital opening in the cloaca (Parker 1931). This fact has an influence on the distribution of freely moving sperms but after 14 days storage in the hen only one of numerous preparations made, contained active spermatozoa and this was made from the infundibulum (hen No. 45).

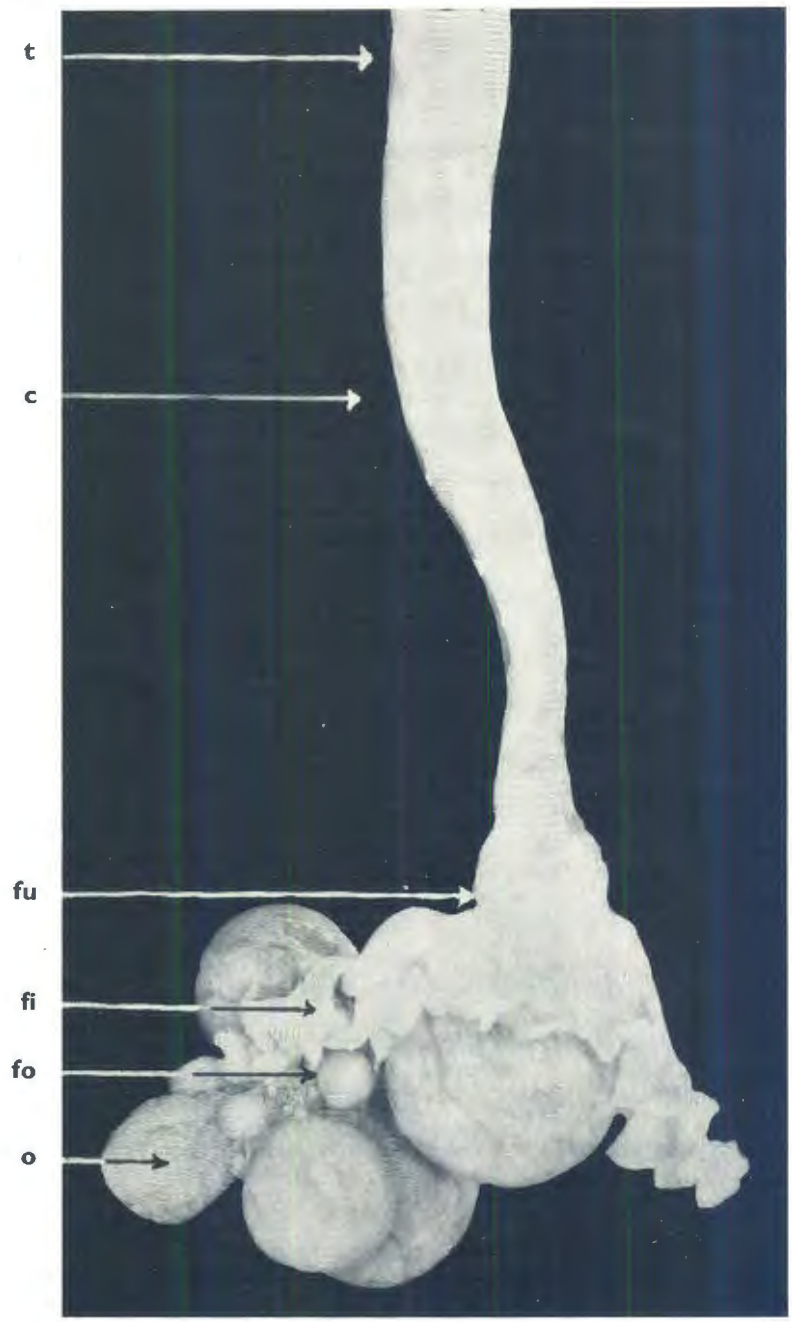
Interesting suggestions may be deducted from a comparison of the findings reported in the literature with the results of investigations on hens Nos. 30, 46 and 45, which in effect will be a comparison between sperm behaviour in the hen after (i) natural copulation (Payne 1914, Ivanov 1924; Warren and Kilpatrick 1929, Anderson 1922; Walton and Wetham 1933); (ii) artificial insemination *per vaginam* (P.M. findings in hen No. 30, three days after introduction of semen and in hen No. 45, 14 days after); (iii) artificial intraperitoneal insemination (P.M. findings in hen No. 46, 11 days after injection of semen).

