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STUDIES ON THE ACCESSORY REPRODUCTIVE ORGANS IN THE COCK

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INTRODUCTION

It has been believed that the reproductive organs of the cock, the male of common barnyard fowl, consist of testis, epididymis, vas deferens and phallus (rudimentary copulatory organ), and that there are no glands corresponding to the seminal vesicle, prostate and bulbo-urethral gland as in mammals. Consequently, it has been thought that the semen in vas deferens is ejaculated without adding fluid to dilute it.

In many passerine and some other birds, the so-called seminal vesicle is a well known organ, but the seminal vesicle in these birds is the posterior part of the vas deferens, enlarged in breeding season or as a result of hormone effects (Riddle, 1927; Bailey, 1953).

In previous papers, the writer described evidences that the vas deferens semen of the cock was diluted with a fluid——*transparent fluid*, so called by the writer in 1951. The transparent fluid is ejected by organs other than the vas deferens and constitutes the greater part of sperm-serum. The ejecting organs as well as the mechanism of the ejection of the fluid were also cleared up by the writer (1950a, 1952c). The accessory reproductive organs* of fowls are similar to accessory reproductive glands of mammals in physiological functions. The mechanism of the ejection of transparent fluid, however, differs from that of secretion of fluids generated by the accessory reproductive glands in mammals, because the transparent fluid is not the mere secretion

* In previous papers, the term "accessory organs of phallus" was used.

of cells but rather it originates from blood and is ejected by the accessory reproductive organs of the cock (Cf. Section 1).

In this paper, the results of these previous experiments related to the accessory reproductive organs of the cock are described in brief, being arranged in order and certain results of further experiments are added.

SECTION I

STRUCTURE OF THE ACCESSORY REPRODUCTIVE ORGANS OF THE COCK

The accessory reproductive organs of the cock consist of two parts or organs, that is, the lymph-fold (by the writer, 1950a) and the vascular body (*Gefässreicher Körper*, after Liebe, 1914; *corpus cavernosum*, after Tannenberg, 1810, and Barkow, 1829—these two authors were cited by Liebe in 1914; *Tannenbergscher Körper*, after Müller, 1908).

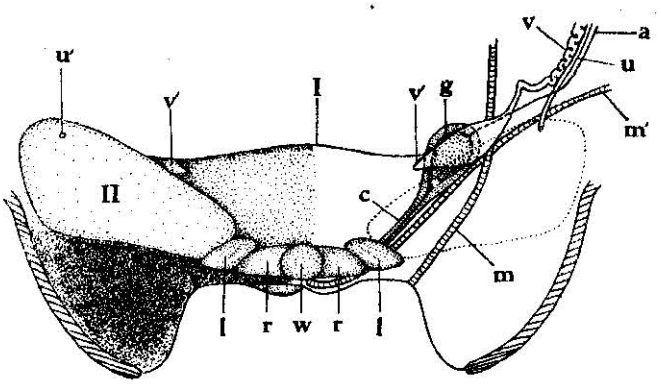

Fig. 1. Diagram of the accessory reproductive organs of the cock in relation to phallus, vas deferens, ureter and muscles. Dorsal wall of the cloaca is cut open and inside of it is shown; left—dorsal view of the cloaca, right—cloacal mucous membrane and a part of cloacal sphincter is removed to show vascular body, vas deferens, ureter and A. pudendalis interna.
a, A. pudendalis interna; c, connecting portion; g, vascular body (Gefässreicher Körper); l, lymph-fold; m, M. retractor penis posterior; m', M. retractor penis anterior; r, round fold; u, ureter; u', opening of ureter; v, vas deferens; v', papillary process of vas deferens; w, white body; I, 1st fold of cloaca; II, 2nd fold of cloaca; r+w+r=phallus; g+c+l=accessory reproductive organs.

The vascular body in the drake is the accessory organ of the penis and its function was demonstrated by Liebe. The lymph generated in the body causes the erection of penis (Liebe, 1914). In the cock, however, only the existence of the vascular body had been determined; the relation between the phallus and the vascular body had remained unknown. In this experiment, it was clearly demonstrated that the vascular body and lymph-fold belonged to one and the same system as the phallus (Fig. 1).

1. *Lymph-fold*: In the "second fold" of the cloaca (so called by Masui et al. in 1925), the writer (1950a) found two triangular or spindle shaped folds, one of each lying on the outside of the "round folds" (Masui et al., 1925), the labia minor (Hashimoto, 1930) or erected copulatory organs (Burrows and Quinn, 1937). The triangular or spindle shaped folds were named "lymph-folds" by the writer. The lymph-fold being about 2.8×1.2 mm. in size is covered with columnar epithelium, and the submucous coat of it consists of loose connective tissue (Cf. Plate 6, 1). Many lymph sinuses are found in this tissue, and they connect with lymph sinuses in the phallus as well as with those in the connecting portion, which is a portion connecting the vascular body with lymph-fold and phallus (Plate 6, 1). The epithelium of the lymph-fold is observed to be a gland, because many secretory granules are found in the cells.

2. *Vascular body*: Cutting open the mucous membrane and cloacal sphincter from inside of the cloaca, two muscles are found, the M. retractor penis posterior and the M. retractor penis anterior, which are considered to be the muscles taking part in coitus in the drake (Liebe, 1914). The former is a slender muscle being 1 mm. in diameter and 22–25 mm. in length, arranged similarly to the arrangement of the same muscle in the drake, and is attached underneath to the white body (white body, after Masui et al., 1925; rudimentary copulatory organ, after Burrows and Quinn, 1937). The latter originates on the basin as a thin aponeuroses, and becomes a small and slender muscle with rounded end on the M. depressor coccygieus and then goes backward in an approximately straight line to attach to one side of the anus and pulls the portion forward (Fig. 1).

