Studies On The Mechanism Of Travel Of Spermatozoa Through The Oviduct In The Domestic Fowl

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STUDIES ON THE MECHANISM OF TRAVEL OF SPERMATOZOA THROUGH THE OVIDUCT IN THE DOMESTIC FOWL

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CONTENTS

Chapter I

ON THE TRAVEL OF SPERMATOZOA INSERTED AT THE UTERUS BY ARTIFICIAL INSEMINATION THROUGH THE OVIDUCT OF THE FOWL IN COELIOTOMY UNDER ANESTHESIA

1. Introduction

In the preceding report (MIMURA, 1939) of this series of study the writer described the results of observations on the time required for spermatozoa, which were inseminated artificially at the ab-ovarian end of the uterus, to travel through the oviduct of the domestic fowl from uterus to infundibulum, and he was led to conclude that some such strong force as was generated by the oviducal muscular movement might be chiefly responsible for the transportation; in addition, if any, to other responsible force generated, for example, by the pro-ovarian ciliary movement which was able to be found in a certain part of the albumen portion of the oviduct (MIMURA, 1937a). And the writer, considering that further observations on the muscular movement of the oviduct were necessary, carried out coeliotomy in some birds under anesthesia to expose the oviduct before the observer's eyes, and observed, in these birds, the oviducal movement, both of peristaltic

and antiperistaltic nature. The mode of pro-ovarian transportation of spermatozoa which were inseminated at the ab-ovarian end of the uterus by artificial insemination was observed in this way.

The present studies were begun in 1934 in connection with the studies on the ciliary movement of the oviduct in the domestic fowl (MIMURA, 1937a) and were performed during the past six years. The majority of the investigations were carried out in recent years and the results of experiments published in the preceding paper (MIMURA, 1939) were, indeed, a part of them.

2. Material and Methods

Nine females in their first or second year of egg production were used in this study; of these, six being White Leghorns, one a crossbred of the White Leghorn with another breed, and the remaining two a Rhode Island Red and a Nagoya. Some of these birds were virgin, for they had never been mated with males, and others had been isolated from males for, at least, nine weeks until the work was carried out. A few matured and fertile males of White and Brown Leghorns were used for obtaining spermatozoa from their vasa deferentia after killing them. All of the birds were reared at our Laboratory.

The general procedure of carrying out the artificial insemination and securing the spermatozoa which were used in this study was the same as the one described in the preceding paper (MIMURA, 1939).

The birds were anesthetized either before or after the artificial insemination. One of them (K28) was anesthetized by inhalation with the vapor of ether, the other three (S6, K85, and K200) with that of a mixture of chloroform and ether, and the remaining five (L16, K207, L56, M151, and M142) were anesthetized with a solution of 5% or 10% Evipan-sodium (BAYER), injected into the wing vein, the average dose being 0.045 gm. per kilogram of body weight. In order to keep the birds under full anesthetic condition it was necessary to repeat frequently the administration of the anesthetics, and in the case of Evipan-sodium additional administrations of smaller dose were performed at intervals of 15 to 20 minutes.

As soon as the bird became anesthetized, it was tied on its back by the wings and legs to an operating board. The feathers of the bird at the left side of the abdomen were then plucked off, and an incision was made antero-posteriorly at this part through the skin, the oviduct being located internally adjacent to the body wall. The oviduct was brought into view by opening the body wall along the incision line and sometimes by putting aside the intestine, and observations on the movement of the oviduct were made macroscopically.

Two birds (S6 and K28) received the artificial insemination before being anesthetized, and each of the other seven (K85, L16, K207, L56, M151, K200, and M142) was anesthetized first and coeliotomized, and then received the artificial insemination. The oviduct was thus exposed before the observer's eyes and its movement was kept under observation. Actual observations of the oviducal movements subsequent to the artificial insemination were made for a certain period of time in this way.

Six birds (K85, K207, L56, M151, K200, and M142) were killed by cutting them in the jugular vein and carotid artery of the neck after the observations had been made, but three birds (S6, K28, and L16) died in the course of observations, as described later on.

In certain cases, the tissue fluid found in the surrounding region of the ovary or uppermost part of the oviduct was taken, prior to dissecting the oviduct out of the body wall, by means of a pipette as a sample for sperm examination which was performed afterwards.

The linear measurements and the sperm examinations were made in the same way as described in the preceding paper (MIMURA, 1939).

The "interval after insemination" is the length of time measured from the moment when the sperm suspension was inseminated till the moment when the different parts of the oviducts were cut into pieces (S6, K85, L16, K207, and L56), or in other cases till the moment when the sperm samples were taken either from the uppermost parts of the intact oviducts (K28 and K200) or from the regions of ovaries of the birds in coeliotomy (M151, and M142).

3. Description of the Results

The results of the examinations on the travel of inseminated spermatozoa through the oviduct of the bird and those of the observations on the oviducal muscular movement are described individually. The situation of the oviduct of each bird is denoted by the schematic illustration in Fig. 1. The length of each of the schematic oviduct is proportional to that of the actual one. The results of linear measurements of the five parts of oviducts of nine birds are given in Table 1.

Table 1

Length (cm.) / Individual	Vagina (A-B)	Uterus (B-C)	Isthmus (C-D)	Albumen portion (D-E)	Infundibulum (E-F)	Total (A-F)
S 6	10.5	8.0	11.5	34.0	10.5	74.5
K 85	7.5	6.0	9.5	28.5	8.0	59.5
K 28	8.0	8.0	10.5	32.5	10.0	69.0
L 16	8.5	9.5	12.0	32.5	10.0	72.5
K 207	10.0	7.0	10.0	27.5	9.5	64.0
L 56	9.5	7.0	10.5	27.0	8.5	62.5
M 151	10.0	8.0	11.0	31.0	11.0	71.0
K 200	7.5	6.0	8.5	26.0	8.0	56.0
M 142	9.0	6.5	10.0	29.5	9.5	64.5

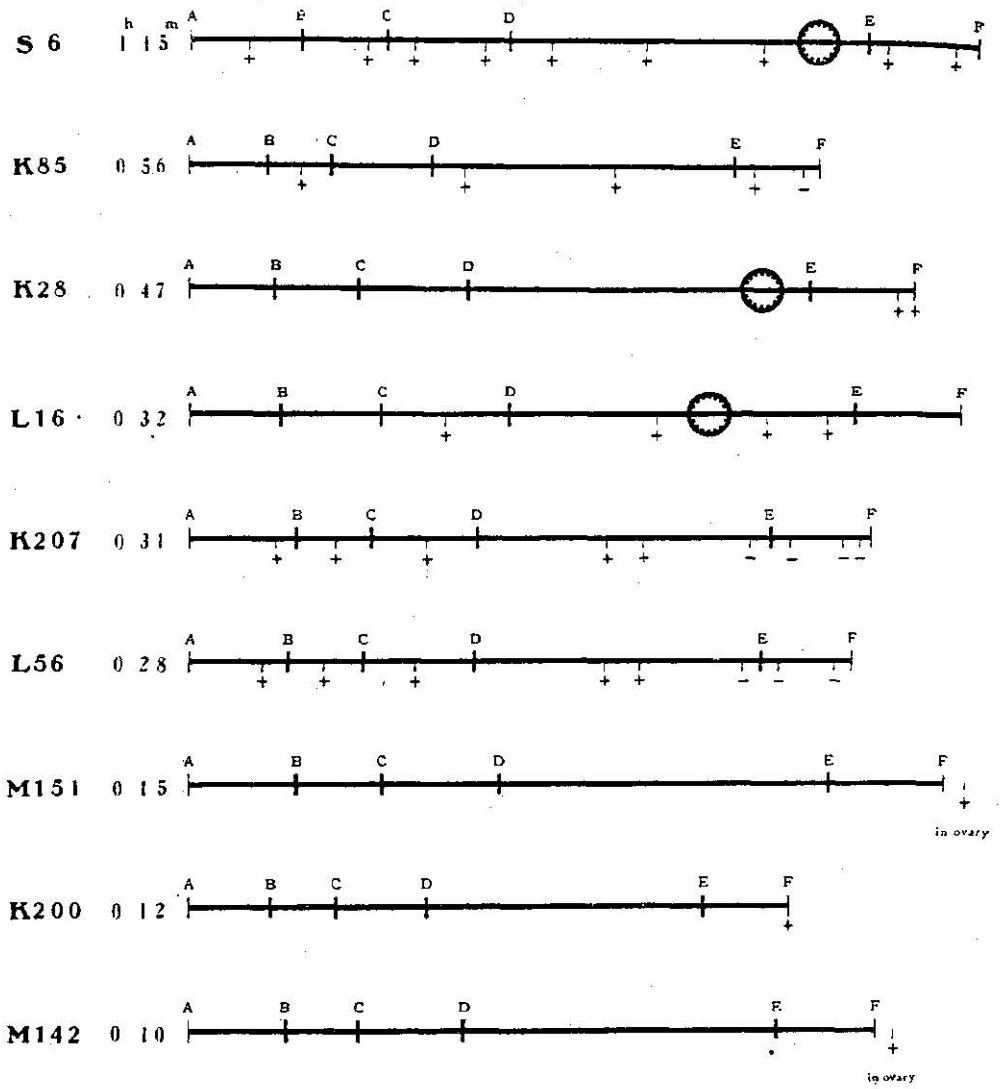


Fig. 1. Schematic oviducts illustrating the places where the sperm samples were taken from the mucous epithelia of the oviducts of birds, S6, K85, K28, L16, K207, L56, M151, K200, and M142, which received the artificial insemination at the uterus in coeliotomy under anesthesia. Figures denoting the time intervals are the "interval after insemination" as explained in the section of Material and Methods. A-B = vagina; B-C = uterus; C-D = isthmus; D-E = albumen portion; E-F = infundibulum. + denotes that the spermatozoa were present in the sample taken from the place; − denotes that the spermatozoa were absent in tbe sample taken from the place. ◯ = an ovum with neither shell nor shell membrane, *viz.*, an ovum or yolk with some surrounding albumen. Positions occupied by the schematic ova on the schematic oviducts correspond to the actual places of the ova found in the oviducts of these birds, sizes of the ova on the schematic oviducts being not proportional to those of actual ones.

S6. The Nagoya, one of Japanese breeds.

The bird laid an egg at 10:55 a.m., and was inseminated with sperm suspension in Ringer's solution at 11:15 a.m., or 20 minutes after the egg laying. Anesthesia was made by letting the bird inhale the vapor of a mixture of chloroform and ether at 12:10 p.m. As soon as the bird was anesthetized the coeliotomy was carried out and the oviduct was exposed before the observer's eyes; and it was found that the oviduct had an ovum in the uppermost part of its albumen portion. The peristaltic movement of the oviduct and the external appearance of the passage of the ovum down through the inner lumen of the oviduct were observed for about 10 minutes, then the bird had a convulsive fit and died at 12:20 p.m. The writer had been absorbed in the observation and had been quite unconscious of the pressure which had been given to the bird on the sternum by the writer's hand keeping the coeliotomized abdomen widely open. The pressure on the sternum probably made the anesthetized bird difficult to breathe and caused the death.

The oviduct was then dissected out of the body wall, cut into pieces, and the inner wall of each piece was opened by an ordinary method. An ovum with some surrounding albumen was found in the anterior part of the albumen portion. The sperm examinations were made at 12:30 p.m., or 1 hour and 15 minutes after the insemination. Sampling sperm smear was made at nine places on the oviduct: one in the vagina, one in the uterus, two in the isthmus, three in the albumen portion, and two in the infundibulum. All of the samples contained the spermatozoa.

K85. The White Leghorn.

The bird laid an egg at 1:35 p.m., and anesthetized itself by inhaling the vapor of a mixture of chloroform and ether at 2:00 p.m. The anesthesia was immediately followed by the coeliotomy, and the artificial insemination was performed in the exposed oviduct by the routine procedure at 2:14 p.m. The direct observation on the antiperistaltic movement was successfully made at the isthmus of the oviduct of this bird. The direction of the wave extending over the oviduct generated by the antiperistaltic movement of oviducal wall was determined by piercing a thread-and-needle into the wall of the oviduct just behind the place where the antiperistaltic wave was observed to have extended over the oviduct, the thread being able to mark the position on the oviduct when it was dissected out of the body wall and was stretched on a dissecting glass-board.

The bird was then killed in the usual way before waking up from the anesthetic condition, and the oviduct was dissected out of the body

wall and the sperm examinations were made at 3:10 p.m., or 56 minutes after the insemination. Sampling sperm smear was made at five places on the oviduct: one in the uterus, two in the albumen portion, and two in the infundibulum. Four samples which were taken from the uterus, albumen portion, and posterior part of the infundibulum contained the spermatozoa, but another one taken from the fimbriae of infundibulum, did not contain any.

K28. The Rhode Island Red.

The bird laid an egg at 1:30 p.m., and was inseminated with sperm suspension at 1:53 p.m., *i.e.*, 23 minutes after the egg laying. Anesthesia was made by allowing the bird to inhale the vapor of ether at 2:00 p.m., and the coeliotomy immediately followed it. The oviduct was found to contain an ovum in its anterior part. Two sperm samples were taken from the fimbriae of the oviduct of the bird in coeliotomy at 2:40 p.m., or 47 minutes after the insemination. All of these samples contained the spermatozoa. The bird died without any struggle at 2:50 p.m., and the immediate cause of the death was considered to be an excessive administration of anesthetics. The oviduct was then dissected out of the body wall, and an ovum with some surrounding albumen was found in the uppermost part of the albumen portion.

L16. The White Leghorn.