In the cases of group (i) Payne (1914) showed that all normal morphologically complete spermatozoa had disappeared from the oviduct of the hen 24 hours after separation from the male. Anderson (1922) found no whole male cells more than 15 hours after insemination. Ivanov (1924) Warren and Kilpatrick (1929) and Walton and Wetham (1933) confirmed these findings; but Parker (1931) in stating that spermatozoa had been found abundantly about the ovaries of both birds and reptiles inferred that other relevant reports exist. Unfortunately these were not available for discussion with the persent work. (ii) In the case of hen No. 30 the spermatozoa were found normal and progressively motile in the upper uterus and in the infundibulum 72 hours after insemination *per vaginam* with 0·3 c.c. of semen during which period no eggs had been laid. In the space of the duct between these regions only the mucous region at the caudal end of the albumen region showed some sperm which were still motile. Hen No. 45 as stated showed active sperm in only one of a number of preparations made from the infundibular region of the oviduct, but not at all in the preparations from other parts and this was at 14 days after 1·0 c.c. mixed semen had been introduced *per vaginam*. (iii) After intraperitoneal injection of semen into hen No. 46, the largest number of active spermatozoa were found in the infundibulum 11 days after the operation. Their abundance was so great that in the observer's opinion it decided the point that some specific site of storage existed in the chalaziferous region of the infundibulum. The possibilty had to be considered, however, that the new method of insemination could be responsible for a difference in the site or method of storage of the male cells in the organs of the hen.

Although the positive findings were confined to isolated cases there was a suggestion that the upper region of the oviduct was more favourable to sperm life than the other regions. But when comparing the results of the specimens from the oviduct alone it seemed important that in the regions of greatest mucous secretion, sperm were found more often than in the albumen secreting region, i.e. the vagina, the mucous region [4 cm. caudal portion of albumen region (Richardson, 1935)], and the infundibulum (mucin secreting, Richardson, 1935, McNally 1942) or chalaziferous region, [see P.M. results on birds Nos. 11 (Table 60 Appendix), and No. 30 (Table 61 Appendix)].

The question of concentration of spermatozoa in storage has an important bearing in this connection. Walton and Wetham (1933) mention it as a factor of prime importance and as mentioned in a preliminary publication (v. Drimmelen, 1946 a) this stimulated work aiming at demonstration of the method of storage and the effect of the different methods of insemination.

FIGURE (xiv).—The Infundibulum of the Fowl's Oviduct.



The infundibulum of the Oviduct of a Fowl Hen in full egg-production (natural size) : (t) : cranial end of albumen secretion part ; (c) chalaziferous region ; (fu) : funnel ; (fi) : fimbriae ; (fo) : follicles ; (o) : Ovary.

(Photo by Mr. Th. Meyer, for the Director of Veterinary Services, Onderstepoort.)

(12) *The effect of intraperitoneal insemination on the position of spermatozoa stored in the fowl hen.*

The results of experiments (5) and (6) stimulated great interest into the question as to whether the intraperitoneal method of insemination had an effect on the position of spermatozoa in the genital organs of a fertile hen, during the period that she remained fertile after insemination or coitus, when all sources of fresh sperm were removed. In order to obtain factual information on this point experiment (7) was planned and carried out with material used in the *fourth* series of inseminations (see pages 36 and 40).

EXPERIMENT (7).

Object.—To determine the effect of the intraperitoneal method of artificial insemination on the position of spermatozoa in the body of the hen.

Material.—The females used in this experiment were all culled from the Onderstepoort poultry plant and the Director of Veterinary Services generously placed facilities at disposal to conduct some of the work at this institution. All birds were pullets just commencing to lay and they were accommodated in a spacious intensive fowl house equipped with trap nests. A few cocks from the plant (Nos. 63 to 67, see Appendix B) were used, but only very small amounts of semen could be obtained from them, and of all the natural matings attempted only one was fertile.

The semen used for the artificial inseminations in this experiment was collected from six males (Nos. 6, 47, 53, 55, 61 and 62) forwarded three weeks before the first insemination to Onderstepoort from Bloemfontein [i.e. moved 300 miles (480 Km.) northwarls at about the same altitude].

Method.—Twenty groups of two to four pullets each were selected out of the 45 available, with the intention of treating them as planned in Table 62. The better producers were placed in the group with the longest interval between coitus or insemination and examination. The birds were inseminated or mated and marked or isolated as described in a previous publication (v. Drimmelen 1946 a). The eggs were collected by the trapnest method, set daily and turned daily and candled when opportunity occurred every two to seven days. After the desired interval following insemination or separation from the male, during which time fertility of the subject was proved if possible, the birds were in turn removed to the operating room and examined for the presence of live, normal spermatozoa as described in a previous publication (v. Drimmelen 1945 b). Body temperature and the blood circulation were maintained by doing the examination under anaesthesia instead of after death, but otherwise the technique did not differ from that of the new procedure described in part (11) (pages 142-150) using the capillary attraction of very fine glass pipettes. Where spermatozoa were found, this procedure was consistently suitable to provide a clear suspension for examination of motility and for the preparation of stained films.

Results.—The number of inseminations and matings carried out are shown in Table 63, but the details and the results of the examinations are set out in Table 64. In these cases, sperm cells of perfectly normal appearance could only be demonstrated after intraperitoneal insemination, when the insemination had been done more than 72 hours previously.