A small body which is egg shaped, flattened, and red colored,

lies in the inside of the cloacal sphincter. This is the vascular body. The anterior part of this body approaches to the crossing portion of two muscles, M. retractor penis posterior and anterior, and also, the location of the vascular body corresponds to the outer side of the ampulla ductus deferentis (Fig. 1; Nishiyama, 1950a, Fig. 4–4). This body is about 7 mm. in length, 1 mm. in thickness and 17–40 mg. (27.6 ± 2.9 mg. on an average) in weight, and is covered with connective tissue capsule. The A. pudendalis interna, lying along vas deferens and ureter, pours into the body. The body consists of lymphoid tissue and numerous capillaries distribute in the medulla, and many blood cells are found in the capillaries by microscopic observations (Plate 6, 4; Nishiyama, 1950a, Fig. 4–8). In the trabeculae of this body, fairly large blood vessels are found (Plate 6, 4). The peripheral lymph sinuses and internal sinuses are found in this body as in the lymph gland, and the peripheral sinuses that are the spaces between vascular body and connective tissue capsule, correspond to the Lymphbildungsraum after Liebe. A slender part of this body (small masses of lymphoid tissues) elongates to the bottom of the lymph-fold and phallus with the lymph sinuses, which encircle the masses of the lymphoid tissues (Fig. 1–c; Plate 6, 5). These lymphoid tissues disappear at the bottom of the lymph-fold and just the lymph sinuses reach the bottom of the phallus (Fig. 5). These lymph sinuses are connected to the sinuses of the phallus and lymph-fold (Plate 6, 1).

In the 3-month-old cockerel, the vascular body is light pink in color and about 8 mg. in weight. During the growth period of the bird, the vascular body gradually increases its weight, finally reaching 27 mg., and the shade of color is also intensified, becoming red in the adult cock (Nishiyama, 1950a, Diagram 1).

In order to confirm the relation of the vascular bodies to lymph-folds as well as phallus, melted gelatin solution was injected into one of the round folds (Nishiyama, 1950a, Figs. 2 and 3). Injected gelatin ran into the lymph-folds and vascular bodies, and also into the lymphatic vessels lying along A. pudendalis interna. From this, it was confirmed that these organs belonged to the same system.

SECTION II

ADDITION OF TRANSPARENT FLUID TO THE VAS DEFERENS SEMEN IN THE COCK

(1) Evidences of addition of transparent fluid

a. Observations of the ejection of transparent fluid: The evidence that the transparent fluid is added to the vas deferens semen can be ascertained by the observations of the ejecting semen in cases where the semen is ejected four times or more in succession from a cock by means of repeated abdominal massages. In these experiments five cocks, J4, N2, K82, P46 and K85, were used.

Generally speaking, the aspects of ejecting semen in repeated collections from a cock are as follows:

In the 1st ejection, a large amount of white semen was collected. When such a large amount of vas deferens semen is ejected as in the 1st ejection, the ejecting semen is apparently white in color and it is difficult to observe the addition of transparent fluid to the vas deferens semen. In some birds, N2 and K82 in this experiment, however, the addition of the transparent fluid to the vas deferens semen was observed; in one bird N2, the first time, a large amount of dense white semen, which contained transparent fluid, was ejected and this was followed by the ejection of transparent fluid only, in all cases. In the other bird K82, the ejection began occasionally by ejection of transparent fluid, and then a large amount of dense semen was added to the fluid.

In the 2nd and 3rd ejections, the evidence of addition of transparent fluid to the vas deferens semen was observed distinctly, that is, in some cases, the ejection of the transparent fluid was followed by the ejection of white semen, and in other cases, white dense semen was added intermittently or momentarily into the ejecting transparent fluid. In an exceptional bird, P46, however, the semen was ejaculated in such a state that the vas deferens semen had entirely mixed with the fluid. The collected semen in the 2nd or 3rd ejection was white or slightly white in color according to the mixture rate of the vas deferens semen and transparent fluid in the collected semen. And generally, the

semen collected in 2nd ejection was denser than the one in the 3rd ejection.

In the 4th, 5th or 6th ejection, either the vas deferens semen was not ejected or it was a very small amount even if it was ejected. On the contrary, the transparent fluid was ejected every time in most cases. Then the collected semen was slightly white in color or transparent in appearance.

These are the general aspects of the ejection of semen, but there is great variance in the amount of ejected transparent fluid and in aspect of addition of transparent fluid to the vas deferens semen both among different cocks, and as between different ejections from same bird. In some cocks, for example, J4, the transparent fluid was ejected at all times, even when the semen was collected consecutively six times or more, there was no great decrease in amount as the number of collections increased; therefore, the total amount of the ejected transparent fluid was very large. On the other hand, in some birds, for example, K85, the amount of transparent fluid in each collection was very small, and frequently the semen (including the transparent fluid) was not ejected after 3rd or 4th collection; therefore, the total amount of transparent fluids was small.

Generally speaking, so far as the cock responds to sexual excitment by means of abdominal massage which causes the erection of phallus, the transparent fluid is ejected at any time and in some birds the ejection of the fluid continues four times or more.

From this evidence and the evidence that the semen in vas deferens is dense and white throughout and totally lacks transparent fluid, there is no doubt that the transparent fluid is added to the vas deferens semen from some other organ than the vas deferens.

b. Density of the collected semen by repeated collections: The density of the semen collected by means of abdominal massages in repeated collections was very low in comparison with that of the vas deferens semen of the same bird (Fig. 2; Nishiyama, 1951b, Tables 2 and 3). The density of the semen which was collected from birds by abdominal massages was not so high as the density of vas deferens semen (Nishiyama, 1951b, Fig. 6).

Therefore, it is concluded that the collected semen is diluted with some fluid after the semen has been ejected from vas deferens.