The bird laid an egg at 10:43 a.m., and was anesthetized by an injection of liquid Evipan-sodium. The initial injection of this anesthetic was made at 11:25 a.m., and additional injections were made until complete anesthesia was produced at 11:55 a.m. The coeliotomy immediately followed it and an artificial insemination was performed in the exposed oviduct in coeliotomy by the routine procedure at 12:08 p.m. An ovum was found, at this time, in the anterior part of the oviduct. Two sperm samples were taken from the fimbriae of the oviduct of the bird in coeliotomy at 12:17 p.m., or 9 minutes after the insemination. The bird died without any convulsion at 12:30 p.m. The oviduct was then dissected out of the body wall and it contained an ovum with some surrounding albumen in the midway of the albumen portion. The sperm examinations were made at 12:40 p.m., or 32 minutes after the insemination. Sampling sperm smear was made at four places: one in the isthmus, one in the albumen portion ab-ovarian to the ovum and two in the albumen portion pro-ovarian to it. All of these samples contained the spermatozoa. But two samples which were taken from the fimbriae 23 minutes previous to these samplings, as described above, did not contain any.

K207. The White Leghorn.

The bird laid an egg at 2:30 p.m., and was anesthetized by an injection of liquid Evipan-sodium. The initial injection of this anesthetic was made at 3:15 p.m. The complete anesthesia was followed by the coeliotomy and an artificial insemination was performed in the exposed oviduct in coeliotomy by the routine procedure at 3:41 p.m. A sperm sample was taken from a surrounding region of the ovary at 4:05 p.m. and examined. The sample did not contain any spermatozoa.

The bird was killed in the usual way before waking up from its anesthesia and the oviduct was dissected out of the body wall. No ovum was found in the oviduct. The sperm examinations were made at 4:12 p.m., or 31 minutes after the insemination, and sampling sperm smear was made at nine places on the oviduct: one in the vagina, one in the uterus, one in the isthmus, three in the albumen portion, and three in the infundibulum, and an additional sampling was made at the ovarian region as described above. Five samples which were taken from the vagina, uterus, isthmus and the middle part of the albumen portion contained the spermatozoa, but the other four taken from the uppermost part of the albumen portion as well as from the infundibulum, did not contain any.

L56. The White Leghorn.

The bird laid an egg at 1:55 p.m., and was anesthetized by an injection of liquid Evipan-sodium at 2:38 p.m. The anesthesia was followed by the coeliotomy and an artificial insemination was performed in the exposed oviduct in coeliotomy by the routine procedure at 2:50 p.m. A sperm sample was taken from a surrounding region of the ovary at 3:10 p.m. and examined, it did not contain the spermatozoa. The bird was killed in the usual way before its waking up from the anesthesia and the oviduct was dissected out of the body wall. No ovum was found in the oviduct. The sperm examinations were made at 3:18 p.m., or 28 minutes after the insemination. Sampling sperm smear was made at eight places: one in the vagina, one in the uterus, one in the isthmus, three in the albumen portion, and two in the infundibulum, and an additional sampling was made at the ovarian region as described above. Five samples which were taken from the vagina, uterus, isthmus, and the middle part of the albumen portion contained the spermatozoa, but the other three taken from the uppermost part of the albumen portion and infundibulum, did not contain any.

M151. The White Leghorn.

The bird laid an egg at 3:35 p.m., and was anesthetized by an in-

jection of liquid Evipan-sodium. The initial injection of this anesthetic was made at 3:50 p.m. The complete anesthesia was followed by the coeliotomy and an artificial insemination was performed in the exposed oviduct in coeliotomy by the routine procedure at 4:00 p.m. Two sperm samples were taken from a surrounding region of the ovary at 4:15 p.m. and 4:20 p.m., *i.e.*, 15 and 20 minutes after the insemination, respectively, and were examined. Both of these samples contained the spermatozoa. The bird was killed in the usual way before its waking up from the anesthesia and the oviduct was dissected out of the body wall. No ovum was found in the oviduct. The sperm examinations on the oviduct were made at 4:40 p.m. Sampling sperm smear was made at several places of the infundibulum and albumen portion of the oviduct. All of these samples contained the spermatozoa.

K200. A crossbred of the White Leghorn.

The bird laid an egg at 1:45 p.m., and was anesthetized by the vapor of a mixture of chloroform and ether at 2:15 p.m. The coeliotomy immediately followed it and an artificial insemination was performed in the exposed oviduct in coeliotomy by the routine procedure at 2:28 p.m. An ovum in the ovary was found just ovulating, and the fimbriae of infundibulum covered the surface of the ovum. A sperm sample was taken from the fimbriae at 2:40 p.m., or 12 minutes after the insemination, and examined. This sample contained the spermatozoa. The bird was then killed in the usual way before its waking up from the anesthesia and the oviduct was dissected out of the body wall. No ovum was found in the oviduct.

M142. The White Leghorn.

The bird laid an egg at 10:30 a.m., and was anesthetized by liquid Evipan-sodium injection at 10:41 a.m. The anesthesia was followed by the coeliotomy, and an artificial insemination was performed in the exposed oviduct in coeliotomy by the routine procedure at 10:55 a.m. Three sperm samples were taken from a surrounding region of the ovary at 11:05, 11:08, and 11:10 a.m., *i.e.*, 10, 13, and 15 minutes after the artificial insemination, respectively, and were examined under a microscope. All of these samples contained the spermatozoa. The bird was killed in the usual way before its waking up from the anesthesia and the oviduct was dissected out of the body wall. No ovum was found in the oviduct. The sperm examinations on the oviduct were made at 11:24 a.m. Sampling sperm smear was made at several places of the infundibulum and albumen portion of the oviduct. All of these samples contained the spermatozoa.

It was found that the Evipan-sodium was quite useful in obtaining satisfactory results in anesthetizing the material birds by injection; while ether or the mixture of chloroform and ether was found to be less convenient in anesthetizing the birds by inhalation, since some difficulties were involved in determining accurately the dose of administration.

All of the birds were kept alive as they were under the influence of anesthetics for certain length of time in order to make direct observations on the movement of oviducts of birds in coeliotomy. The ovum was found passing down through the inner lumen of the oviduct in each of the three birds, S6, K28, and L16, and it was actually observed that the ovum was carried down through the oviduct by its peristaltic movement. It was recognized, in the bird K85, that an antiperistaltic movement of the oviduct developed at the isthmus shortly after the artificial insemination, and continued for a while. In the remaining birds, the oviducts usually exhibited slight movements when they were first exposed by coeliotomy, and when the artificial inseminations were performed, greater movements were able to be observed, especially in the parts of the uterus and the isthmus, although the movement did not indicate the nature of an antiperistalsis as clearly as in the bird K85.

The direct observation on the oviduct in this study revealed that the struggle of death was not responsible for the visible change that occurred in the movement of the oviduct of the bird S6 and it was learned that some movement of the oviducts persisted for a while (5–15 min.) after the bird's death.

It was of interest to learn that the spermatozoa found in the samples which were taken from the albumen portion of each of the four birds, S6, K85, L16, and M142, were found to be covered by a dense albumen layer, the layer being prolonged in the form of a continuous cord or strand in this portion of the oviduct. The albumen cord or strand which is usually secreted abundantly in advance of the ovum was found especially in the oviducts of these birds.

It was of importance in considering the mechanism of travel of spermatozoa through the oviduct to know that the time required for the spermatozoa inserted at the ab-ovarian end of the uterus by the artificial insemination to travel through the oviduct

from uterus to infundibulum or even to the ovarian region was only 10 minutes after the insemination in the bird M142, although the oviduct of this bird was found to be of medium length as compared with those shown in Table 1 in the present paper as well as those reported in the previous one (MIMURA, 1937b); the whole length of the oviduct was 64.5 cm., and that of the vagina 9.0 cm., so the length from the posterior end of uterus to the pro-ovarian end of the oviduct was to be 55.5 cm. The spermatozoa traversed, therefore, the distance of 55.5 cm. through this region of the oviduct within 10 minutes after the artificial insemination.

For the convenience' sake, the relations between the time interval after the artificial insemination and the existence of spermatozoa at various portions of oviducts or ovarian region where the sperm samplings were made are summarized and shown in Table 2.

Table 2. Table showing the relations between the time intervals after insemination and existence of spermatozoa at various portions of the oviducts or ovarian region where the sperm samplings were made.

Individual	In coeliotomy				At autopsy					
	Ovarian region		Fimbriae		Fimbriae		Infundibulum		Albumen portion	
	Time interval (min.)	Sperm presence (+) or absence (−)	Time interval (min.)	Sperm presence (+) or absence (−)	Time interval (min.)	Sperm presence (+) or absence (−)	Time interval (min.)	Sperm presence (+) or absence (−)	Time interval (min.)	Sperm presence (+) or absence (−)
S 6							75	+(A.P.)		
K 85					56	−	56	+(P.P.)		
K 28			47	+						
L 16			9	−					32	+(A.P.)
K 207	24	−					31	−	31	+(M.P.)
L 56	20	−					28	−	28	+(M.P.)
M151	15	+					40	+	40	+(A.P.)
K 200			12	+						
M142	10	+					29	+	29	+(A.P.)

A.P.=anterior part; M.P.=middle part; P.P.=posterior part.

4. Consideration

The time of ovulation of any ovum which was actually found in a certain part of the oviduct at autopsy could, as stated in the preceding paper (MIMURA, 1939), approximately be estimated, and the place where the ovum, if any, was found in the oviduct of each bird, at the time of artificial insemination, was estimated as shown in Table 3.

Table 3. Table showing the positions of the ova and the stages of their formation in the oviducts at the time of insemination as well as at autopsy.

Individual	Time interval between egg laying and the insemination	Ovum found actually in the oviduct at autopsy		The estimated position and the stage of formation of that ovum in the oviduct or in the ovary *at the time of insemination*, which was actually found in the oviduct at autopsy		Remarks	
		Position in the oviduct	Stage of formation	Position in the oviduct or ovary	Stage of formation		
S 6	20 min.	Alb. p.	Y+A	Ovary, (before ovulation)	Ovum	The uppermost part where the spermatozoa found in coeliotomy or at autopsy, and the time interval	infundibulum, 75 min. (at autopsy)
K 85	39						infundibulum, 56 min. (at autopsy)
K 28	23	Alb. p.	Y+A	Ovary, (before ovulation)	Ovum		infundibulum, 47 min. (in coeliotomy)
L 16	85	Alb. p.	Y+A	Infundibulum or Alb. p.	Y+		uppermost part of alb. p., 32 min. (at autopsy)
K207	71						middle part of alb. p., 31 min. (at autopsy)
L 56	55						middle part of alb. p., 28 min. (at autopsy)
M151	25						ovary, 15 min. (in coeliotomy)
K200	43						infundibulum, 12 min. (in coeliotomy)
M142	25						ovary, 10 min. (in coeliotomy)

Alb. p. = albumen portion; Y + A = ovum or yolk with some surrounding albumen; Y+ = ovum or yolk with no albumen, or yolk only.

It will be seen from Fig. 1 and Table 3 that (i) when the artificial insemination was performed in a bird shortly after the egg laying, and when it was estimated that its oviduct contained no ovum in it, rapid progress of spermatozoa was able to be observed (S6 and K28) as was already shown in the preceding paper (MIMURA, 1939), and that (ii) when the oviduct was estimated to contain an ovum in the lower part of the infundibulum or in the uppermost part of the albumen portion at the time of insemination the spermatozoa inserted showed immediate advancement and reached the portion just posterior to the ovum in the oviduct (L16).

WARREN and SCOTT (1935) stated that the activity of the musculature of the oviduct was largely involuntary and the movement of this sort was not affected by an anesthesia, and the writer, too, was convinced of the truth of that statement from the observations on the oviducts of nine birds used in this study, which were all anesthetized. Even the death struggle which occurred under observations in the bird S6 in coeliotomy, as described above, did not give any apparent stimulation or disturbance to the movement of the musculature of the oviduct. Furthermore, in spite of the fact that nine birds used in this study were all anesthetized and coeliotomized as well, and were logically considered to have suffered severer trauma than the birds used in the preceding work then received artificial inseminations without undergoing either anesthesia or coeliotomy (MIMURA, 1939), the time intervals which were observed to be spent by the spermatozoa, inseminated in the oviduct at the ab-ovarian end of the uterus and destined to travel upwards through the oviduct from the uterus to reach the infundibulum or even to the ovarian region, were found to be shorter in the former (M151, K200, and M142) than in the latter (*e.g.*, J60). From the results of these observations it might be considered that such trauma caused by anesthesia and coeliotomy had little or no effect in retarding the rate of the progress of the spermatozoa travelling through the oviduct, if the activity of the moving oviducts, either peristaltic or antiperistaltic, persisted for the entire duration of observations. However, when the exposure of the oviducts of the birds in coeliotomy for observation continued for a long time (*e.g.*, K207 and L56) with the result of drying the oviducal wall in the air and also of lessening the body heat,

the gradual fall of the activity of moving oviducts was found to become somewhat manifest, and more delayed transportation of the spermatozoa was considered to occur.