The data collected in experiment (7) suggested very strongly, that the spermatozoa of the hen received some form of protection and immobilization during storage. This stimulated further investigation and as reported in a preliminary publication (v. Drimmelen, 1946 a), examination of serial sections led to the discovery of " spermnests " or multiple concentrations of spermatozoa in the mucous membrane of the infundibulum. [See figure (vvi).] This work was carried out as experiment (8).

TABLE 62.

Plan of Experiment (7).

Number of Groups.	Method of Treatment.	Period of Interval.	Day in Period of Experiment.		Remarks.
			Treated.	Examined.	
1	Intraperitoneal insemination.....	2–6 hrs.	5	5	4 Birds.
2	„ „	6–12 hrs.	2	2	2 „
3	„ „	12–24 hrs.	16	17	3 „
4	„ „	2 days	10	12	2 „
5	„ „	4 days	5	9	2 „
6	„ „	8 days	3	11	2 „
7	„ „	12 days	6	18	2 „
8	„ „	16 days	4	20	2 „
9	„ „	20 days	3	23	2 „
10	„ „	28 days	2	30	2 „
11	Natural mating.....	2–12 hrs.	14	14	3 „
12	„ „	12–24 hrs.	9	10	2 „
13	„ „	4 days	9	13	2 „
14	„ „	12 days	13	25	2 „
15	„ „	20 days	4	24	2 „
16	Insemination *per vaginam*.....	2–12 hrs.	6	6	3 „
17	„ „	12–24 hrs.	3	4	2 „
18	„ „	4 days	12	16	2 „
19	„ „	12 days	16	28	2 „
20	„ „	20 days	6	26	2 „

EXPERIMENT (8).

Object.—(*a*) To determine if a special relation could be demonstrated between spermatozoa stored *in vivo* in the infundibulum of the fertile hen and the tissues of that organ.

(*b*) To determine if spermatozoa introduced during natural coitus occupied a similar position in relation to the tissues of the oviduct as those introduced by intraperitoneal injection.

Material and Method.—Specimens of the cranial parts of the oviducts from hens Nos. 905, 902, 912 and 933 were removed and fixed immediately after slaughter and serial sections of 5 μ thick were examined microscopically. (See v. Drimmelen, 1946 a.) Later hens Nos. 2933, inseminated *per vaginam* and 2903, naturally mated, were similarly examined.

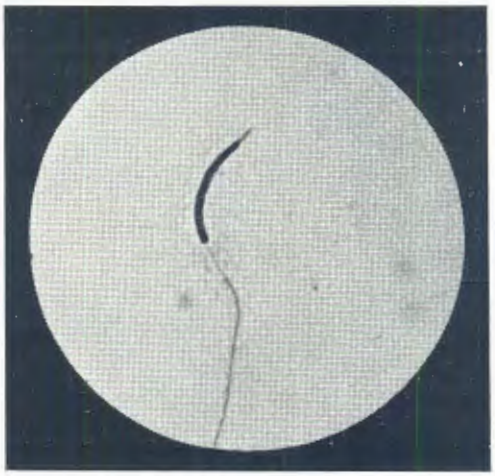
Results.—A statement of the results is given in Table 65. It was observed that the case inseminated by intraperitoneal injection (hen No. 902), which had stored the spermatozoa longer than the hens Nos. 912 and 2903 inseminated by natural coitus, and which had laid roughly as many eggs in the interval, showed numerous large concentrations of spermatozoa in the mucosa of the chalaziferous region in the so called " spermnests ". The hen with naturally admitted semen, showed only just the presence of a few sperm in the " spermnests " found. The structures were photographed [see figures (xv) to (xvii)]. This finding was interpreted to suggest: (*a*) that a definite, consistent and unique arrangement existed in nature whereby the spermatozoa of the fowl are concentrated and immobilized during storage in the mucous membrane of the cranial terminal portion of the oviduct; (*b*) that this arrangement was functioning in a similar way whether the bird was inseminated by intraperitoneal injection of semen or by natural coitus.

TABLE 63.

Inseminations Attempted for the purpose of Determining the Influence of the Method of Insemination on the Site of Storage of Spermatozoa in the Fowl Hen in the Fourth *Series of Inseminations.*

Line.	Method of Insemination.	Instruments used for Insemination.	Number of Inseminations performed.	Number of Birds Inseminated.	Number of Birds Fertilized.	Number of Birds slaughtered for Examination.	Number of Cases in which parts of Normal Sperm were located.	Number of Cases in which Active Sperm were located.
	Column (1)	Column (2)	Col. (3)	Col. (4)	Col. (5)	Col. (6)	Col. (7)	Col. (8)
a	Artificial intraperitoneal insemination	Coarse, long needle [type (vii)] with glass syringe	16	11	4	7	1	2
b		Fine, long, blunt needle [type (ix)] with glass syringe	4	2	2	2	0	0
c		Glass bacterial pipette.....	3	3	2	1	0	0
d		Modified " Holborn " inseminator and glass syringe [type (ii)]	18	9	1	1	0	0
e	Artificial insemination *per vaginam*	Glass syringe.....	1	1	1	1	0	0
f		Glass bacterial pipette.....	9	5	2	4	0	2
g		Modified " Holborn " inseminator and glass syringe [type (ii)]	1	1	0	0	0	0
h	Natural coitus.....	—	9 (matings)	9 (matings)	1	4	1	1
i	Total.....	—	61 (or more natural)	39 (or less natural)	13	20	2	5

FIGURE (XV).—A Fowl Spermatozoon stored Eight Days *in vivo* in the Hen.