In repeated collections, the density of the semen became lower as the number of collections increased. Shibata et al. (1938), who collected the semen by abdominal massage, also reported a similar aspect. It is impossible to explain this aspect with the idea that the semen is ejected without adding some fluid; the density of the semen involved in the anterior part of the vas deferens is higher than that which is involved in the posterior part and the semen in the ampulla has the lowest density of all (Nishiyama, 1951b, Table 3). Therefore, it must be considered that the semen which is collected four times or more in succession from a bird, will increase in density in direct proportion to the number of collections. The objection that may be raised against the above consideration is that the secretion from vas deferens may increase greatly when the semen is ejected, especially when ejections are repeated. However, the densities of the semen in ampullae ductuum deferentium collected from the birds which were killed just after repeated collections of semen by abdominal massages were similar to the densities of the semen in ampullae ductuum deferentium of controls, in which the semen was not collected before autopsy. From these evidences, it is probable that the density of the semen which is ejected from vas deferens of a bird is almost the same even if the ejections be repeated, for the semen in upper part is diluted slightly by secretion from the cells of vas deferens.

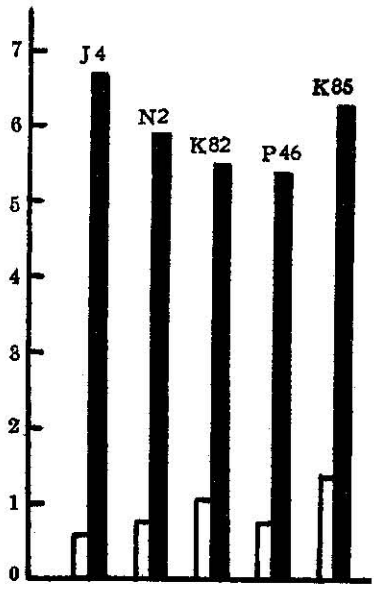

Fig. 2. Density (number in million of spermatozoa in 1 cub. mm.) of the collected semen before and after operation (Cf. p. 285). | | before operation, ▬ after operation.

Consequently, it must be considered that the shift of the

semen density is caused by the dilution of vas deferens semen with some fluid originating from a part other than the vas deferens, or that as the number of collections increases, the transparent fluid increases in proportion to the vas deferens semen.

c. Amount of the collected semen in repeated collections: The amount of the semen stored in vasa deferentia in these experimental birds was 0.39–0.74 ml. (Nishiyama, 1951b, Table 4). When a bird ejaculates semen, only a part of the semen stored in the vas deferens is ejaculated, and even after the repeated ejaculations have terminated, there is no reduction in the amount of semen in the anterior part of the vas deferens, and only a slight reduction is observed in the posterior part although the ampulla ductus deferentis is almost emptied.

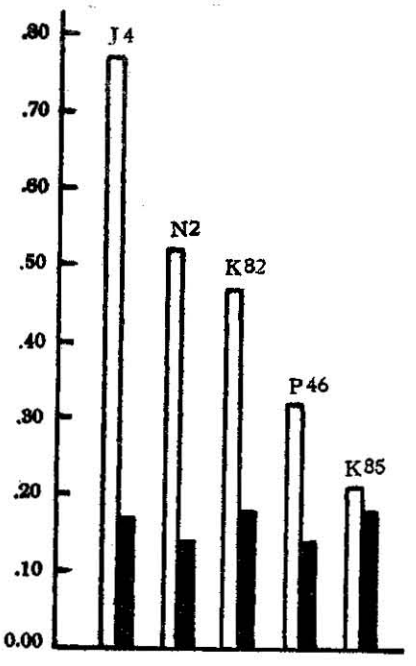

Fig. 3. Amount (cc) of the collected semen before and after operation (Cf. p. 285).
before operation, after operation.

On the other hand, the volume of the semen collected by means of abdominal massage was very large (0.21–0.77 ml. on an average from each bird) (Fig. 3), and in two birds, J4, K82, the ejected volumes were frequently larger than the total volume stored in the vasa deferentia of each bird (Nishiyama, 1951b, Tables 1 and 4).

Considering these facts, we may say that the ejaculated semen of a cock is a mixture of the dense semen in the vas deferens with the transparent fluid.

The maximum amounts of the collected semen which have been reported by several authors (Burrows and Quinn, 1937; Burrows and Titus, 1939; Shibata et al., 1938; Wheeler and Andrews, 1943) reached 2–4 ml., and it is impossible to consider that this amount

of semen was ejected from the vasa deferentia themselves. Therefore, from the result of this experiment, the writer presumes that the semen collected by these authors also consisted of vas deferens semen and transparent fluid.

It has been reported by some authors (Penquite et al., 1930; Hutt, 1939; Parker et al., 1940), that if the semen was collected repeatedly within a short time from a cock in a state of normal copulation, the density of the collected semen became lower as the number of collections increased. From this evidence it appears that the transparent fluid is also added to the semen in the case of normal copulation.

(2) Amount of the transparent fluid added to the vas deferens semen and the density of the collected semen

After such observations mentioned above had been made, the phalli and lymph-folds of these birds, J4, N2, K82, P46 and K85, were cut out with scissors and then the remainder was cauterized electrically. After the wound had healed, the semen was again collected from each bird. It was found that the transparent fluid was not ejected at all, thus making the aspect of ejection much the same in each bird; although there were differences in amounts at each collection, the semen ejected from operated birds was always dense and white regardless of the number of collections. The greater part of the semen which was collected was ejected in the first ejection, and the bulk of the semen was reduced markedly to a smaller amount in 2nd ejection. In 3rd or 4th collection, the semen was either ejected in a small amount or not ejected at all. The collected semen was, therefore, dense and white in color. This semen was probably the vas deferens semen with no addition of the transparent fluid. The density of this semen was slightly lower than that of the semen in the ampulla ductus deferentis of each bird (Nishiyama, 1951b, Tables 2 and 3). However, it seems probable that the difference in density is due to the difference in collecting dates and is especially due to the fact that the semen was not collected from the birds for several days before the autopsies. Thus, the difference between the mean value in the amount of collected semen before and after the operation indicates the volume of the added transparent fluid, and the proportions of

the transparent fluid to total volume of the semen collected from each experimental bird before the operation are calculated to be 78%, 73%, 62%, 56% and 14%, respectively.