With regard to the length of time required for the passage of inseminated spermatozoa through the oviduct, the writer observed in the previous study (MIMURA, 1939), that it took 26 minutes at least to pass through the oviduct from uterus to infundibulum, the rate of progress calculated being 2.4 cm. per minute or 400 μ per second, and he was led to conclude that some such strong force as was generated by the oviducal muscular movement, instead of the motility of spermatozoa themselves, might be chiefly responsible for the transportation of spermatozoa through the oviduct in the fowl. The length of time required to pass through the oviduct, according to the present study, was reduced markedly and it was observed that the spermatozoa inserted at the posterior end of the uterus reached the ovarian region through the oviduct within 10 minutes in the bird M142, the rate of progress being about 5.55 cm. per minute or 925 μ per second. In the observations on the rate of locomotion of spermatozoa which will be described later, it was determined that the maximum velocity of spermatozoön was 36 μ per second in liquid egg albumen. As the intervals mentioned above were too short for the spermatozoa to progress by their own locomotion from the uterus to the uppermost part of the oviduct where they were found, the writer concluded that the muscular activity of the oviduct must have been chiefly responsible for the transportation of the spermatozoa. And further, during this study, the direct observation was made on the actual movement of antiperistalsis at the isthmus of the bird K85 immediately after the artificial insemination and the results of sperm examinations of the oviduct showed a rapid progress of spermatozoa from uterus to infundibulum. This observation favored an assumption that the pro-ovarian transportation of spermatozoa through the oviduct should be accomplished chiefly by this antiperistaltic movement of the oviduct which invariably developed and extended over the entire length of the oviduct from uterus to infundibulum. It might therefore be inferred that the antiperistaltic movement developed in the oviducts of all the birds used in this study and that the spermatozoa inserted at the posterior end of each uterus of these birds were rapidly transported towards

ovary and were found to have reached the midway of the albumen portion (K207 and L56), the uppermost part of it (L16), the infundibulum (S6, K85, K28, and K200), or even the ovarian region itself (M151 and M142) by this antiperistalsis of the oviduct. The direct observation on the movement of the oviduct carried out in each of three birds, S6, K28, and L16 in coeliotomy indicated that an ovum had been transported by the normal peristaltic movement of the oviduct through the albumen portion, and this observation favored an assumption that the peristalsis was one of the important factors in the transportation of an ovum through the oviduct of the bird.

The writer estimated that in the case of the artificial insemination at the uterus of the bird L16, whose oviduct contained an ovum in the posterior part of the infundibulum or the uppermost part of albumen portion at the time as shown in Table 3, the ovum ovulated was normally transported by the peristaltic movement of the oviduct till the artificial insemination was performed. On the other hand the spermatozoa inserted at the uterus of the bird was thought to have been transported towards the ovary through the oviduct and reached the uppermost part of albumen portion by the antiperistaltic movement of the oviduct which was excited by the act of artificial insemination, but the persistence of the antiperistalsis of the oviduct was not so long in this case that the normal peristalsis was produced again and the ovum was carried down through the albumen portion and was found in the midway of this portion at autopsy.

It could be supposed in conclusion that, in domestic fowls, the travel of spermatozoa through the oviduct from uterus to infundibulum was accomplished mainly by the antiperistaltic movement of oviducal musculature and that the downward passage of ovum through the entire length of the oviduct was performed chiefly by the peristaltic movement of the oviduct.

5. Summary

1) The birds used in this study were anesthetized either before or after the artificial insemination, and the anesthesia was immediately followed by the coeliotomy, and all of them were kept alive as they were under the influence of anesthetics for a certain

length of time, and the direct observations on the movement of the oviducts were carried out.

2) Liquid Evipan-sodium (Bayer) injected intravenously was found to be a more satisfactory basal anesthetic for this study than ether and chloroform inhalated.

3) The gross observations of the oviducts indicated that the mode of movement of the oviducts of anesthetized birds was normal, and it was determined that the activity of the oviducal musculature was not in any way stimulated by the death struggle of birds.

4) The direct observation on the antiperistaltic movement of oviduct was made at the isthmus of a bird after the artificial insemination, and an ovum passing down through the oviduct by normal peristaltic movement was observed in other birds.

5) At different intervals of time after the artificial insemination, the birds were killed and the routine examinations of the presence of spermatozoa in various parts of the oviduct were made.

6) It was found in a bird that the spermatozoa inserted at the posterior end of the uterus reached the ovarian region through the oviduct within 10 minutes.

7) It was confirmed that the oviducal muscular movement must have necessarily been responsible for the pro-ovarian transportation of the spermatozoa through the oviduct in the fowl, that is to say, the travel of the spermatozoa through the oviduct from uterus to infundibulum was accomplished mainly by the antiperistaltic movement of the oviducal musculature, and that the downward passage of an ovum through the entire length of the oviduct was made also by the oviducal muscular movement, especially by the peristaltic movement of the oviduct.

Chapter II

ON THE TRAVEL OF INANIMATE SUBSTANCES OTHER THAN SPERMATOZOA INJECTED INTO THE LUMEN AT VARIOUS PARTS THROUGH THE OVIDUCT OF THE FOWL IN COELIOTOMY UNDER ANESTHESIA

1. Introduction

In Chapter I, it was shown that the mode of muscular movement of the oviducts observed in anesthetized birds was not different from that in non-anesthetized normal birds, that the ovum, therefore, ovulated from the ovary and accepted in the oviduct in bird could pass down through the oviducal lumen by the peristaltic movement of the oviduct, that the spermatozoa inserted artificially at the uterus could also be transported rapidly upwards to the uppermost part of the oviduct or even to the ovary through the oviducal lumen by, presumably, the antiperistaltic movement of the oviduct; and the actual movement of the antiperistalsis developed and extended over a certain length of some part of the oviduct was also described.

In the present Chapter, descriptions are made of the results obtained from the experiments on the travel of the substances of inanimate nature, instead of spermatic fluid, such as a solution of methylene blue or a suspension of carbon particles in Ringer's solution or in egg albumen, injected into the lumen at various parts, through the oviduct, the birds being anesthetized and coeliotomized; these experiments were performed in order to examine the bearing which the travel of such inanimate substances in non-spermatic fluid had upon the oviducal movements of birds.

2. Material and Methods

Thirty-one birds, namely, twenty-three White Leghorns, three Brown Leghorns, one Rhode Island Red, two Barred Plymouth Rocks, a crossbred of the Barred Plymouth Rock with the Rhode Island Red and a crossbred of the White Leghorn with another breed, were used in this study. All of the birds were in their first or second year of egg production, and were reared at our Laboratory.

Every bird was anesthetized by an injection of a solution of

10% Evipan-sodium into the wing vein and coeliotomized in the same way as described in Chapter I.

The oviduct exposed received injections of one or both of two substances, as described below, into its inner lumen through the wall at its several parts with a hypodermic needle and syringe. The very part of the oviduct where the injection was performed was marked off by an insertion of a thread into the oviducal wall with a needle. After the injections were performed, the open body wall was closed and the skin was pulled together, thus enabling the body cavity to conserve the humidity and body heat of the bird. When it was desired to prolong full anesthesia, the bird was given additional inhalations of ether at short intervals of time.

Two kinds of substance were used for the injection in this study, namely, (i) charcoal carbon particles, obtained by grinding charcoal in an iron mortar, and suspended in Ringer's solution, the same as used in the work reported in the preceding paper (MIMURA, 1939), and (ii) a solution of methylene blue dissolved in Ringer's solution. In some cases, liquid egg albumen obtained from a fresh hen's egg was used instead of Ringer's solution. A small amount, 0.2–1.0 cc. of a solution of methylene blue and 0.1–2.0 cc. of a suspension of carbon particles in Ringer's solution, were injected into the lumen of the oviduct. Both the solution and the suspension were injected one by one into the oviduct of the same bird in separate portions in fifteen birds out of thirty-one in this study.

At different intervals of time after the injection was made, the birds were killed, and the linear measurements of the oviducts were performed at autopsy. The whole oviduct of each bird was then opened by splitting it longitudinally, and the state of spreading of injected substances on the surface of the epithelium of the oviduct was observed macroscopically. In any part where the injected substances were scattered sparsely, making it so difficult to detect macroscopically, they were examined under a binocular microscope. The opening of the oviduct was performed with the utmost precaution so as to avoid the conveyance of the injected substances from the very portion of spreading to further areas, as the substances were apt to flow drifting upwards and downwards.

The solution of methylene blue injected stained the cells of the mucous epithelium of the oviduct, and infiltrated into the yolk of an ovum through its vitelline membrane and stained the yolk spherules, if it was found to be contained in the upper part of the oviduct and had no thick albumen envelope. Methylene blue which impregnated the mucous epithelium or yolk spherules did not discharge the color for a sufficient length of time of the observations, even though more secretion followed in the epithelium.

The carbon particles injected, either adhered loosely to the surface of the mucous epithelium or intermixed with the egg albumen secreted by the mucus. These particles of carbon tended, in most cases, to flow driftingly up or down to some distances in the oviduct, according to the direction of the ciliary movement*. The dye of methylene blue impregnated the epithelial tissue or the yolk spherules in an ovum and the coloration lasted long enough to make the observations possible, as denoted above, while it is worth-while to note that the carbon particles having been spread before the observations began to move along the surface of the mucous epithelium by ciliary movement, which make it imperative to make prompt observations.

The time "interval after injection" in this Chapter means the time which elapses from the moment of injection of either of two substances till the moment when the oviduct was opened by splitting it longitudinally.

3. Description of the Results

The time intervals after the injection of each of the birds are given in Fig. 2, and the data concerning the rate of dispersion of the injected substances are denoted by the schematic illustration in the same figure. The length of each of the schematic oviducts is proportional to that of the actual one. And the results of linear measurements of the five parts of oviducts of the birds studied are given in Table 4.

* The writer (1937) demonstrated that the most convenient method of observing the ciliary movement of the mucous epithelium was to watch the movement of small particles of charcoal powder sprayed on the surface of ciliated epithelium.

Table 4

Length (cm.) / Individual	Vagina (A-B)	Uterus (B-C)	Isthmus (C-D)	Albumen portion (D-E)	Infundibulum (E-F)	Total (A-F)
K 104	9.0	6.0	9.5	23.5	9.0	57.0
M 32	8.0	6.0	10.0	29.0	8.0	61.0
M 216	10.0	7.5	11.0	38.0	11.0	77.5
K 17	7.0	5.5	7.5	25.0	8.5	53.5
M 246	7.5	6.5	7.5	28.0	10.0	59.5
M 18	8.5	5.5	9.0	27.0	10.5	60.5
L 7	10.5	8.0	9.0	29.0	10.0	66.5
K 2	8.0	6.0	9.0	25.5	9.0	57.5
K 204	9.5	7.0	9.0	32.0	9.0	66.5
K 205	11.0	7.0	11.0	32.0	11.5	72.5
M 144	8.5	7.0	12.5	31.5	11.5	71.0
M 219	8.5	6.0	10.0	27.0	11.5	63.0
M 218	9.0	6.5	9.5	27.5	13.0	65.5
M 241	8.0	7.0	11.0	32.0	13.5	71.5
M 67	8.5	6.5	10.0	25.0	10.5	60.5
M 117	8.0	6.0	9.5	31.5	12.5	67.5
Y 3	8.5	7.0	11.0	31.5	9.0	67.0
M 137	8.0	6.5	9.0	27.0	11.5	62.0
K 27	9.0	8.0	10.0	27.0	10.0	64.0
M 251	8.0	6.5	9.5	31.0	9.5	64.5
K 176	9.0	7.0	11.0	30.0	9.5	66.5
M 217	9.5	6.5	11.5	31.0	10.5	69.0
L 95	8.5	7.0	9.0	30.5	11.0	66.0
L 23	9.0	7.5	9.5	30.0	9.0	65.0
K 105	8.0	6.5	7.0	22.5	8.0	52.0
L 17	9.0	8.0	10.0	26.5	9.5	63.0
K 196	9.0	10.5	9.5	27.5	11.0	67.5
K 201	10.0	7.0	7.0	27.5	11.0	62.5
L 40	10.5	7.5	11.0	33.0	9.5	71.5
K 203	9.5	7.0	10.0	29.0	10.0	65.5
K 202	11.5	8.0	10.0	32.5	10.5	72.5

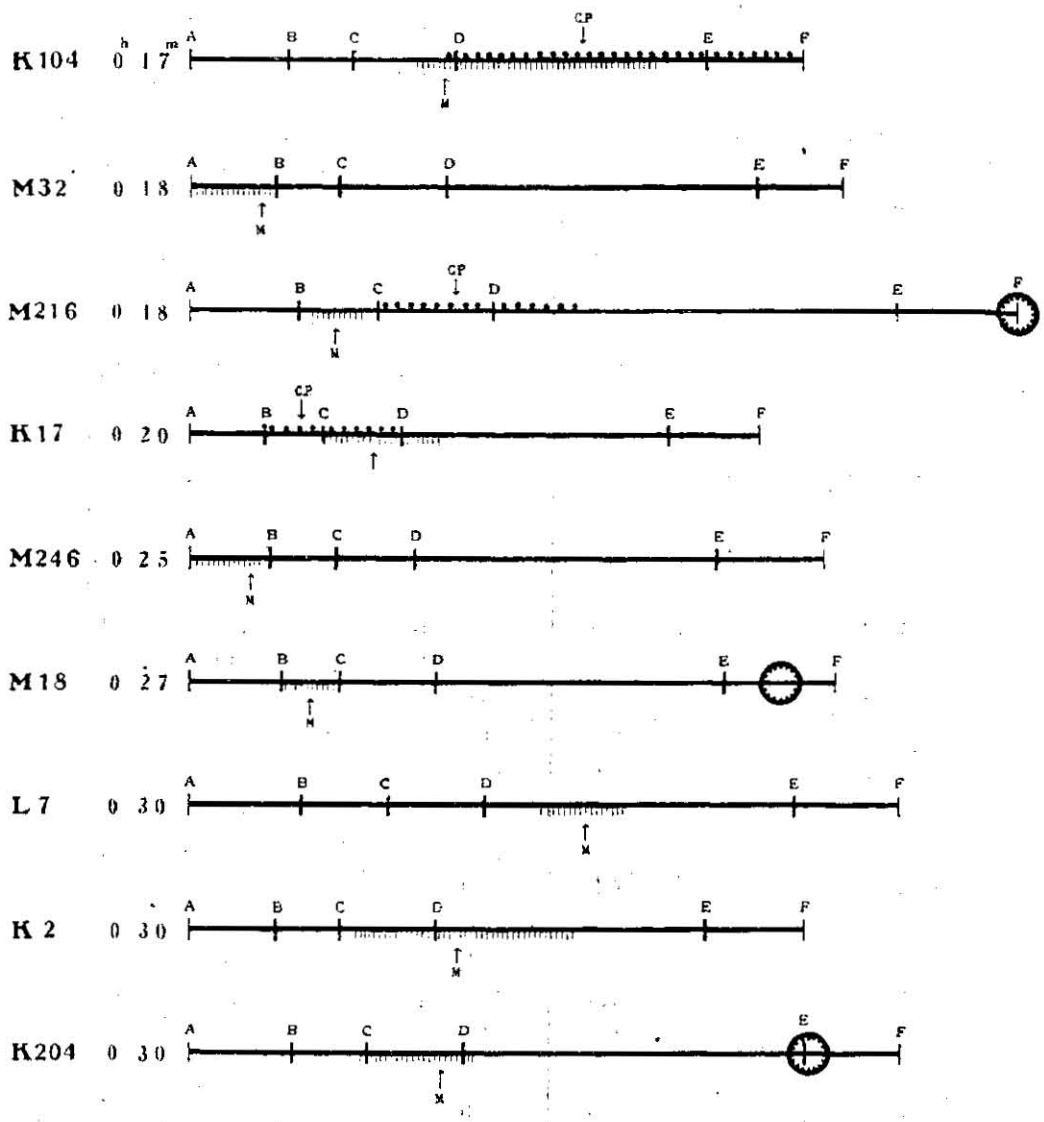


Fig. 2. Schematic oviducts illustrating the rate of dispersion of injected substances in the inner lumen of the oviducts of birds, K104, M32, M216, K17, M246, M18, L7, K2, and K204, which received the injection with inanimate substances into the oviduct. Figures denoting the time intervals are the "interval after injection" as explained in the section of Material and Methods.