Microphotograph of one of a large number of sperm cells seen in a dry stained film of fluid from the infundibulum of hen No. 902, slaughtered eight days after intraperitoneal insemination with semen from cock No. 6. Note the well defined, clear middlepiece, with central longitudinal structure. (X 1400) ; (Stained deeply with ordinary-carbol-fuchsin-eosin, and partly decolorized to show intact structure of middlepiece).

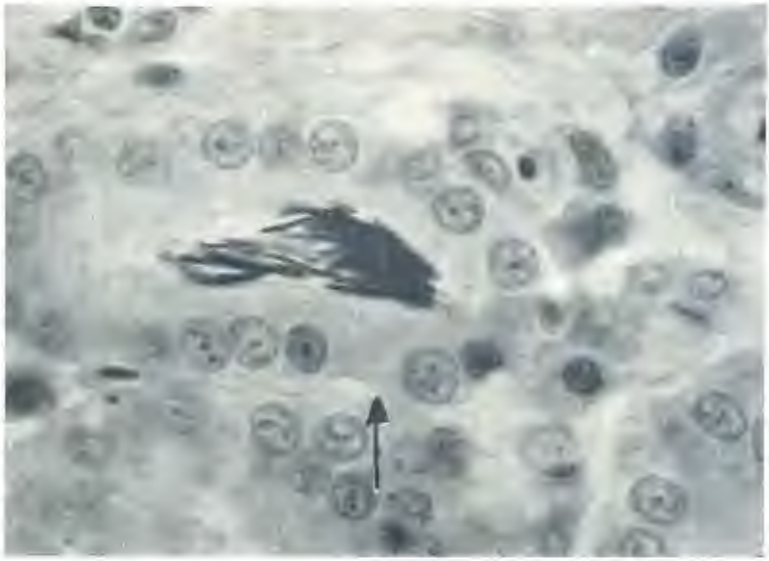
(*Photo by Mr. Th. Meyer, for the Director of Veterinary Services, Onderstepoort.*)

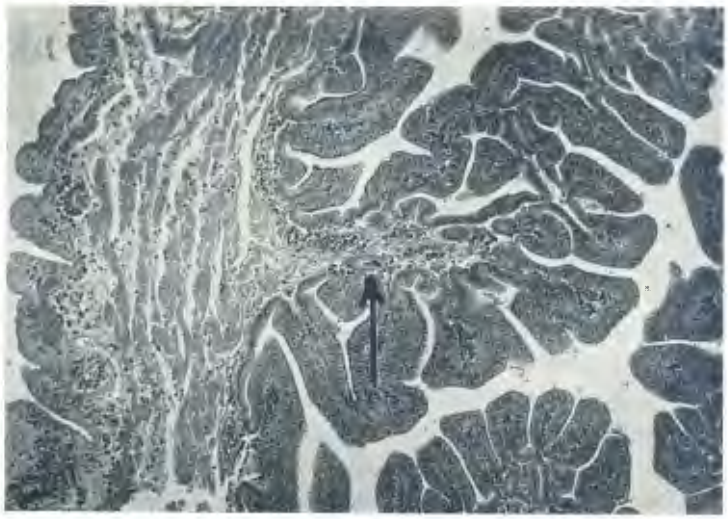
TABLE 64.—(See Appendix C, Table 64.)

The Results of Examination of the Genitalia of Twenty Fowl Hens at Different Times after Insemination by Various Methods in the Fourth *Series of Inseminations Performed.*

Line.	Method of Insemination.	Number of Hens Inseminated; One Case Each.	Localities in which indications of Live Sperm were found.	Interval between Insemination and Examination.
	Column (1)	Column (2)	Column (3)	Column (4)
a	Intraperitoneal insemination...	11	Infundibulum..	Once one day; once eight days and once twelve days.
b	Insemination *per vaginam*.....	5	Uterus.....	Once six hours and once one day.
c			Vagina.....	Once six hours and once one day.
d			Cloaca.....	Once six hours.
e	Natural mating.....	4	Infundibulum..	Once one day and once four days.

FIGURE (xvi) (*a*).—A " Spermnest " or Group of Fowl Sperms stored in the Hen.