On the other hand, there is not a great variance, among birds, in the amount of semen ejected from vas deferens, as indicated by the fact that there is no significant difference in the bulk of the semen collected from each experimental bird after operation (Fig. 3), and that the volumes of spermatozoa of the centrifuged semen, the semen being collected from each bird before and after operation, appear to be almost same in amount (Cf. centrifuged semen, Nishiyama, 1951b, Figs. 2 and 4). Thus, the larger amount of more dilute semen is collected from the individual which ejects a greater amount of the transparent fluid.

These considerations clearly explain the evidences that the density of the semen collected from the bird which ejected the larger amount of semen was lower, and the rate of increase in the density of the semen before and after operation reached 989% in bird J4, which ejected the largest amount of the fluid of birds, while it was only 365% in the smallest one, P46 (Nishiyama, 1951b, Table 2).

When the semen collections are carried out by repeated massages, the amount of vas deferens semen is very large at the first time, and thereafter it undergoes marked reduction, as mentioned before, while the amount of transparent fluid suffers no considerable reduction even if the semen collections are repeatedly performed, therefore the density of ejaculated semen which depends on the proportion of amounts of both vas deferens semen and transparent fluid becomes lower as the number of collections increases.

Section III

Mechanism of the Ejection of Transparent Fluid

The writer reported in a previous paper (Nishiyama, 1950b) that the erection of phallus of the cock was brought about by similar means as the erection of the penis in the drake, which had been described by Liebe in 1914. Namely, in the cock, the erection of phallus is caused by means of flowing in of the lymph or a fluid similar to lymph; this fluid is generated from the tissue

of the vascular body by sexual excitement and flows into the lymph sinuses of the phallus.

On the other hand, the lymph-folds belong to one and the same system as the phallus as well as the vascular bodies, and the lymph sinuses of the lymph-folds connect to the lymph sinuses in the phallus and to those in the vascular bodies through the connecting portions, as mentioned in Section 1. When Ringer solution or melted gelatin solution was injected into one of the vascular bodies, the injected fluid penetrated into the lymph sinuses of the lymph-folds, phallus and opposite vascular body, and these organs swelled up. In these conditions, it was also observed that the injected solution flowed out of swelled lymph-folds.

Thus, it seems to be logical that the lymph which causes the erection of phallus flows out of the lymph-folds while the phallus maintains erection (Fig. 5). Actually, the transparent fluid is ejected simultaneously with the erection of phallus. If the connecting portions are bound tightly with surgical threads through the cloacal wall at both sides, and the connections between the vascular bodies and phallus as well as lymph-folds are intercepted, the operated birds which ejected the transparent fluid and erected the phallus by abdominal massage before operation, do not eject the transparent fluid and do not erect the phallus, even if abdominal massage is tried.

Since the tissue of vascular body is a lymphoid tissue (Cf. Section 1), the fluid which is generated in the tissue of the vascular body may be lymph or a fluid similar to lymph. Thus, the fluid ejected from lymph-folds, i.e., the transparent fluid, is considered to be *lymph*. From the evidence that the transparent fluid is ejected six times or more with no significant decrease in amount, in so far as the cock responds to sexual impulses (Cf. Section 2), it may also be assumed that the transparent fluid originates not in secretion but in lymph.

To demonstrate clearly the evidence that the transparent fluid originates in blood, 0.8 mc. of isotope P^{32} was injected intravenously into a cock which had been obstructed from contamination of the semen in vas deferens, by means of binding the anterior vasa deferentia with surgical threads and destruction of the openings of vasa deferentia with electric cautery, in order to collect the

pure transparent fluid. The opening of vas deferens is seen as a papillary process on the wall of cloaca (Fig. 1).

The specific activity (counts/cc/min.) of transparent fluid at 10 minutes after the injection was higher than that of the blood at same time. A severe decrease in the specific activity of the fluid was observed for six hours after injection and thereafter, the decrease became much slower. There was not a temporary increase at all in the decreasing curve of the specific activity. Moreover, the decreasing curve of the specific activity of transparent fluid resembled considerably to that of the plasma; the decreasing curve of the specific activity of plasma was determined by injecting intravenously 2 mc. of isotope P^{32} into other cock (Fig. 4).

From these evidences, the writer believes that the transparent fluid is a fluid of blood origin and presumably lymph.

For the purpose of ascertaining the portion ejecting the transparent fluid, the following physiological experiments were performed: the phallus, lymph-folds or both of them were cut off from five cocks (Cf. Nishiyama, 1952c, Table 1), and the aspect of ejection was investigated with these operated birds (Cf. Nishiyama, 1952c, Tables 2–6). The conclusions of these experiments are as follows:

1. The amount of ejected transparent fluid diminishes as a result of cutting out the phallus, and if the lymph-folds have been cut out previously, the ejection of the transparent fluid stops by cutting out the phallus. Similar effects are also induced by cutting out the small parts of both sides of the phallus.

2. If the lymph-folds are cut out, the amount of ejected transparent fluid will diminish, and in cases where the small parts of both sides of the phallus have been cut out previously, the transparent fluid is not ejected after cutting the lymph-folds.