↑ or ↓ denotes the place where the injection was performed; M = a solution of methylene blue; C.P. = a suspension of carbon particles; • • • = carbon particles dispersed in the inner lumen of the oviduct; ıılıı = dye of methylene blue dispersed in the inner lumen of the oviduct; ◌ = an ovum or yolk without surrounding albumen. Other notations are the same as in Fig. 1.

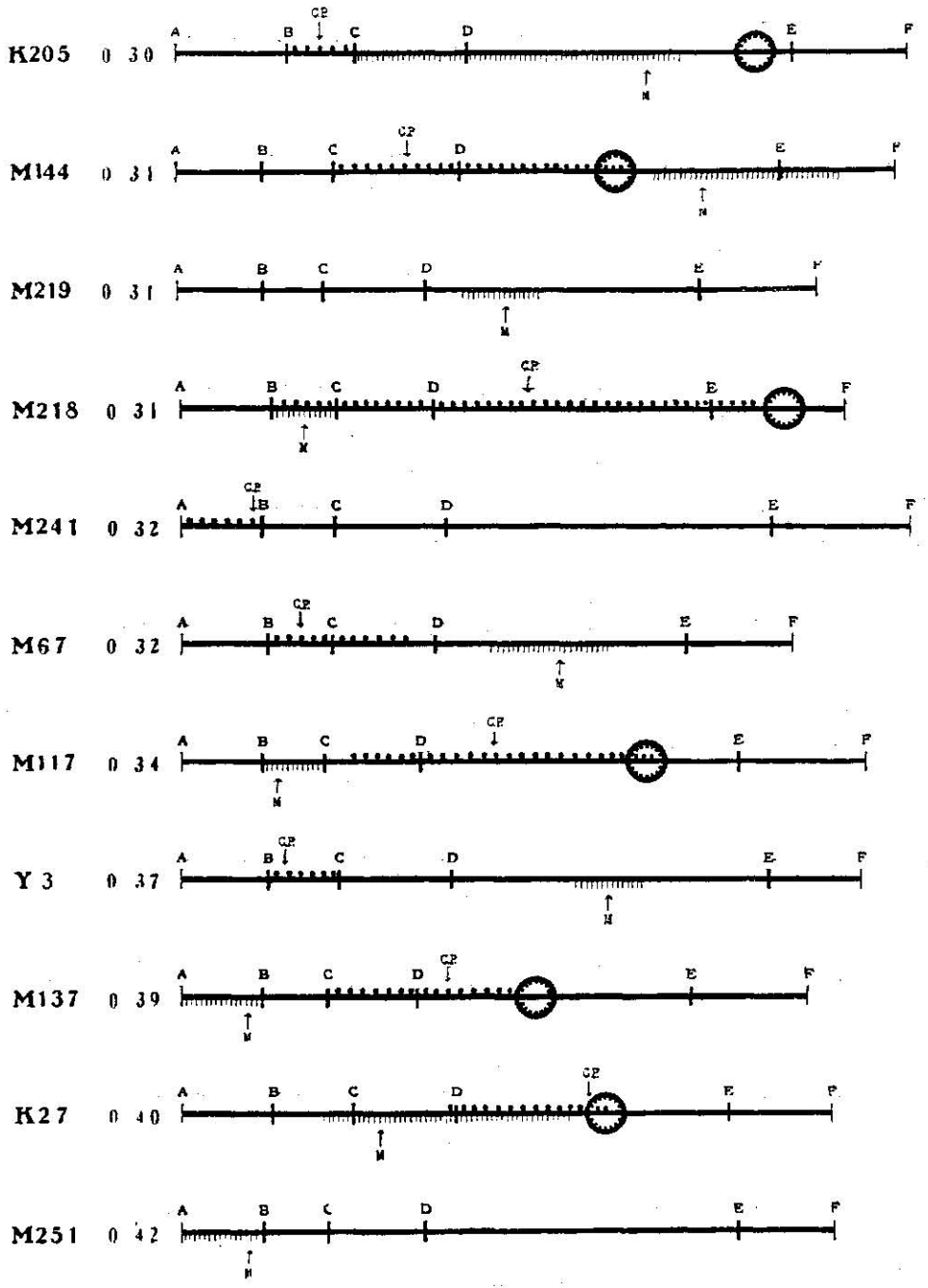


Fig. 2—(*Continued*). Schematic oviducts illustrating the rate of dispersion of injected substances in the inner lumen of the oviducts of birds, K205, M144, M219, M218, M241, M67, M117, Y3, M137, K27, and M251, which received the injection with inanimate substances into the oviduct.

○ = an ovum or yolk with some surrounding albumen (K205, M144, M117, M137, and K27) or without it (M218).

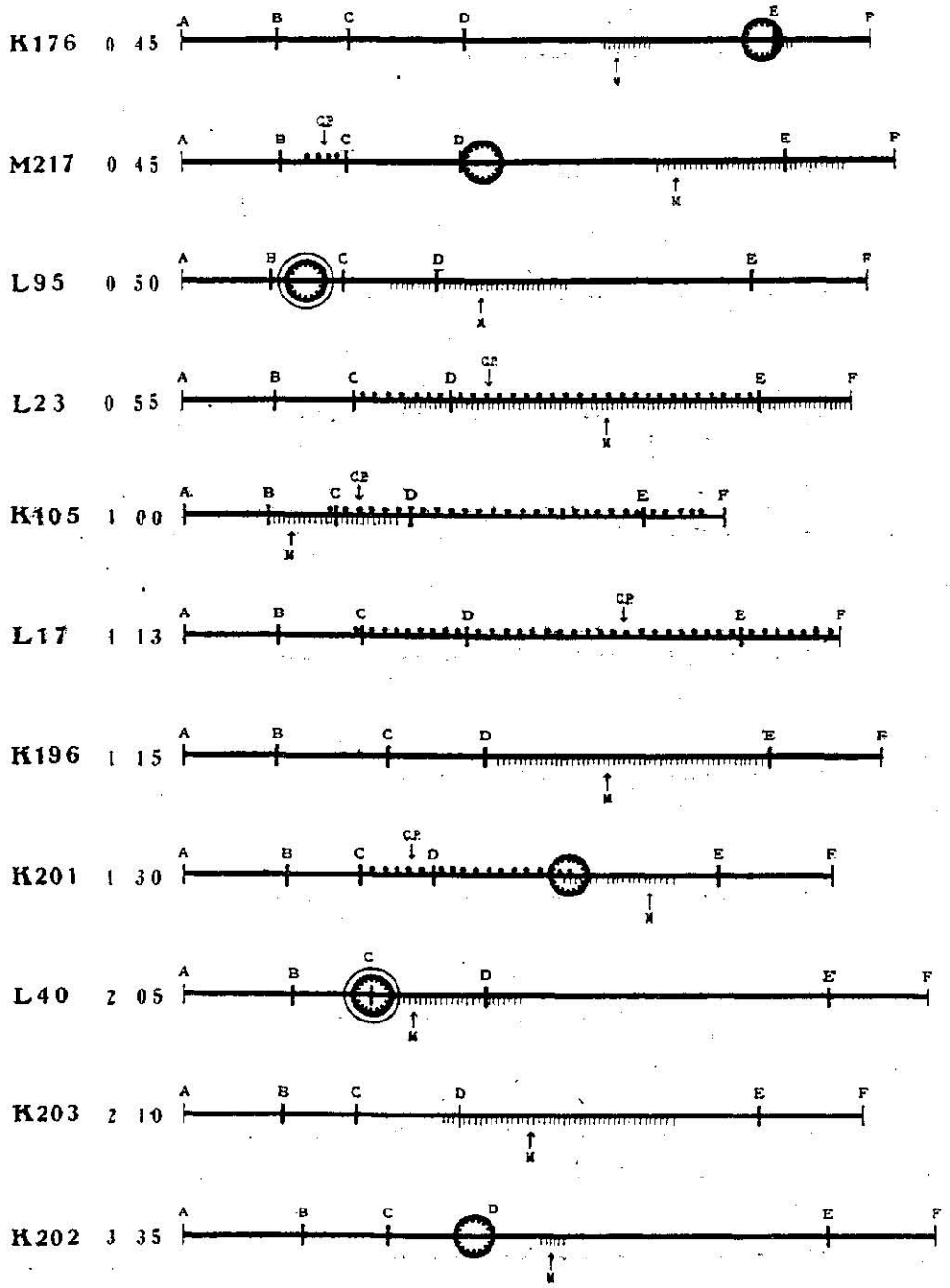


Fig. 2—(*Concluded*). Schematic oviducts illustrating the rate of dispersion of injected substances in the inner lumen of the oviducts of birds, K176, M217, L95, L23, K105, L17, K196, K201, L40, K203, and K202, which received the injection with inanimate substances into the oviduct. ◯ = an ovum or yolk with some surrounding albumen (M217, K201, and K202) or without it (K176); ◎ = an ovum with albumen and shell membrane (L40) or that with albumen, shell membrane and calcareous shell (L95).

K104. The Brown Leghorn. (*Cf.* Fig. 2 and Tables 8 and 9)

The bird was brought to the laboratory and was anesthetized by the routine procedure at 10:55 a.m. The coeliotomy immediately followed it and it was found that the uterus contained an egg with a hard shell. 1.0 cc. of a suspension of carbon particles was injected into the lumen of the middle part of the albumen portion at 11:03 a.m., and in addition to it, 0.5 cc. of a solution of methylene blue was also injected into the isthmus immediately after the former injection. The bird unexpectedly laid an egg under this condition at 11:07 a.m. It was killed and observations of the inner lumen of the oviduct were made at 11:20 a.m., or 17 minutes after the injections. No egg or ovum was found in the oviduct at autopsy. It was observed that carbon particles introduced into the oviduct had been scattered both up and down the oviduct from the place of injection, and that the methylene blue had passed through the oviduct mostly upwards, and little downwards.

M32. The White Leghorn. (*Cf.* Fig. 2. and Table 6)

The bird was anesthetized in the usual way at 9:28 a.m., and the coeliotomy immediately followed it and a single injection with 0.25 cc. of a solution of methylene blue was made into the inner lumen of the vagina at 9:50 a.m. It was found that some quantity of methylene blue flew out of the cloaca after the injection. Observations of the oviduct were made at 10:08 a.m., or 18 minutes after the injection. No egg or ovum was found in the oviduct at autopsy. Methylene blue introduced into the vagina was found to be diffused throughout the vagina, but had not passed up the oviduct.

M216. The White Leghorn. (*Cf.* Fig. 2 and Table 5)

The bird laid an egg at 10:10 a.m., and was anesthetized at 10:27 a.m. It died just prior to being tied by the bird's wings and legs to the operating board, subsequently observations were made on this bird, too. After the coeliotomy of the bird, 4 minutes after the death, double injections were made with 0.25 cc. of a solution of methylene blue into the uterus, and with 0.7 cc. of a suspension of carbon particles into the isthmus, respectively, at 10:37 a.m. Observations of the oviduct were made at 10:55 a.m., or 18 minutes after the injections. An ovum was found to have just ovulated, a part of which being wrapped by the margin of the fimbriae. Some carbon particles had become scattered in the oviduct both up and down to some distance, and the methylene blue was retained in the injected part only, having made no progress.

K17. The Brown Leghorn. (*Cf.* Fig. 2 and Tables 7 and 8)

The bird laid an egg at 1:05 p.m., and was anesthetized at 1:20 p.m.

The coeliotomy immediately followed it, and double injections were made with 0.5 cc. of a suspension of carbon particles into the uterus, and with 0.5 cc. of a solution of methylene blue into the isthmus, respectively, at 1:27 p.m. Observations of the oviduct were made 1:47 p.m., or 20 minutes after the injections. No egg or ovum was found in the oviduct at autopsy. It was observed that carbon particles introduced into the uterus had passed up the oviduct and were scattered through the whole length of both uterus and isthmus, and that the methylene blue introduced into the isthmus had also passed up the oviduct to a short distance though some had been carried down the oviduct to the anterior end of the uterus.