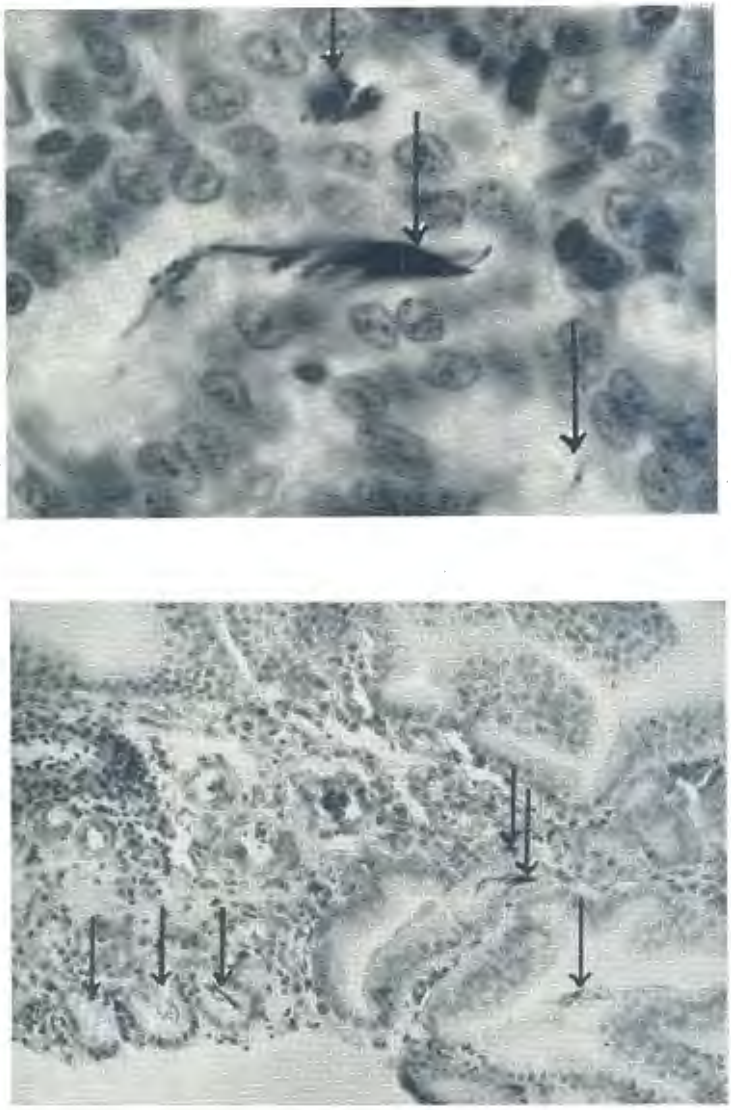




Microphotographs of a section of the chalaziferous region of the oviduct of hen No. 902, slaughtered eight days after intraperitoneal insemination. Note the regular arrangement of the sperm nuclei in the lumen between the epithelial cells, and the position of the " Spermnest " in the folds near the wall of the oviduct : (X 1200 and X 140) ; (Stain : haemalum-eosin).

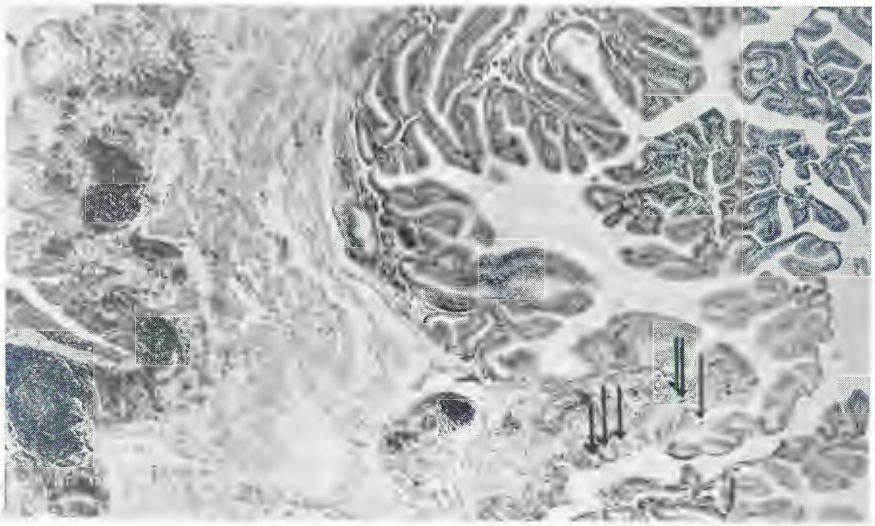
(*Photos by Mr. Th. Meyer, for the Director of Veterinary Services, Onderstepoort.*)

FIGURE (xvi) (*b*).—" Spermnests " or Groups of Fowl Sperms stored in the Hen.



Microphotograph of several " spermnests " in a section of the oviduct of hen No. 902. (X 1200 and X 450) ; (Stain : von Gieson's method).