From these data, the transparent fluid is apparently ejected from both phallus and lymph-folds. However, this is not true; and it seems probable that the transparent fluid is not ejected from phallus but from the lymph-folds themselves. This conclusion is induced from the results of the gelatin injection experiment and microscopic observation of the tissue joining phallus and lymph-fold. In the physiological experiments, this tissue was unconsciously cut out together with the phallus, but it was afterward found,

by means of gelatin injection experiment and microscopic observation, that the tissue belonged to the lymph-fold, being a part of the lymph-fold and joining it with the phallus. In the gelatin injection experiment, the injected gelatin flowed out of this tissue in the same manner as out of lymph-fold. The injected solution, however, did not flow out of the phallus at all.

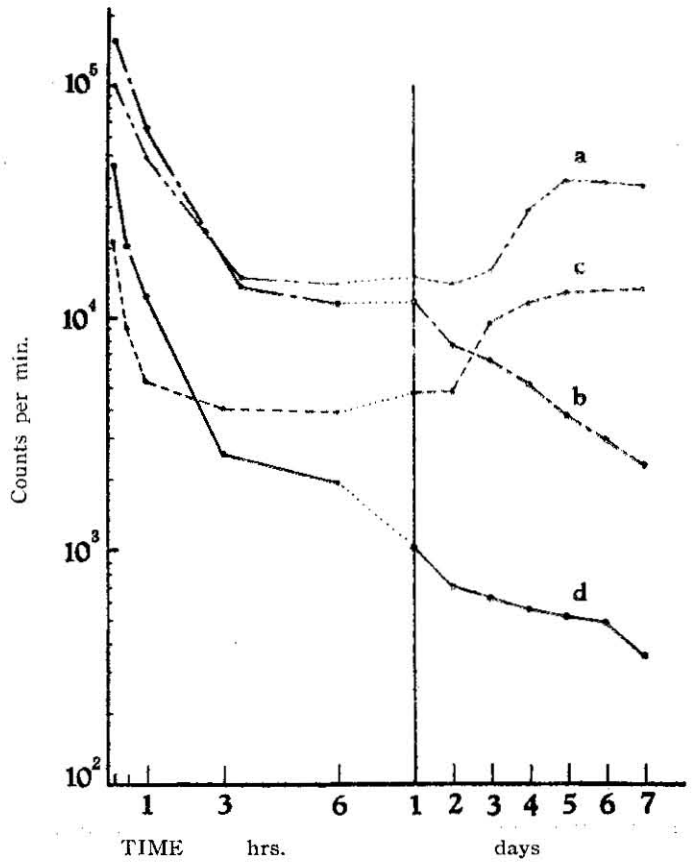

Fig. 4. Logarithmic changes in specific activities of blood, blood plasma and the transparent fluid of cocks after intravenous injection of labeled phosphate.

a, Specific activity of blood of bird No. 1, which received 2 mc. of isotope P^{32}; b, Specific activity of blood plasma of bird No. 1; c, Specific activity of blood of bird No. 2, which received 0.8 mc. of isotope P^{32}; d, Specific activity of the transparent fluid of bird No. 2.

Further investigation by microscopic observation was performed for the purpose to ascertain more clearly the portion ejecting the transparent fluid, with special reference to the phallus and lymph-fold including the tissue above-mentioned between the phallus and so called lymph-fold in Section 1. Dilute solution of melted gelatin containing a little Indian ink, was injected into one of the vascular bodies. The phallus, lymph-folds and vascular bodies swelled up as a result of injection, and the injected solution flowed out of the lymph-folds. In this condition, the specimen was put in cold 10% formalin solution. The lymph-fold and phallus were cut off from the specimen after the gelatin curdled, and then the pieces were fixed with formalin. The preparations of these tissues were made by routine method. In these preparations, the injected gelatin which had penetrated in the tissues was observed clearly owing to the existence of the Indian ink particles. The lymph sinuses in the submucous coat of the lymph-fold had been packed with gelatin and the cells of connective tissue lying beneath the columnar epithelium had been surrounded with it. It was also clearly ascertained that the gelatin had penetrated into the intercellular spaces of the epithelium and passed out of it (Plate 6, 2). The penetration of the gelatin was markedly conspicuous at the tops and sides of the fine crevices of the fold, although the gelatin had not penetrated into the epithelium at the bottom of them. In the flat parts of the epithelium, the gelatin had passed out of the epithelium throughout the parts but the degree of extrusion of the gelatin in these parts was lower than that in the crevices (Nishiyama, 1952c, Plate 1, Fig. 3).

In the phallus, the injected gelatin had also filled up the lymph sinuses in the submucous coat similarly as in the lymph-fold. The gelatin, however, had not penetrated at all into the stratified epithelium of the phallus (Plate 6, 3).

From the evidence mentioned above, the mechanism of the ejection of the transparent fluid may be summarized as follows: When a cock responds to sexual impulses, the lymph or a fluid similar to lymph is separated from blood copiously in the tissue of vascular bodies, and the lymph drains from the hind opening of them into the phallus and lymph-folds, through the lymph sinuses connecting the vascular bodies to the lymph-folds and the phallus. Lymph pressure in the phallus increases greatly, and

thus, the erection of phallus is caused (Cf. Nishiyama, 1950b). The lymph flowing into the lymph sinuses of lymph-folds reaches the loose connective tissue underlying the columnar epithelium, and still flows out of the lymph-folds through the intercellular spaces of the epithelium (Fig. 5). The transparent fluid, which is added to the vas deferens semen, is considered to be the lymph

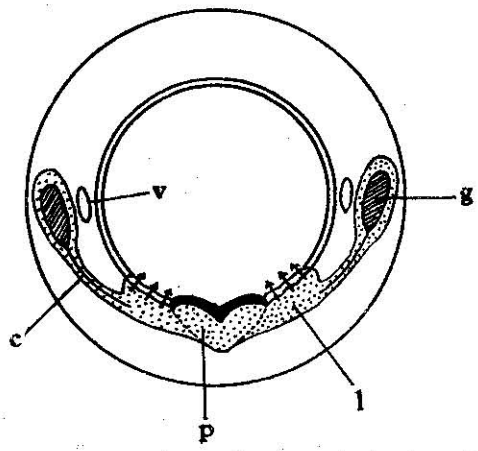

Fig. 5. Diagram to illustrate the mechanism of ejection of the transparent fluid.
c, connecting portion; g, vascular body; l, swelled lymph-fold which is ejecting the transparent fluid; p, erected phallus; v, ampulla ductus deferentis; ↑ denotes the ejection of the transparent fluid.

flowing out of the lymph-folds in the manner above-mentioned, and it might be true that very small amount of secretion from the epithelial cells is added to the lymph to make up the transparent fluid, since the epithelium of the lymph-fold is seemed to be a gland (Cf. Section 1).