M246. The White Leghorn. (*Cf.* Fig. 2 and Table 6)

The bird laid an egg at 8:40 a.m., and was anesthetized at 11:46 a.m. The coeliotomy immediately followed it, and a single injection was made with 0.2 cc. of a solution of methylene blue into the anterior part of the vagina at 12:05 p.m. Observations of the oviduct were made at 12:30 p.m., or 25 minutes after the injection. No egg or ovum was found in the oviduct at autopsy. It was observed that the methylene blue introduced into the vagina had diffused itself through the whole length of the vagina, but had not passed up to the uterus.

M18. A crossbred of the White Leghorn with another breed. (*Cf.* Fig. 2 and Table 7)

The bird laid an egg at 10:38 a.m., and was anesthetized by the usual procedure. The initial injection of an anesthetic was made at 11:05 a.m. and additional injections of it were made when (11:20 a.m.) a complete anesthesia was produced. The bird fell into a syncopic state and ceased to breathe, through the overdosage of anesthetic, but it was revived soon after by artificial respiration. Then the coeliotomy was performed at 11:30 a.m., and a single injection was made with 0.65 cc. of a solution of methylene blue into the uterus at 11:35 a.m. Observations of the oviduct were made at 12:02 p.m., or 27 minutes after the injection. An ovum or yolk without any surrounding albumen was found in the infundibulum, and it was noted that the methylene blue was retained in the injected part only.

L7. The White Leghorn. (*Cf.* Fig. 2 and Table 5)

The bird laid an egg at 11:10 a.m., and was immediately anesthetized in the usual way. The bird died due to an excessive administration of anesthetics at 11:14 a.m., the process of routine observations was made on this bird, too. A single injection with methylene blue was made

at the middle part of the albumen portion at 11:30 a.m., and observations of the oviduct were carried out at noon, or 30 minutes after the injection. It was found then that the largest follicle in the ovary was just going to be ovulated and the follicular rupture had practically begun at the part of the stigma. The methylene blue had not passed neither up nor down the oviduct but remained in the injected part only, as is shown in Fig. 2 and Table 5.

K2. The White Leghorn. (*Cf.* Fig. 2 and Table 9)

The bird was removed from the trapnest in which she was sitting for egg laying and was anesthetized in the usual way, complete anesthesia being produced at 10:23 a.m. The coeliotomy immediately followed it, and the bird laid an egg, in this condition, at 10:34 a.m. A single injection with methylene blue was made at the albumen portion at 10:38 a.m., and observations of the oviduct were carried out at 11:08 a.m., or 30 minutes after the injection. No egg or ovum was found in the oviduct at autopsy. It was observed that the methylene blue introduced into the posterior part of the albumen portion of the oviduct had been carried upwards and been diffused throughout its interior up to the middle part of this portion.

K204. The White Leghorn. (*Cf.* Fig. 2 and Table 5)

The bird laid an egg at 10:25 a.m., and was anesthetized at 10:50 a.m. The coeliotomy immediately followed it, and the bird accidentally died during coeliotomy at 10:53 a.m. The cause of this death was considered to be an excessive administration of anesthetics. The bird underwent the subsequent process of routine observations as other birds studied, and 0.25 cc. of a solution of methylene blue was injected into the anterior part of the isthmus at 11:00 a.m., and its observations were made at 11:30 a.m., or 30 minutes after the injection. An ovum or yolk without any surrounding albumen was found at the margin of the infundibulum and the albumen portion. The methylene blue introduced into the isthmus was found not to have passed up the oviduct, though downward passage of it to a short distance was observed.

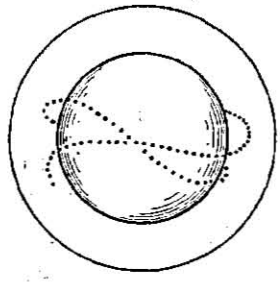
K205. The White Leghorn. (*Cf.* Fig. 2-(*Continued*) and Tables 7 and 9)

The bird laid an egg at 11:45 a.m., and was anesthetized at 11:55 a.m. The coeliotomy immediately followed it, and double injections were made with 0.5 cc. of a suspension of carbon particles into the uterus, and with 0.5 cc. of a solution of methylene blue into the albumen portion, respectively, at 12:10 p.m. Observations of the oviduct were made at 12:40 p.m.,

or 30 minutes after the injections. An ovum with some surrounding albumen was found in the anterior part of the albumen portion. It was found that carbon particles introduced into the uterus were retained in the injected part only, and methylene blue introduced into the middle part of the albumen portion had been carried down through the oviduct and had become diffused throughout its interior to the posterior end of the isthmus. Some amount of methylene blue was noted to constitute the core of an elongated dense albumen cord which was extended from the anterior part of the albumen portion to the posterior end of the isthmus.

M144. The White Leghorn. (*Cf.* Fig. 2-(*Continued*) and Tables 8 and 9)

The bird laid an egg at 9:35 a.m., and was anesthetized at 11:30 a.m. The coeliotomy was performed at 11:40 a.m. Double injections were made with 0.7 cc. of a suspension of carbon particles into the isthmus, and with 0.4 cc. of a solution of methylene blue into the part just anterior to the ovum which was found at the anterior part of the albumen portion in coeliotomy, respectively, at 11:51 a.m. Observations of the oviduct were made at 12:22 p.m., or 31 minutes after the injections. An ovum with some surrounding albumen was found in the albumen portion. Carbon particles introduced into the isthmus were found to have been dispersed so as to make the core of an elongated cord formed of the dense albumen and had made much pro-ovarian progress through the oviduct. The upper end of the elongated albumen cord containing carbon particles joined the ovum with some surrounding albumen also contained carbon particles which were arranged so as to make a ring engirdling the yolk, as shown in Text-Fig. 1. Methylene blue introduced into the pro-ovarian region with respect to the ovum was found to have been carried up to a greater extent through the oviduct.



Text-Fig. 1.

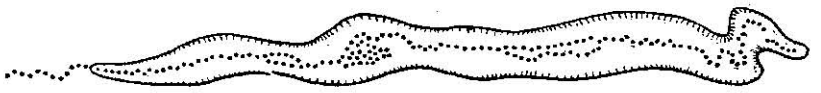
M219. A crossbred of the Barred Plymouth Rock with the Rhode Island Red. (*Cf.* Fig. 2-(*Continued*) and Table 5)

The bird laid an egg at 12:40 p.m., and was anesthetized at 1:05 p.m. The coeliotomy immediately followed it, during which the bird accidentally died at 1:12 p.m. The bird underwent the subsequent observations in the same way as other birds studied. 0.5 cc. of a solution of methylene blue was injected into the posterior part of the albumen por-

tion at 1:15 p.m., and observations of the oviduct were made at 1:46 p.m., or 31 minutes after the injection. No egg or ovum was found in the oviduct at autopsy. Methylene blue introduced into the albumen portion was retained in the injected part only: no progress of it was found.

M218. The Barred Plymouth Rock. (*Cf.* Fig. 2-(*Continued*) and Tables 7 and 9)

The bird laid an egg at 10:25 a.m., and was anesthetized at 10:47 a.m. The bird instantly fell into a syncopic state and ceased to breathe, but it was revived soon afterward by artificial respiration. The coeliotomy followed it, and double injections were performed with 0.5 cc. of a solution of methylene blue into the uterus, and with 0.9 cc. of a suspension of carbon particles into the albumen portion, respectively, at 11:09 a.m. Observations of the oviduct were made at 11:40 a.m., or 31 minutes after the injections. An ovum or yolk without any surrounding albumen was found in the infundibulum. Methylene blue introduced into the uterus was retained in the injected part only, no progress being noted. Most of the carbon particles injected were found in an inner core of a dense albumen cord, in which they appeared in the form of a string composed of a dense swarm of small carbon particles



Text-Fig. 2.

as shown in Text-Fig. 2. The string of the carbon particles extended from the anterior part of the albumen portion to the posterior end of the uterus, and the layer of dense albumen surrounding the elongated string was thick in the albumen portion and distinctly thin in the parts of the isthmus and the uterus.

M241. The White Leghorn. (*Cf.* Fig. 2-(*Continued*) and Table 6)

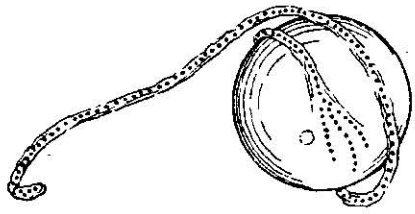
The bird laid an egg at 10:45 a.m., and was anesthetized at 1:25 p.m. The coeliotomy immediately followed it, and a single injection was made with 0.4 cc. of a suspension of carbon particles into the anterior part of the vagina at 1:45 p.m. Observations of the oviduct were made at 2:17 p.m., or 32 minutes after the injection. No egg or ovum was found in the oviduct at autopsy. Plenty of carbon particles introduced into the vagina were drifted down thence and minute particles had been scattered throughout the whole length of the vagina.

M67. The White Leghorn. (*Cf.* Fig. 2-(*Continued*) and Table 5)

The bird was removed from the trapnest in which she was sitting for egg laying, and was anesthetized in the usual way. The bird accidentally died from an unknown cause without any struggle during the injection of the anesthetic, at 10:35 a.m., but it underwent the subsequent process of routine observations as the other birds. Double injections were made with 0.5 cc. of a solution of methylene blue into the albumen portion, and with 0.1 cc. of a suspension of carbon particles into the uterus, respectively, at 10:44 a.m., and they were examined at 11:16 a.m., or 32 minutes after the injections. No egg or ovum was found in the oviduct at autopsy. It was observed that the methylene blue introduced into the middle part of the albumen portion had passed neither up nor down the oviduct and mostly remained in the injected part only, while the carbon particles introduced into the uterus had travelled up the oviduct and had extended themselves throughout the whole length of both the uterus and the isthmus. The writer was impressed by the fact that the injected carbon particles might have flowed through the oviduct in a pro-ovarian direction, as a result of the slightest local pressure of the manipulation, indicating that the substances were driven in one direction as the result of an accidental pressure.

M117. The Barred Plymouth Rock. (*Cf.* Fig. 2-(*Continued*) and Tables 7 and 9)

The bird laid an egg at 9:27 a.m., and was anesthetized at 10:05 a.m. The coeliotomy immediately followed it. Double injections were made with 0.45 cc. of a solution of methylene blue into the uterus, and with 0.8 cc. of a suspension of carbon particles into the posterior part of the albumen portion, respectively, at 10:17 a.m. Observations of the oviduct were made at 10:51 a.m., or 34 minutes after the injections. An ovum with some surrounding albumen was found in the anterior part of the albumen portion. Carbon particles introduced into the albumen portion were found to have been scattered in the form of a core of an elongated cord of the dense albumen and to have made much pro-ovarian progress through the oviduct. The upper end of the albumen cord containing carbon particles joined with the surrounding dense albumen of the ovum, and this surrounding albumen also contained carbon particles which were arranged so as to make a ring engirdling the yolk, as shown in Text-Fig. 3. The methylene blue introduced into the uterus was retained there, no progress being found.



Text-Fig. 3.

Y3. The White Leghorn. (*Cf.* Fig. 2-(*Continued*) and Table 5)

The bird laid an egg at 12:15 p.m., and was anesthetized at 2:03 p.m. The bird died in the course of the injection of anesthetic but it underwent the subsequent process of routine observations as the other birds. The coeliotomy was performed at 2:10 p.m., and double injections were made with 0.9 cc. of a solution of methylene blue into the middle part of the albumen portion, and with 2.0 cc. of a suspension of carbon particles into the uterus, respectively, at 2:13 p.m. and observations of the oviduct were made at 2:50 p.m., or 37 minutes after the injections. No egg or ovum was found in the oviduct at autopsy. It was observed that substances injected had not moved neither up nor down the oviduct and were retained in the injected part only.

M137. The White Leghorn. (*Cf.* Fig. 2-(*Continued*) and Tables 6 and 9)

The bird laid an egg at 9:30 a.m., and was anesthetized at 11:25 a.m. The coeliotomy immediately followed it, and double injections were made with 0.75 cc. of a suspension of carbon particles into the posterior end of the albumen portion, and with 0.6 cc. of a solution of methylene blue into the anterior part of the vagina, respectively, at 11:36 a.m. Observations of the oviduct were made at 12:15 p.m., or 39 minutes after the injections. An ovum with some surrounding albumen was found in the middle part of the albumen portion. Some particles of carbon introduced into the albumen portion were found to have moved up the oviduct and to have entangled themselves with the dense albumen surrounding the ovum, and the other particles of carbon introduced had been carried down the oviduct and had been scattered in the whole length of the isthmus. Methylene blue introduced into the vagina was observed to have been diffused throughout the vagina, but had not moved up the uterus.

K27. The Rhode Island Red. (*Cf.* Fig. 2-(*Continued*) and Tables 8 and 9)

The bird laid an egg at 10:30 a.m., and was anesthetized at 10:50 a.m. The coeliotomy immediately followed it, and double injections were made with 0.1 cc. of a suspension of carbon particles into the middle part of the albumen portion, and with 0.5 cc. of a solution of methylene blue into the isthmus, respectively, at 11:18 a.m. Observations of the oviduct were made at 11:58 a.m., or 40 minutes after the injections. An ovum with some surrounding albumen was found in the middle part of the albumen portion. Carbon particles were noted in the core of an elongated cord or strand of dense albumen, the upper end of the elongated cord joining with the surrounding dense albumen or the ovum, and the pos-

terior end reaching the anterior part of the isthmus. Methylene blue was found to have well moved up the oviduct, its downward passage extending to a short distance only.