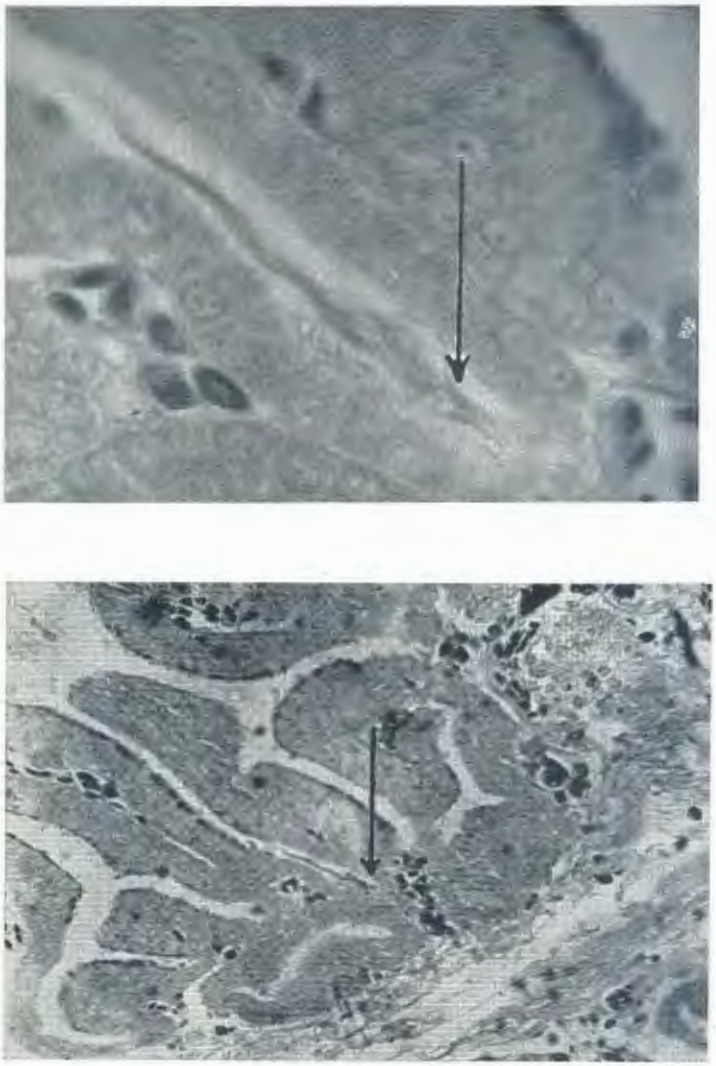
FIGURE (xvi) (*b*)—(*continued*).



The position of several " spermnests " in the mucosa of the infundibulum of hen No. 902, slaughtered eight days after insemination by the intraperitoneal method. Note the near proximity of the nests to the vascular stroma of the folds. (X 90) ; (Stain : von Gieson's method).

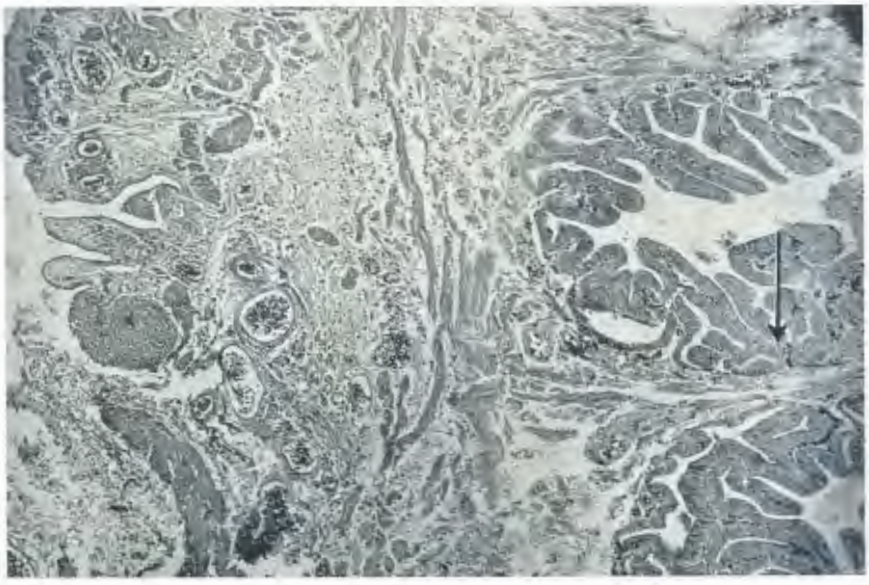
(Photos by Mr. Th. Meyer, for the Director of Veterinary Services, Onderstepoort.)

FIGURE (xvi) (*c*).—Longitudinal Section of a " Spermnest ".



Microphotographs of a " spermnest " in longitudinal section in the oviduct of hen No. 902, showing the arrangement of the spermatozoa in the fundus of a short epithelial duct. Note position of tails in regular bundle and the large and small blood vessels near the fundus. (X 1200 and X 540) ; [Stain : Giemsa's method (slow)].