The vas deferens semen is ejected simultaneously with the ejection of the transparent fluid and both the fluid and the semen are ejected along the longitudinal groove of the erected phallus to the outside of the body (Fig. 6).

The lymph in the phallus and the lymph-folds runs off within a short time, e.g., a few seconds, through the same passage flowing in the opposite direction, from the phallus and the lymph-folds to the vascular bodies and finally runs into the lymphatic vessels lying along the A. pudendalis interna; then, the phallus regains

its original quiescent condition and the ejection of the transparent fluid ceases (Cf. Nishiyama, 1950b).

So far as the cock responds to sexual impulses, the erection of phallus and ejection of transparent fluid are caused every time regardless of whether the vas deferens semen is ejected or not, and in some birds they are repeated four times or more.

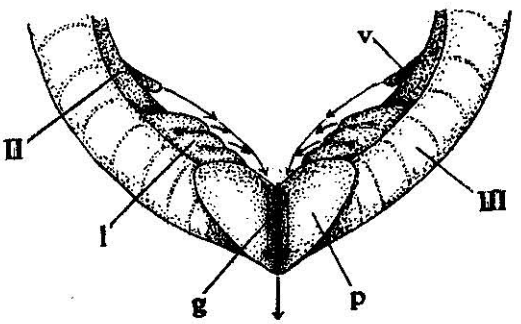

Fig. 6. Diagram showing the ejaculation of the semen of the cock.
g, longitudinal groove of the erected phallus; I, swelled lymph-fold; p, erected phallus; v, papillary process of vas deferens; II, 2nd fold of cloaca; III, 3rd fold of cloaca, i.e., anus; ↓ denotes the ejection of vas deferens semen from v, and outflow of transparent fluid from I, as well as the ejaculation of the semen (the mixture of vas deferens semen with transparent fluid) along g to the outside of anus.

Section IV

The Influence of Androgen on the Accessory Reproductive Organs of the Cock*

The accessory reproductive organs of cocks eject the transparent fluid, and add the fluid to the vas deferens semen at the moment when the semen in vas deferens is ejected (Cf. Section 2). If an early castration is performed in the cock, these organs degenerate. From these facts, it appears that the accessory reproductive organs of the cock correspond to the accessory reproductive glands of mammals in regard to their physiological function.

* The results of this work was reported more fully before the Paper Reading Sessions of Tenth World's Poultry Congress held in Edinburgh, 13th-21st August, 1954 (Section Papers, p. 88, Xth World's Poultry Congress).

Table 1. Hormones and the periods of injection.

Periods of injection (in 1951)	Testosterone acetate		Oestrone benzoate		Reference
	Daily dose in crystal (mg.)	Daily dose of 1 or 5 mg. Amolisin (A)* (cc)	Daily dose in crystal (γ)	Daily dose of 1000 I.U. Ovahormone benzoate** (cc)	
Feb. 17–21	0.5	0.25 cc of 1 mg. A.	16	0.08	Injected daily at 10:00 a.m.
Feb. 22–26	1.0	0.5 cc of 1 mg. A.	30	0.15	„
Feb. 27–Mar. 8	2.0	0.5 cc of 1 mg. A. twice a day	60	0.15 twice a day	Injected daily at 9:00 a.m. & 5:00 p.m.
Mar. 9–13	5.0	0.5 cc of 5 mg. A.	150	7.5	Injected daily at 10:00 a.m.

* 1 mg. or 5 mg. Amolisin (A) is a preparation which contains 1 mg. or 5 mg. testosterone acetate in 0.5 cc of sesame oil, respectively.

** 1000 I.U. Ovahormone benzoate is an oil solution containing 0.1 mg. of oestrone benzoate.

On the other hand, it is well known that the degenerated accessory reproductive glands of an early castrated mammal increase their size and weight (Deanesly and Parkes, 1936; Korenchevsky et al., 1932, 1936, 1937, 1939; Tschopp, 1935; Zuckerman and Parkes, 1936, 1938) and their functions are also recovered by androgen administration (Moore and Gallagher, 1930; Shapiro, 1937; Moore and Price, 1938).

For the purpose of ascertaining how the accessory reproductive organs of the fowl respond to the androgen administration, a daily dose of 0.5–5 mg. of testosterone acetate (total dosage, 52.5 mg.) was injected intramuscularly for 25 days with oestrone benzoate, the amount of it being about one thirtieth of testosterone acetate, into three capons which had been castrated 87 days after hatching and whose vasa deferentia openings had been seared and destroyed with an electric cautery before experiment in order to block the contamination of the secretion in vas deferens, and the data from these injected capons were compared with those from their three controls as shown in Table 1.*

The phalli, lymph-folds and vascular bodies of the injected birds recovered their size and weight (Fig. 7; Nishiyama, 1954,

Fig. 7. Left vascular body of injected birds (upper column) and left vascular body of control birds which were covered with the connective tissue capsule (lower column). All specimens were fixed with formalin and photographed. (From Nishiyama, 1954).

* This table is the same as that reported before the Tenth World's Poultry Congress.