M251. The White Leghorn. (*Cf.* Fig. 2-(*Continued*) and Table 6)

The bird laid an egg at 1:30 p.m., and was anesthetized at 1:54 p.m. The coeliotomy immediately followed it, and a single injection was made with 0.3 cc. of a solution of methylene blue into the anterior part of the vagina at 2:06 p.m. Observations of the oviduct were made at 2:48 p.m., or 42 minutes after the injection. No egg or ovum was found in the oviduct at autopsy. Methylene blue introduced into the vagina was found to have been diffused throughout the vagina and had shown no sign of pro-ovarian progress.

K176. The White Leghorn. (*Cf.* Fig. 2-(*Concluded*) and Table 9)

The bird laid an egg at 10:30 a.m., and was anesthetized at 10:50 a.m. The coeliotomy was then performed, and a single injection was made with 0.3 cc. of a solution of methylene blue into the middle part of the albumen portion at 11:10 a.m. Observations of the oviduct were made at 11:55 a.m., or 45 minutes after the injection. An ovum was found at the margin of the infundibulum and albumen portion, a part of its vitelline membrane being stained slightly with methylene blue. Methylene blue introduced into the albumen portion was found to have been carried up the oviduct and had stained the mucous epithelium of the posterior part of the infundibulum; a mass of dense albumen of this color was, on the other hand, found in the isthmus and it demonstrated that the methylene blue had spread out to this part of the oviduct.

M217. The White Leghorn. (*Cf.* Fig. 2-(*Concluded*) and Tables 7 and 9)

The bird laid an egg at 9:10 a.m., and was anesthetized at 11:21 a.m. The coeliotomy immediately followed it, and an ovum was found in the posterior part of the albumen portion. Double injections were made with 0.5 cc. of a solution of methylene blue into the pro-ovarian part of the albumen portion with respect to the ovum, and with 0.2 cc. of a suspension of carbon particles into the uterus, respectively, at 11:43 a.m. Observations of the oviduct were made at 12:28 p.m., or 45 minutes after the injections. An ovum with some surrounding albumen was found in the posterior end of the albumen portion. Methylene blue introduced into the albumen portion was found to have well moved up the oviduct, but no sign of ab-ovarian progress was noted. Carbon parti-

cles were retained in the injected part only: no progress of it was found.

L95. The White Leghorn. (*Cf.* Fig. 2-(*Concluded*) and Table 9)

The bird was removed from the trapnest in which she was sitting for egg laying, and was anesthetized in the usual procedure at 9:50 a.m. The coeliotomy immediately followed it, and an egg with a hard shell was found in the uterus. A single injection was made with a solution of methylene blue into the posterior part of the albumen portion at 10:00 a.m. Observations of the oviduct were made at 10:50 a.m., or 50 minutes after the injection. It was found that an egg with a hard shell was yet in the uterus and methylene blue had made much pro-ovarian progress and a little ab-ovarian progress. Most of methylene blue noted was being confined to the state of dense albumen strand and extended into the whole length of albumen portion of the oviduct. The posterior head of the strand was located in the middle part of the isthmus.

L23. The White Leghorn. (*Cf.* Fig. 2-(*Concluded*) and Table 9)

The bird laid an egg at 10:30 a.m., and was anesthetized at 10:44 a.m. The coeliotomy immediately followed it, and double injections were made with 0.3 cc. of a solution of methylene blue into the middle part of the albumen portion, and with 0.5 cc. of a suspension of carbon particles into the posterior part of the same region of the oviduct, respectively, at 11:11 a.m. Observations of the oviduct were made at 12:06 p.m., or 55 minutes after the injections. No egg or ovum was found in the oviduct at autopsy. Methylene blue introduced into the albumen portion was found to have well moved up the oviduct and had reached even the ovary and had stained some follicles there, while some had been carried down the oviduct and diffused into the isthmus. Carbon particles introduced into the posterior part of the albumen portion were also found to have made much pro-ovarian progress through the oviduct, but its ab-ovarian progress extended to a short distance.

K105. The Brown Leghorn. (*Cf.* Fig. 2-(*Concluded*) and Tables 7 and 8)

The bird laid an egg at 1:30 p.m., and was anesthetized at 3:25 p.m. The coeliotomy immediately followed it, and double injections were made with 0.5 cc. of a solution of methylene blue into the uterus, and with 1.0 cc. of a suspension of carbon particles into the isthmus, respectively, at 3:30 p.m. Observations of the oviduct were made at 4:30 p.m., or 1 hour after the injections. No egg or ovum was found in the oviduct at autopsy. Carbon particles introduced into the isthmus were found to

have reached well up the oviduct and had been scattered throughout its interior up to the pro-ovarian end of the oviduct. Methylene blue was also found to have moved up the oviduct to a short distance and had been diffused throughout the uterus and isthmus.

L17. The White Leghorn. (*Cf.* Fig. 2-(*Concluded*) and Table 9)

The bird laid an egg at 11:50 a.m., and was anesthetized in the usual way. The initial injection of an anesthetic was made at 12:15 p.m., and additional ones were made until complete anesthesia was produced at 12:42 p.m. The coeliotomy immediately followed it, and a single injection was made with 0.2 cc. of a suspension of carbon particles into the middle part of the albumen portion at 12:52 p.m. Observations of the oviduct were made at 2:05 p.m., or 1 hour and 13 minutes after the injection. No egg or ovum was found in the oviduct at autopsy. It was observed that carbon particles introduced into the albumen portion had been carried up along the whole length of the oviduct and had been scattered throughout its interior, but some had been carried down the oviduct and dispersed throughout its interior to the anterior end of the uterus.

K196. The White Leghorn. (*Cf.* Fig. 2-(*Concluded*) and Table 9)

The bird laid an egg at 1:10 p.m., and was anesthetized in the usual way. The initial injection of an anesthetic was made at 1:35 p.m. The bird fell into a temporary suspension of respiration but revived soon afterward during the routine observations. After the coeliotomy, a single injection was made with 0.2 cc. of a solution of methylene blue into the middle part of albumen portion at 3:00 p.m. Observations of the oviduct were made at 4:15 p.m., or 1 hour and 15 minutes after the injection. No egg or ovum was found in the oviduct at autopsy. Methylene blue introduced into the albumen portion was observed to have well travelled both up and down the oviduct, and the dye which had been carried downwards was noted to have been entangled with a dense albumen.

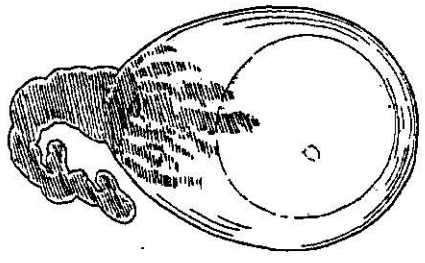
K201. The White Leghorn. (*Cf.* Fig. 2-(*Concluded*) and Tables 8 and 9)

The bird laid an egg at 10:00 a.m., and was anesthetized at 10:30 a.m. The coeliotomy was then performed, and double injections were made with a suspension of carbon particles into the isthmus, and with a solution of methylene blue into the anterior part of the albumen portion, respectively, at 10:50 a.m. Observations of the oviduct were made at 12:20 p.m., or 1 hour and 30 minutes after the injections. An ovum with some surrounding albumen was found to have been distinctly stained

with methylene blue. The methylene blue introduced into the albumen portion showed no sign of pro-ovarian progress. The carbon particles injected were found to have become the core of the dense albumen cord. The core was arranged in the form of a string composed of a dense swarm of carbon particles. The anterior end of the cord containing carbon particles was found to have joined with the dense albumen surrounding the ovum, its posterior end reaching the anterior end of the uterus. The arrangement of these contents of the oviduct indicated evidently that the core of carbon particles which were injected into the isthmus had moved up into the albumen portion where they became enclosed in the dense albumen and had then returned without reversing their long axis to the place where they were found at autopsy.

L40. The White Leghorn. (*Cf.* Fig. 2-(*Concluded*) and Table 8)

The bird laid an egg at 10:40 a.m., and was anesthetized at 1:25 p.m. The coeliotomy immediately followed it, and a single injection was made with 0.7 cc. of a solution of methylene blue into the isthmus at 1:35 p.m. Observations of the oviduct were made at 3:40 p.m., or 2 hours and 5 minutes after the injection. An abnormal egg with a peculiar external appearance was found at the isthmo-uterine junction, and the abnormal egg contained a normal yolk in it, but had a very thin layer of dense albumen around the yolk, and had a short tubular attachment or a short stalk of dense albumen with a curved end at the ab-ovarian pole of the egg; this egg was of such irregular shape and its whole surface was covered with a thin granular layer, as shown in Text-Fig. 4. A dense albumen surrounded the ab-ovarian pole of the ovum and filled the stalk which was stained deeply with methylene blue; but the yolk was not stained at all. Methylene blue also stained the mucous epithelium of the oviduct extending from the part at which this dye had been injected at the isthmus to the posterior part of the albumen portion. From these facts, it seemed that the injected solution of methylene blue might have been driven up to the albumen portion where it became enclosed in the dense albumen, meeting with an descending ovum. It had then returned into the uterus with the dense albumen enclosing the yolk.



Text-Fig. 4.

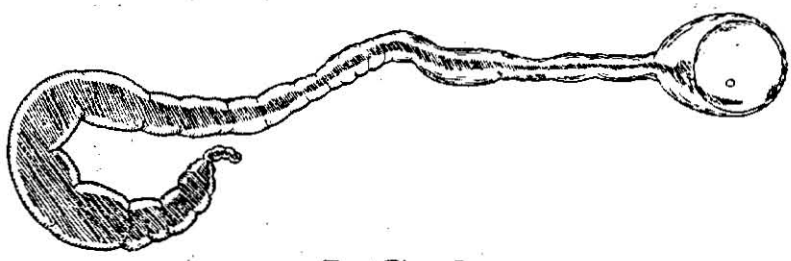
K203. The White Leghorn. (*Cf.* Fig. 2-(*Concluded*) and Table 9)

The bird laid an egg at 1:27 p.m., and was anesthetized at 1:40 p.m.

The coeliotomy immediately followed it, and a single injection was made with 1.0 cc. of a solution of methylene blue into the posterior part of the albumen portion at 2:00 p.m. Observations of the oviduct were made at 4:10 p.m., or 2 hours and 10 minutes after the injection. No egg or ovum was found in the oviduct at autopsy. Methylene blue introduced into the albumen portion was found to have been carried both up and down the oviduct.

K202. The White Leghorn. (*Cf.* Fig. 2-(*Concluded*) and Table 9)

The bird laid an egg at 10:25 a.m., and was anesthetized at 11:00 a.m. The coeliotomy was performed, and an ovum was found in the anterior part of the oviduct. A single injection was made with 0.7 cc. of a solution of methylene blue into the posterior part of the albumen portion at 11:25 a.m. Observations of the oviduct were made at 3:00 p.m., or 3 hours and 35 minutes after the injection. An ovum or yolk with a dense albumen was found in the uppermost part of the isthmus, its peculiar appearance was much like that of the ovum which was found in the oviduct of the bird L40, and it had a much larger stalk; a dense albumen surrounding the yolk was extended at its ab-ovarian pole so as to form an elongated stalk, and the posterior end of



Text-Fig. 5.

the stalk reached the ab-ovarian end of the uterus, the very end bending backwards, as shown in Text-Fig. 5. The whole surface of the stalk was covered with a continuous layer of shell membrane, and the part of the stalk bent backwards was covered, furthermore, with a thin layer of granular material of calcareous shell, and the part of the ovum or yolk was surrounded by the dense albumen and was free from either shell or shell membrane. The whole albumen forming the elongated stalk was stained with methylene blue in its core, and some coloration of this dye was found on one end of the yolk stretching the stalk. It was supposed that this coloration occurred when the ovum just entered the infundibulum or the anterior end of the albumen portion.

When an injection was made into the lumen of the oviduct of the bird the injected substance showed the sign of spreading along the inner lumen to some extent both up and down the oviduct due to the pressure generated by the act of injection. The extent of the spreading was conditioned by the amount of substance injected as well as the inner dimension of the lumen of the oviduct, and yet it was measured to be, in the majority of cases, within the limit of 4.5 cm. in length from the point at which the injection was performed, and so, if the injected substance could be seen to have travelled in the lumen of the oviduct, either up or down, more than 5 cm. in length longitudinally from the point of injection, the writer assumed that the occurrence of the transportation of the injected substances through the oviduct was due to a force or forces other than the pressure generated by the act of injection. The data are shown in Tables 5–9.

In summarizing the results of observations it is convenient to divide thirty-one birds above described into five groups as follows.

Group 1. Six birds, M216, L7, K204, M219, M67, and Y3, which died accidentally during or just after the initial administration of anesthetic belong to this group. These birds, in spite of their death, underwent the subsequent processes of observations like other birds studied, *i.e.*, the coeliotomy, the injection of some substances, and observations of the inner lumen of the oviduct. In these birds the injected substances were mostly retained in the injected part only, or were not dispersed beyond the distance over 5.0 cm. from the places of injections, and they were assumed to have in no way advanced up or down through the oviduct with three exceptions (M216, K204, and M67), data being shown in Table 5. The carbon particles introduced into the uterus of the bird M67 were seen, at autopsy, to have moved along up and down the oviduct; the writer, however, was aware that the suspension of carbon particles were forced to advance both up and down through the oviduct by an additional pressure which was applied by the writer himself at the time of injection. The carbon particles introduced into the isthmus of the bird M216 were seen, at autopsy, to have also moved along up and down the oviduct, but the writer did not recognize that such an additional pressure, as noted above in the case of the bird M67, was applied. It was learned in these series of investigations that some

movements of oviduct persisted for a while (5–15 min.) after the bird's death, thus enabling the writer to see the movement even after the oviduct was dissected out of the body wall by removing their attached ligaments; the carbon particles in the oviduct of the bird M216 and the methylene blue in the oviduct of the bird K204 were presumably carried up or down by such a force as was generated by the movements of oviduct after the bird's death.