FIGURE (xvi) (*c*)—(*continued*).



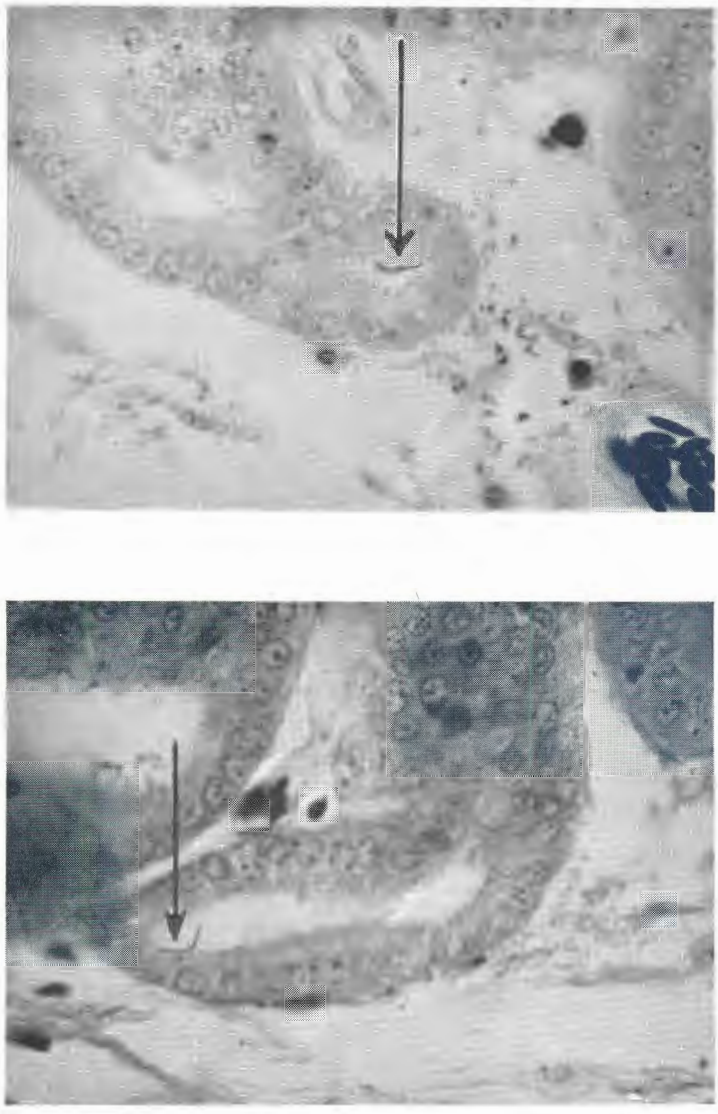
The position of the " spermnest " cut in longitudinal section in the chalaziferous portion of the oviduct of hen No. 902, in relation to the wall of the duct. Note layers of loosely arranged circular and longitudinal muscle fibres as described by Bradley (1928), and vascularity of the organ. (X 90) ; [Stain : Giemsa's method (slow)].

(*Photos by Mr. Th. Meyer, for the Director of Veterinary Services, Onderstepoort.*)

Discussion.

The results of experiment (7) confirm the findings in experiment (6) in that the progressively motile spermatozoa were only demonstrated in the infundibulum of a fertile hen. Only during a short period after insemination or coitus could they be found in the other parts. The evidence collected does not prove conclusively whether a difference in the locality of storage *in vivo* can exist between hens fertilized via the vagina and by intraperitoneal injection. For proof of this a larger number of birds and more favourable working circumstances would be required. The amount of work could be reduced by making much less intensive search of each preparation, since whenever the spermatozoa were found motile they were seen within a few minutes of placing the preparation under the lens of the microscope. If a number of co-operative microscopists could all be supplied with a portion of the genitalia of a hen at slaughter, the examination of a bird could be completed in 20 to 30 minutes and very little preparation would be necessary. The results of experiment (8) were fortunate in this connection as the discovery of " spermnests " in two naturally mated hens and in an intraperitoneal insemination case suggests that *no difference* in locality of sperm storage *in vivo* exists in the fowl hen.

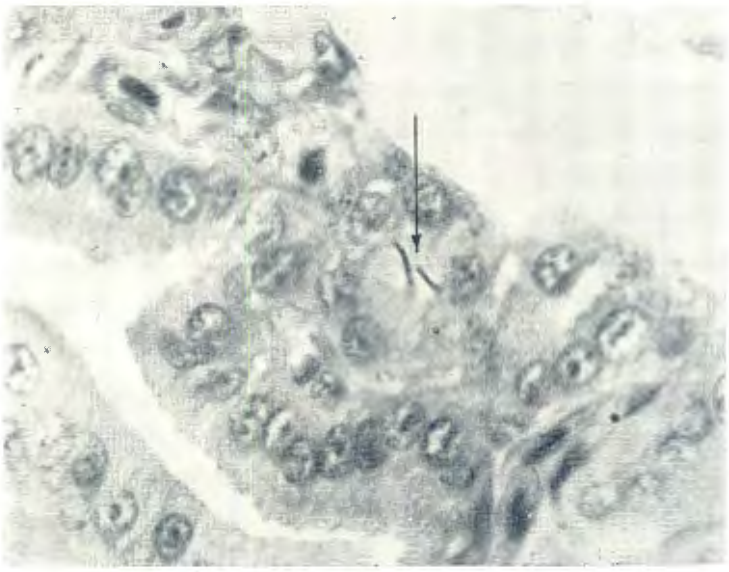
FIGURE (xvii).—" Spermnests " in the Oviduct of a Naturally Mated Hen.