Table 3, Fig. 4), and their histological appearances also recovered from markedly degenerated states of capons to the states of normal cocks (Nishiyama, 1954, Plate 14, Figs. 1–5) by means of androgen administration.

The injected birds ejected the transparent fluid as a result of recovering of their function; very little amount of transparent fluid was ejected for the first time on 7th–12th day by abdominal massage. The amounts mostly increased day after day and the maximum amounts of the fluid which was collected from each experimental bird by abdominal massage as well as with semen collector reached 0.21, 0.19 and 0.185 ml., respectively (Nishiyama, 1954, Fig. 3 and Table 2).

Thus, the influences of the androgen on the accessory reproductive organs of cocks are quite similar to those of androgen on the accessory reproductive glands in mammals.

Considering the physiological function and responses to the castration as well as androgen administration, it may be thought that the accessory reproductive organs of cocks and the accessory reproductive glands of mammals are analogous.

Section V

Some Characteristics of the Transparent Fluid

(1) Some of the physical and chemical characteristics

The pH value of the cock's semen has been reported by several authors, that is, 7.0 by Shibata et al. in 1938, 7.15 by Zagami in 1939, 6.3–7.8 by Lambert and McKenzie in 1940, 7.2 and 7.4 by Parker et al. in 1940, 7.04 by Wheeler and Andrews in 1943, 7.0 by Nishikawa in 1944 and 7.3–7.8 by Gallein in 1948.

In this experiment, the pH value of normal semen, which was white in color and was called "white semen" by the writer, was measured with a potentiometer with quinhidrone electrode, and was observed to be 7.2 ± 0.13 on an average. On the other hand, the pH value of the transparent semen was observed to be 7.9 ± 0.13 on an average (Nishiyama, 1952a, Table 2), it showed that it was higher than that of white semen. The transparent semen is the semen which will be collected in 4th, 5th or 6th collection

in case of repeated collections and will appear transparent, so that the semen consists, for the most part, of transparent fluid.

Considerable large variations of pH value were observed among the semen in both white and transparent one. However, should we compare the pH value of the white semen with that of transparent semen which was collected in a course of repeated collections, the pH values of the white semen were higher, in almost all of the cases, than those of the transparent semen collected in the same course.

The pH value of an ejaculated semen is, therefore, influenced by the mixture rate of the transparent fluid to the semen in vas deferens, and as the mixture rate increases, the pH value of the ejaculated semen shows a shift toward alkalinity.

The protein content of the transparent fluid (which was collected from the bird which had been obstructed from the contamination of vas deferens semen as mentioned in Section 3) measured with a protein refractometer was less than 1%.

The viscosity of transparent fluid measured with a Hess viscosimeter was lower than that of blood serum and the values were 1.1 and 1.5, respectively. The difference was probably due to their protein contents.

(2) The influence on the motility of the spermatozoa of the cock

As the diluter of cock semen, several solutions, i.e., the physiologic saline solution, egg albumen, embryonic extract, serum etc. have been reported (Motohashi and Moritomo, 1927; Ishikawa, 1930; Nikitina, 1932; Grodzinski and Marchlewski, 1935; Hayashi, 1938). Of these diluters, the Ringer solution is thought to be a representative diluter of cock's semen. The duration of sperm motility in the transparent fluid (this transparent fluid was secured from the transparent semen, after the agglutination of the spermatozoa which were involved in the semen had been completed) was much the same with that in the Ringer solution (Nishiyama, 1951a, Table 1), and the increase of the abnormal spermatozoa in the course of storage in both fluid and solution was also similar to each other.

It was also observed that the activity or motility of spermatozoa just after collection depended on the density of semen; the spermatozoa in dense semen ejaculated showed highest motility and

they were more vigorous than the spermatozoa in the vas deferens semen. On the contrary, in the transparent semen, the greater part of which being consisted of transparent fluid, the motility of the spermatozoa was very low. Therefore, it seems probable that when a small amount of the transparent fluid is mixed with the vas deferens semen, the spermatozoa increases their motility, but when considerable amount of it is mixed, the spermatozoa markedly decrease their motility.

(3) Agglutination of spermatozoa in the transparent fluid

Mammalian spermatozoa agglutinate in various solutions and fluids (Dittler, 1920; Guter, 1922; Fogelson, 1926; Krzyszkowsky and Pawlow, 1927; Yamane, 1921; Kato, 1932, 1936). Grodzinski and Marchlewski (1938) reported that the cock spermatozoa agglutinated in the blood serum of the cock or of the masculinised hen.

The writer found that the cock spermatozoa also agglutinated in the transparent fluid.

If the collected semen was left standing in test tube, the spermatozoa which had swum freely just after collection, agglutinated in various intensities within 10–15 minutes after collection. The intensity of sperm agglutination appeared to be lowest in white semen and highest in faint white colored semen. In transparent semen the intensity appeared to be lower than that in the faint white colored semen.

The number of freely swimming spermatozoa, however, decreased as the semen became more dilute, and finally, almost all of the spermatozoa were led to adhere with one another in the most dilute semen, i.e., in the transparent semen. Then, it seems probable that intensity of sperm agglutination depends on the number of spermatozoa and on the amount of a certain substance as well, which apparently induces sperm agglutination. The substance is considered to be an organic matter similar to fibrin, as will be described later.