Table 5. Table showing the data concerning the injections performed in the bird died.

Individual	Intervals of time between the death and the injection	Intervals of time after the injection	Place of injection and substance injected	Rate of dispersion of injected substances observed at autopsy		Ovum or egg found actually in the oviduct at the time of autopsy	
				Downward passage	Upward passage	Position in the oviduct	Stage of formation
	min.	min.		cm.	cm.		
M216	4	18	Uterus, M. Isthmus, C.P.	2.0 7.0	2.0 13.5		
L 7	16	30	Alb. p., M.	4.0	3.0		
K 204	7	30	Isthmus, M.	7.5	3.0	Infundibulum-Alb. p.	Y+
M219	3	31	Alb. p., M.	4.0	3.5		
M 67	9	32	Uterus, C.P. Alb. p., M.	3.0 7.5	11.0 5.0		
Y 3	10	37	Uterus, C.P. Alb. p., M.	1.0 3.5	5.5* 3.5		

C.P. denotes that the injection performed with a suspension of carbon particles.
M. denotes that the injection performed with a solution of methylene blue.
Alb. p.= albumen portion; Infundibulum-Alb. p.= the margin of the infundibulum and the albumen portion; Y+= ovum or yolk with no albumen or yolk only.
an exceptional case.

The results of observations carried out in the other twenty-five birds which received the injections into their oviducts are described in Groups 2–5, divided according to the parts of oviduct at which the injections were performed.

Group 2. Five birds, M32, M246, M241, M137, and M251 belong to this group.

In these birds the injections were performed at the vaginae of the oviducts. The bird M241 was injected with a suspension of carbon particles and other four birds with a solution of methylene blue, and in all birds without exception the injected substances showed no progress upwards into the uterus, but some progress downwards and were scattered throughout the vagina, as described above individually.

Table 6. Table showing the data concerning the injections performed in the vagina.

Individual	Intervals of time after the injection	Substance injected	Rate of dispersion of injected substances observed at autopsy		Ovum or egg found in the oviduct				Remarks
					Ovum or egg found actually in the oviduct at the time of autopsy		Ovum or egg which was estimated or actually found in the oviduct at the time of injection		
			Downward passage	Upward passage	Position in the oviduct	Stage of formation	Position in the oviduct	Stage of formation	
	min.		cm.	cm.					
M 32	18	M.	6.7	1.3					No egg laying
M246	25	M.	6.0	1.5					No ovulation
M241	32	C.P.	7.0	1.0					No ovulation
M137	39	M.	6.5	1.5	Alb. p.	Y+A	Alb. p.	Y+A	
M251	42	M.	6.5	1.5					

Y+A=ovum or yolk with albumen, the deposition of the dense albumen not complete, or ovum or yolk with some surrounding albumen.

Other notations are the same as in Table 5.

In the bird M32 no egg laying occurred and in birds M246 and M241 no ovulation occurred on that day, and in these circumstances the injected substances into the vagina had in no way advanced upwards through the vagina. In the bird M137 the injection of methylene blue was performed and an observation was made from outside on an inserted ovum which was being passed down through the albumen portion of the oviduct. No sign of progress of injected substance upwards through the vagina was observed in this bird.

It was learned from this and other investigations that the carbon particles injected into the vagina almost entirely ran out of the vagina before the autopsy was performed, and also learned that in fact the volume of injected solution or suspension was excessive in relation to the capacity of the vagina, and as the sphincter muscle at the utero-vaginal margin hindered its upward passage the injected substance was forced out. The active ab-ovarian ciliary movement in the vaginal epithelium was considered to give effect to some extent in driving out the injected substances.

Group 3. Seven birds, K17, M18, K205, M218, M117, M217, and K105 belong to this group, and the injections were performed at the uteri of the oviducts. It was found in birds K17 and K105, that the injected substances were carried upwards in the oviduct and were diffused in the isthmus, but in other birds, M18, K205, M218, M117, and M217, the substances injected did not move,

Table 7. Table showing the data concerning the injections performed in the uterus.

Individual	Intervals of time after the injection	Substance injected	Rate of dispersion of injected substances observed at autopsy		Ovum or egg found in the oviduct				Remarks
					Ovum or egg found actually in the oviduct at the time of autopsy		Ovum or egg which was estimated or actually found in the oviduct at the time of injection		
			Downward passage	Upward passage	Position in the oviduct	Stage of formation	Position in the oviduct	Stage of formation	
	min.		cm.	cm.					
K 17	20	C.P.	3.0	9.0					No ovulation
M 18	27	M.	2.5	2.5	Infundibulum	Y+	Ovary or Fimbriae	Ovum or Y+	
K205	30	C.P.	3.0	3.0	Alb. p.	Y+A	Infundibulum	Y+	
M218	31	M.	3.5	3.0	Infundibulum	Y+	Ovary or Fimbriae	Ovum or Y+	
M117	34	M.	1.5	4.5	Alb. p.	Y+A	Infundibulum	Y+	
M217	45	C.P.	1.5	1.5	Alb. p.	Y+A	Alb. p.	Y+A	
K105	60	M.	2.5	10.0					No ovulation

Notations are the same as in Tables 5 and 6.

but were retained in the injected part only. In the former, it was noted that no ovulation occurred during the day.

The substances injected into the uterus, or into the part more pro-ovarian, were drifted down to the posterior end of the uterus, but they had not at all run out from the uterus to the vagina before the autopsy was performed. A strong sphincter muscle was located at the margin of the uterus and the vagina, as described above, and it was considered that a suggestion could be made from these series of observations that the sphincter was responsible for the prevention of the passage of fluid from uterus to vagina or inversely from vagina to uterus, during the time of observations.

Group 4. Seven birds, K104, K17, M144, K27, K105, K201, and L40 belong to this group. The injections were made at the isthmuses, and in all birds without exception the substances injected into the oviduct showed pro-ovarian progress, prominent one in most cases though ab-ovarian progress was not remarkable. No ovulation occurred in two birds K17 and K105 on that day, but in the other five, K104, M144, K27, K201, and L40, it had taken place before the injections were performed, thus the substances were injected into the oviduct in the pro-ovarian region (K104) or in the ab-ovarian (M144, K201, K27, and L40) with respect to the inserted ovum.

Group 5. Seventeen birds, K104, K2, K205, M144, M218, M117, M137, K27, K176, M217, L95, L23, L17, K196, K201, K203, and K202 belong to this group. The injections were made in the albumen portions of the oviducts, and the results of observations of the inner lumen of the oviduct, proved to be quite complex. It was observed that the injected substances had made much pro-ovarian progress through the oviduct in thirteen birds, namely, K104, K2, M144, M218, M117, M137, M176, M217, L95, L23, L17, K196, and K203. In the other four, namely, K205, K27, K201, and K202, however, the injected substances in the oviduct were found in the ab-ovarian region with respect to the place where they were injected, but it was demonstrated from the available evidence that they had been first forced up through the oviduct and had been then carried down to the place where they were found at autopsy, with an exception in the bird K205 which failed to exhibit any pro-ovarian progress of the injected substance through the oviduct at all. It was estimated that no ovulation

Table 8. Table showing the data concerning the injections performed in the isthmus.

Individual	Intervals of time after the injection	Sub-stance injected	Rate of dispersion of injected substances observed at autopsy		Ovum or egg found in the oviduct				Remarks
					Ovum or egg found actually in the oviduct at the time of autopsy		Ovum or egg which was estimated or actually found in the oviduct at the time of injection		
			Downward passage	Upward passage	Position in the oviduct	Stage of formation	Position in the oviduct	Stage of formation	
	h. min.		cm.	cm.					
K 104	0 17	M.	3.0	19.5			Uterus	Y+A+SM+S	Egg laying occurred 4 minutes after the injection
K 17	0 20	M.	5.0	6.0					No ovulation
M144	0 31	C.P.	7.5	22.0	Alb. p.	Y+A	Alb. p.	Y+A	
K 27	0 40	M.	6.0	18.5	Alb. p.	Y+A	Alb. p.	Y+A	
K 105	1 00	C.P.	3.0	33.5					No ovluation
K201	1 30	C.P.	6.0	17.0	Alb. p.	Y+A	Infundi-bulum	Y+	
L 40	2 05	M.	8.5*	10.0	Isthmo-Uterine	Y+A+SM+	Alb. p.	Y+A	

Y+A+SM+S=ovum or yolk with albumen, shell membrane, and shell (complete egg or about so); Y+A+SM+=ovum or yolk with albumen and shell membrane; Isthmo-Uterine.=the isthmo-uterine junction.

Other notations are the same as the Tables 5, 6, and 7.

* *Cf.* Description of the Results for the bird *L40* in p. 201.

Table 9. Table showing the data concerning the injections performed in the albumen portion.

Individual	Intervals of time after the injection	Substance injected	Rate of dispersion of injected substances observed at autopsy		Ovum or egg found in the oviduct				Remarks
					Ovum or egg found actually in the oviduct at the time of autopsy		Ovum or egg which was estimated or actually found in the oviduct at the time of injection		
			Downward passage	Upward passage	Position in the oviduct	Stage of formation	Position in the oviduct	Stage of formation	
	h. min.		cm.	cm.					
K 104	0 17	C.P.	12.5	20.0			Uterus	Y+A+SM+S	Egg laying occurred 4 minutes after the injection
K 2	0 30	M.	9.5	11.0					
K 205	0 30	M.	29.0	3.5	Alb. p.	Y+A	Infundibulum	Y+	
M144	0 31	M.	5.0	13.5	Alb. p.	Y+A	Alb. p.	Y+A	
M218	0 31	C.P.	24.0	22.5	Infundibulum	Y+	Ovary or Fimbriae	Ovum or Y+	
M117	0 34	C.P.	14.5	16.5	Alb. p.	Y+A	Infundibulum	Y+	
M137	0 39	C.P.	12.5	7.5	Alb. p.	Y+A	Alb. p.	Y+A	
K 27	0 40	C.P.	13.5	2.0	Alb. p.	Y+A	Alb .p.	Y+A	
K 176	0 45	M.	23.5*	17.0	Infundibulum-Alb. p.	Y+	Ovary	Ovum	
M217	0 45	M.	2.0	16.0	Alb. p.	Y+A	Alb. p.	Y+A	
L 95	0 50	M.	9.0	25.0*	Uterus	Y+A+SM+S	Uterus	Y+A+SM+S	
L 23	0 55	C.P. M.	13.0 20.0	25.5 26.5					Prob. no ovulation
L 17	1 13	C.P.	26.0	20.5					No ovulation
K 196	1 15	M.	11.0	16.0					No ovulation
K 201	1 30	M.	12.0	2.0	Alb. p.	Y+A	Infundibulum	Y+	
K 203	2 10	M.	8.5	13.0					No ovulation
K 202	3 35	M.	22.5*	1.0	Isthmus	Y+A+ (SM)	Alb. p.	Y+A	

Y+A+(SM)=ovum or yolk with albumen and shell membrane, the formation of the shell membrane not complete.
Other notations are the same as in Tables 5, 6, 7, and 8.
* *Cf.* Description of the Results for the birds *K176*, *L95*, and *K202*, in pages 198, 199, and 202, respectively.

occurred on that day in birds L17, K196, and K203, and also it was probably so in the bird L23. In each of birds M218 and K176 an ovum was observed in the anterior part of the oviduct at autopsy, since these birds had substances injected into the oviduct just before the ovulation. In birds K205, M117, and K201, the injections were performed just after the ovulation had occurred. In each of the birds K104, M144, M137, M217, L95, and K202, the ovum actually found in the oviduct was taken into consideration, and the injection was made at the pro-ovarian region in each of K104, M144, M217, and L95, and ab-ovarian region in each of M137 and K202, with respect to the inserted ovum, respectively.

At different interval of time after egg laying, the injections were performed; for example, the injection was made in the bird K2 four minutes after egg laying. Furthermore, the injection in the bird K104 was made not long (4 minutes) before egg laying. In all cases with one exception, the propulsion of the injected substances through the oviduct occurred with no relation to that of ovulation or egg laying.

The substances introduced into the albumen portion by injection made remarkably great pro-ovarian progress through the oviducts of most birds, and their ab-ovarian progress was also prominent in the oviducts of many birds.

4. Consideration

From the results of observations made in the study on the travel of inanimate substances such as a solution of methylene blue or a suspension of carbon particles it was shown that these inanimate substances, when they were injected in the various parts of the oviduct except in the part of the vagina, moved up through the oviduct toward the ovary. As will be discussed in Chapter IV, the factors which might have worked for this passage of injected substances were able to be considered, as follows: (i) contraction of the wall of the oviduct, (ii) capillary attraction of the oviducal lumen, (iii) ciliary action of the oviducal epithelium, and (iv) the mutual pressure of the viscera. Among these items, however, the capillary attraction was not sufficient to explain the difference of passage between living and non-living birds, that is, passage in living birds was observed to have occurred while it was not the

case with most of dead birds; and the ciliary action of the oviducal epithelium was also not adequate to explain the pro-ovarian passage, because the injected substances made pro-ovarian progress either in the region where the pro-ovarian ciliary movement existed or in the region where it did not exist. The mutual pressure of the viscera was not the factor as will be discussed in Chapter IV. And so, the writer was led to conclude that the pro-ovarian passage of the injected substances through the oviduct was accomplished by the contraction of the wall of the oviduct or the muscular movement which was observed to have developed in the intact oviduct in response to the stimulus brought upon it by the process of injection. The muscular movement just referred to was an antiperistaltic one, the same as observed in the study described in Chapter I, and this antiperistalic movement developed at the region where the injection was made, and extended thence upwards to the pro-ovarian region of the oviduct.