Microphotograph of sections of the infundibulum of hen No. 912, slaughtered four days after separation from the male. Note the sperm heads (nuclei) in the typical position, and the prominence of the blood vessels. (X 750) ; (Stained by Dr. C. Kunst : Heidenhain's iron-haematoxylin).

(*Photos by Mr. Th. Meyer, for the Director of Veterinary Services, Onderstepoort.*)

FIGURE (xviii).—" Spermnest " in the Oviduct of a Naturally Mated Hen, six days after Separation from the Male.



Microphotograph of a section of the infundibulum of hen No. 2903, slaughtered six days after separation from the cock. Two sperm heads (nuclei) can be seen in the spermnest. (X 900); (Stain : Haemalum-eosin).

(*Photo by Mr. Th. Meyer, for the Director of Veterinary Services, Onderstepoort.*)

The significance of the discovery of "spermnests" in experiment (8) was briefly discussed in the earlier publication cited, (v. Drimmelen 1946 a) the main point being that this finding supplies an acceptable explanation for the phenomenon of long duration of fertility in the fowl hen after separation from the male. The results of Munro (1938) on the temperature medium interaction on fowl sperms suggest that these cells are immotile in the upper genital tract of the living hen; but the presence of sperm nests implies some motility of the sperm in this region in order to attain the position in which they were found [see figure (xvi)]. This discrepancy will have to be clarified by further observations. It would be extremely interesting to attempt to fertilize hens of which the chalaziferous region of the oviduct had been removed as was done by Burmester and Card (1939) and others.

The rapid replacement of old sperm by fresh in a fowl hen successively mated to different males (Crew, 1926; Warren and Kilpatrick, 1929; Warren and Gish, 1943) and the favourable influence on fertility brought about by conditioning of the oviduct for egg-production (Lamoreux 1940) are problems on which the demonstration of " spermnests " has an important bearing. It may also give a useful lead to research on storage of fowl sperm, which though viable in the female for periods very much longer than in most mammals, retain their fertilizing power when kept *in vitro* for only a fraction of the period that ram and bull semen can be stored effectively.

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TABLE 65.

The Results of Microscopical Examination of Sections of the Cranial Parts of the Oviducts of Fertile Fowl Hens.

Line.	Number of hen.....	902	905	933	2933	912	2903
	Column (1)	Column (2)	Column (3)	Column (4)	Column (5)	Column (6)	Column (7)
	Insemination:—						
a	Method of insemination	Intraperitoneal.	Intraperitoneal.	Intraperitoneal.	*Per Vaginam*	Natural Coitus	Natural Coitus.
b	Donor of semen.....	Fowl cock No. 6	Cocks Nos. 47 and 53	Cock Number 6	Cock Number 6	Cock No. 63	Cock No. 68.
c	Amount of semen.....	0·5 ml.	0·6 ml.	0·4 ml.	0·6 ml.	—	—
d	Instruments of insemination	Coarse, sharp, metal needle and all-glass syringe	Modified "Holborn" inseminator	Coarse, sharp, metal needle and glass syringe	Glass pipette	—	—
	Production record:—						
e	Days observed before insemination.....	15	5	2	25	12	11
f	Eggs laid in this period	8	3	2	17	4	6
g	Days observed after insemination.....	8	4	16	8	4	7
h	Eggs laid in this period	4	1	10	5	2	4
i	Number of eggs proved fertile.....	3	0	1	33	2	4
	Post Mortem Examination findings:—						
	Sperms seen in smear preparations of:—						
j	The funnel.....	Many	None	None	None	None	None
k	The cranial chalaziferous region.....	Many	Doubtful	None	None	None	None
l	The middle chalaziferous region.....	Some	None	None	None	None	None
m	The caudal chalaziferous region.....	Some	None	None	None	None	None
n	The albumen region.	None	None	None	None	None	None
o	Spermatozoa seen in "Spermnests" in mounted and stained sections of the oviduct	Very numerous in the chalaziferous region; "Spermnests" containing large numbers of spermatozoa	Not found	Not found	Not found (incomplete search)	Several "Spermnests" found containing few spermatozoa	Numerous "Spermnests" found containing several spermatozoa.