The mode of the antiperistaltic movement was found to be peculiar in each of four parts of the oviduct, namely, the vagina, the uterus, the isthmus, and the albumen portion.

In birds M32, M246, M241, M137, and M251 the inanimate substances were injected in the part of vagina and no pro-ovarian progress of the injected substances through it was found to occur. Failure of pro-ovarian progress of the spermatozoa inseminated into the vagina had been frequent in the case of the oviducts of birds into which artificial insemination had been made, if this was performed before the sphincter muscle at the margin between the vagina and uterus relaxed in response to the stimulus brought upon it by the mechanical pressure added by the forefinger inserted in the vagina (MIMURA, 1939). These facts indicated that the antiperistalic movement in the vagina did not develop so easily and acutely as in other parts of oviduct, in response to the stimulus or the irritation induced by the solitary act of injection.

When the injection was carried out in the uterus, on the other hand, the propulsion of the injected substances through the oviduct was well demonstrated in some birds (K17 and K105), but, in others it was not so evident.

The writer observed in his preceding study (MIMURA, 1939) that if the oviduct contained an ovum or yolk in some part, the

spermatozoa inserted by an artificial insemination would be retaine in the inseminated part only, and would make no upward progress through the oviduct, but if the oviduct was estimated to contain no ovum or yolk, rapid progress of the inseminated spermatozoa was able to be observed. The data presented in the present paper were similar to those just referred to, and they indicated that when the injection was made in the uterus of a bird, whose oviduct was estimated to contain an ovum or yolk in some part, the injected substances showed no upward advancement and were found to have been retained in the injected part only, but when the bird was estimated to contain no egg or ovum in its oviduct at the time of injection, a little progress of the injected substances was found. It could be presumed from these results that if an injection was made in the uterus of a bird whose oviduct contained an ovum or yolk in some part, the antiperistalsis was not prominently induced or was ineffective to urge the injected substances to advance upwards through the oviduct of the bird.

It was revealed, from the results shown in Chapter I, that the spermatozoa inserted in the uterus by artificial insemination advanced remarkably and rapidly up to the infundibulum or even to the ovary, and were dispersed throughout the whole length of the oviduct. However, it was observed in the present study that only a little progress of the injected substances, or rather failure to advance through the oviduct occurred, though they were injected similarly into the lumen of the uterus. The difference between marked progress of the inserted spermatozoa upwards through the oviduct in the case of the artificial insemination, and less marked progress of the injected substances upwards through it in the case of the injection was, according the writer's conception, made by the quality of antiperistalsis, which might be expected to be more intense in the former cases than in the latter.

Furthermore, when the injection was made in either the isthmus or the albumen portion of the oviduct a more prominent dispersion of the injected substances was observed than in the case when the injection was performed in the vagina and the uterus, as the intensity of the antiperistaltic movement induced by the stimulus of injection in the former parts of the oviduct was observed to be higher than that of the latter parts.

In each of birds M144 and M137, the injection of a suspension

of carbon particles was made at the ab-ovarian region of the oviduct with respect to the ovum contained in the albumen portion. The dispersion of the injected substances was found to have reached the place adjacent to the ovum, and an antiperistalsis was thought to have developed in response to the stimulus induced by the process of injection, and moved through the oviduct upwards to the place where the ovum was found. In each of birds M144, M217, and L95, the injection was made at the pro-ovarian region with respect to the ovum contained in the albumen portion or the uterus, and an antiperistalsis developed at the region of the oviduct where the injection was performed, and extended thence through the oviduct upwards to its pro-ovarian end.

The antiperistaltic movement was always brought upon the wall of the oviduct when the injection was made and when it stimulated the tissues in the wall of the oviduct, and the inanimate substances injected were found to have been transported through the oviduct by the antiperistalsis. But the antiperistaltic movement of the oviducal wall was probably not persistent and diminished its intensity of the movement in a short time, and a peristaltic movement took the place of it, so that the injected substances, which first progressed upwards to the pro-ovarian direction, were forced to move backwards to the opposite direction, as shown in birds K27, K201, L40, and K202; and this phenomenon was perceptible by the fact that the injected substances first travelled upwards and reached the ovum which was contained in the upper part of the oviduct, and adhered to it, and then this ovum with the substances adhered had passed by the place of injection downwards when the bird was autopsied.

HARTMAN (1932) indicated, in a problem of transportation of spermatozoa through the oviduct, that it was not necessary to postulate antiperistaltic action of the uterus in fowls for, with the cloacal sphincter closed, peristaltic contractions were quite sufficient to effect a thorough mixture of the contents of the uterus. It was, however, inevitably concluded from the data of birds M217, and L95, that the pro-ovarian transportation of injected substances through the oviduct did not result from the reverse effect of normal peristaltic movement of the oviduct.

The investigations reported in the present Chapter were made partly in order to demonstrate how the transportation of injected

substances could be influenced by the ciliary movement of the oviduct, and the description of the results will be made here in the following paragraphs.

The writer observed (MIMURA, 1937a) two systems of ciliary movement, *i.e.*, pro-ovarian in the albumen portion and ab-ovarian in all parts of the oviduct in the domestic fowl. These movements were active in those oviducts which had been dissected from the body wall and kept at room temperature, and were more vigorous in those oviducts which had been kept in the body cavity and yet retained their body heat though the bird had died. The oviducts of the dead birds, as previously described, showed that the injected substances were mostly retained in the injected part only, and that there was little or no advancement of them in the oviducts. The peculiar dispersion of the injected substances in the inner lumens of the oviducts of the dead birds was probably due to the cessation of mucous secretion from the epithelia, and the resistance due to the viscousness on the surface of the mucous epithelia was the factor which limited the extent of the dispersion of the injected substances in the inner lumens of the oviducts. Thus the substances injected into the lumens of the oviducts adhered or accumulated on the surface of the mucous epithelia in these circumstances, and little or no advancement took place.

In the living birds, fluid and mucus were secreted by the glands of the oviducts into their lumens and were destined to flow along the surface of the mucous epithelium by means of the ciliary movement of the epithelium.

As already set forth, the ab-ovarian system of the ciliary movement of the oviduct developed in the entire length of it, and it was inferred that the ciliary current extending throughout the oviduct from infundibulum to vagina was capable of accomplishing the transportation of the fluid or mucous contents of the oviduct through its entire length. The downward passage of the injected substances through the oviduct was undoubtedly accomplished by the ab-ovarian ciliary movement to some limited extent, especially in the part of the vagina, but their passage was considered to be mainly due to the peristaltic movement of the oviduct, especially in its upper parts than the isthmus. The pro-ovarian system of the ciliary movent was restricted in a certain part of the albumen portion of the oviduct in the fowl as already described. Thus the

upward passage of the injected substances through the albumen portion was accomplished by the pro-ovarian ciliary system to some limited extent, but it showed no peculiar mode of passage of the injected substances in the albumen portion as compared with that in all of other parts of the oviduct. It was, therefore, ascertained, that so far as the pro-ovarian progress of the injected substances through the oviduct was concerned, the ciliary movement did not play an important rôle in the transportation of the oviducal contents through the oviduct.

PARKER discovered two systems of ciliary movement, one for ab-ovarian direction and the other for pro-ovarian direction, in the oviduct of the turtle (1928) as well as in the pigeon (1930) and supposed that the spermatozoa inserted into the lower parts of the oviduct reached the pro-ovarian end of it by transportation afforded by the pro-ovarian ciliary movement. Furthermore, the same author (1931) assumed that the cavity of the uterine tube in the rabbit was broken up by its folds and muscles into a system of temporary longitudinal compartments, on the walls of which there were ciliary currents (ab-ovarian) and in the centres of which there were counter-currents (pro-ovarian). Thus, they furnished opportunity for frequent exchange of its contents. He also maintained that, in consequence of the currents and counter-currents in the system of tubal compartments, spermatozoa and other small particles were transported in a somewhat accidental way up or down the tubes. Spermatozoa that entered the lower end of tube from the uterus might eventually be discharged at the upper end next to the ovary. The above explanations appeared to be unsatisfactory in the cases treated by the writer, since his observations did not support these views.

In almost all flock of domestic fowls abnormal eggs of various forms were produced owing to some physiological disturbances of the oviduct or ovary of the bird. PARKER (1906) gave a full discussion of some abnormal eggs, and presumed that some of them were produced in the oviduct in consequence of an antiperistalsis having been exerted in it. PEARL and CURTIS (1914) showed experimentally that if the lower end of the oviduct of a fowl was ligated, eggs even with a fully formed shell might be discharged into the body cavity by the abrupt antiperistalsis of the oviduct. CURTIS (1916) also considered the formation of various kinds of

abnormal eggs and reported that an examination of her data gave little evidence that the oviducal glands were excited by an egg accidentally moving up the oviduct to pour out their secretion. Furthermore, the suggestions were made that perhaps the egg moved very rapidly up the oviduct and that there was not sufficient time for the stimulus to become effective, though the mechanism of the movement of the oviduct, which forced an egg backwards, was not discussed at all.

In the present study, the rapid advancement of the injected substances upwards to the upper part of the oviduct accomplished by antiperistalsis was observed; and these injected substances, which were found in the cord of dense albumen attached to the ovum in the form of a core of the cord, made their way to the isthmus and uterus, and the former secreted the shell membrane and the latter the calcareous shell deposited on the surface of the albumen cord, consequently forming a peculiar kind of abnormal egg. Such abnormal eggs were found in birds K201, L40, and K202 at autopsy.

Double injections with a solution of methylene blue as well as a suspension of carbon particles in one and the same oviduct, and the effect of the frequency of injection on the transportation of injected substances was observed, and no difference between them was discovered.

5. Summary

1) The birds used in this study were first anesthetized and then coeliotomized, and the oviduct exposed before the observer's eyes was injected, by means of a hypodermic needle and syringe, with a solution of methylene blue or a suspension of carbon particles into the inner lumen of their oviduct through the oviducal wall. At various intervals of time after the injection, the birds were killed and observations on the injected substances in the inner lumen of oviduct were carried out.

2) Some birds accidentally died during the initial administration of the anesthetic or just after it, but these underwent the subsequent process of the routine examination as the other birds did. The dead birds showed that the injected substances were mostly

retained in the injected part only and had made little or no progress through the oviduct.

3) In the living birds, the substances injected into the inner lumen of the oviduct were dispersed through the oviduct, thereby proving that the birds were actually alive and its oviducts were functional.

4) It was determined that the injected substances, such as a solution of methylene blue or a suspension of carbon particles moved up the oviduct, and that this passage was considered to have been accomplished by oviducal muscular movement, especially antiperistaltic one, which was brought upon the wall of the oviduct in response to the stimulus induced by the act of injection.

5) It was actually observed that the antiperistaltic action was brought upon the region in which the injection was made, and was extended upwards to the pro-ovarian end of the oviduct.

6) The mode of antiperistaltic movement which developed in response to the stimulus induced by the act of injection was found to be peculiar in each of the four parts of the oviduct.

7) When the injection was performed in the vagina of the oviduct, no pro-ovarian progress of the injected substances through the oviduct was observed. The fact indicated that the antiperistaltic movement was not easily or acutely brought upon the wall by the act of injection in the region of vagina.

8) The injection was performed at the uterus, and the propulsion of the injected substances through the oviduct was well demonstrated in a few of the birds, but, in other birds, it was not demonstrated as clearly as above that pro-ovarian progress of the injected substances had made their way upwards to the upper part of the oviduct.

9) When the injection was made in either the isthmus or the albumen portion, more prominent dispersion of the injected substances through the oviduct in pro- or ab-ovarian direction was observed than in the cases where the injections were performed in either the vagina or the uterus of the oviduct. This fact indicated that in the parts of the isthmus and the albumen portion the antiperistalsis was easily produced, which affected to cause the rapid progress of the injected substances through the oviduct in the pro-ovarian direction.

10) The antiperistalsis excited by the act of injection had no sufficient force to move up the inserted egg or ovum through the oviduct; furthermore, the persistence of the antiperistaltic action of the oviduct was not so long, for, it lost its intensity of action in a short time.

11) The downward passage of the injected substances through the oviduct was considered to be due to the peristaltic action of the oviduct augmented by the ciliary movement of the oviduct.

Chapter III

ON THE TRAVEL OF SPERMATOZOA DEPOSITED IN THE VAGINA BY NORMAL COPULATION THROUGH THE OVIDUCT OF THE FOWL

1. Introduction

It was shown in Chapter I that the spermatozoa inserted at the posterior end of the uterus by artificial insemination made a prompt and rapid upward progress, when the oviducts did not contain any egg in the albumen portion or in the more posterior parts of the oviduct at the time of insemination. Even the inanimate substances inseminated at the posterior end of the uterus advanced upwards through the oviduct very rapidly (MIMURA, 1939), and such inanimate substances injected at various parts of the oviducts other than the vagina made their progress upwards to the upper parts of the oviducts as shown in Chapter II. And the writer considered that oviducal movement by the muscles, that is, antiperistaltic movement of the oviduct was the primary factor which made the spermatozoa and inanimate substances such as carbon particles and the dye of methylene blue proceed upwards. To obtain further information on the relations of the travel of spermatozoa to the antiperistaltic movement of the oviduct, observations were made on the progress of spermatozoa deposited in the vaginae of the fowls by normal copulation, and the results of them are described here in the present Chapter.