The forms of sperm agglutination depend upon the activity and number of the spermatozoa as well as on the amount of the transparent fluid mixed with the vas deferens semen. In the white semen which is the densest one and contains innumerable vigorous spermatozoa, they agglutinate forming clumps of large irregular shape and minute spherical shape, as well, but the number of

clumps appeared in the semen is very small. In more dilute semen, which is faint white in color, the forms of the clumps are similar to those of the white semen, but number of the clumps is very large. And frequently, the spermatozoa adhere to the wall

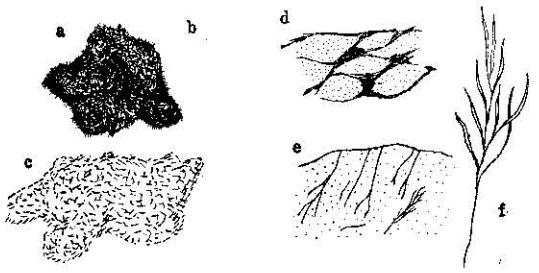

Fig. 8. Illustration of various forms of clump made of cock's spermatozoa in the semen.
a, large irregular shaped; b, spherical; c, cloudy; d, reticulum; e, threads formed by spermatozoa; f, ditto, enlarged.

of the test tube which contains the semen. In the transparent semen, the spermatozoa also adhere to the wall of the test tube and frequently agglutinate, sometimes to form a very loose cloudy mass, and sometimes to form reticulum or threads (Fig. 8), and all of the spermatozoa which do not agglutinate show a tendency to adhere with one another. Generally speaking, as the semen becomes more dilute, adhesion of spermatozoa which forms a clump becomes weaker and the number of adhering spermatozoa becomes smaller.

In the sea urchin, the spermatozoa agglutinate by "fertilizin" (Lillie, 1915). In mammals, according to Kato (Kato, 1936), the spermatozoa agglutinate under the influence of certain hydrogen ion concentrations, of the secretions from female genital tract and of other body fluid, and the cause of the agglutination is the action of salts contained in these substances.

In the cock, one of the causative factors inducing sperm agglutination which should be contained in the transparent fluid, is thought to be of organic nature, as indicated in the following experiments. The transparent fluid of the cock diminishes its sperm agglutinating power by filtration through filter paper, and the supernatant which is prepared by adding charcoal powder or dense semen to the transparent fluid and centrifuging it after 30

minutes, shows lower agglutinability than the original transparent fluid. This diminution of agglutinability shows that one of the causative factors of agglutination is neither the hydrogen ion concentration nor electrolytes, but presumably an action of organic substance as shown in the following paragraphs.

From the above evidence and the evidence that after the collected semen has completed the sperm agglutination in certain intensities according to its characteristics, that is, to the mixing rate of transparent fluid to the vas deferens semen, the intensity of agglutination does not increase even if new dense semen be added to the semen (Nishiyama, 1952a), it may be considered that the transparent fluid contains the sperm agglutinating substance in a certain amount.

The chalaza-like substance (Nishiyama, 1952b)—fragment of chalaza-like appearance—arises frequently in the transparent semen, within 10–20 minutes after collection. The interior fine white part of this fragment consists mostly of numerous spermatozoa, and the outer part of it consists of translucent adhesive substance which appears to be like dilute gelatin. The chalaza-like substance also arises in the transparent fluid which is collected from the bird which has been obstructed from contamination of the vas deferens semen. In this case, the substance appears translucent, for there is no interior white part. If a little of sodium citrate is put into the collecting tube first, and the collected transparent fluid is perfectly mixed with it immediately after collection, the chalaza-like substance will not arise in the fluid at all.

From these observations and the evidence that the transparent fluid is of blood origin, it may be thought that the rising of the chalaza-like substance is due to a matter similar to fibrinogen. It seems probable that in some type of agglutination, i.e., agglutination forming clump of large irregular shape or cloudy mass, the phenomenon of the sperm agglutination or sperm clumping in the fowl has a certain connection with the rising of this chalaza-like substance. Because, it is seen that the spermatozoa, in this shape of clump, adhere to a adhesive matter which is stainable with eosin. And, if a little of the sodium citrate solution is mixed perfectly with the collected semen immediately after the collection, this type of clump scarcely arises. Then, a

causative matter of sperm agglutination which seems to be contained in certain amount in the transparent fluid and to be of organic nature as mentioned before, may be considered to be a similar matter as fibrin, and then, it is probable that the large irregular shaped clump or cloudy mass is not due to sperm agglutination but is merely adhesive mass to an adhesive substance which arises in the semen by minute partial coagulation of the transparent fluid.

The minute spherical clump in the semen of the fowl is similar in shape to the rosset of mammalian sperm agglutination, and the course of agglutination is also similar to mammalian one (Krzyszkowsky and Pawlow, 1927; Kato, 1936). Then, it seems probable that this type of clumping is presumably true agglutination.

Summary and Conclusion

The accessory reproductive organs of the cock consist of two parts or organs, that is the lymph-fold and the vascular body. The lymph-folds are two triangular or spindle shaped folds, lying on the outside of the round folds, one on each side. The vascular bodies which are egg shaped, flattened and red colored, lie in the inside of cloacal sphincter, one on either side of the cloacal wall, and are covered with connective tissue capsules. The vascular body consists of lymphoid tissues, and the vascular body, lymph-fold and phallus are connected to one another by lymph sinuses, these organs belonging to one and the same system.

The accessory reproductive organs of the cock eject a fluid similar to lymph in the following manner, and the fluid constitutes greater part of the sperm-serum in the case where repeated collections of the semen are performed. When a cock responds to sexual impulse the lymph, or a fluid similar to lymph, is separated from the blood copiously in the tissue of the vascular body, and then the lymph runs off from the hind opening of the body into the phallus and lymph-fold through the lymph sinuses connecting the vascular body with lymph-fold and phallus. Lymph pressure in the phallus increases greatly, thus, causing the erection of the phallus. The lymph which flowed into the lymph sinuses of the lymph-fold reaches the loose connective tissue underlying

the columnar epithelium, and still flows out of the lymph-fold through the intercellular spaces of the epithelium. This lymph or a fluid similar to lymph ejected from lymph-fold is called transparent fluid by the writer, and it might be true that the transparent fluid contains a very small amount of secretion from the epithelial cells of lymph-fold. The vas deferens semen is ejected simultaneously with the transparent fluid, and both the fluid and the semen are ejaculated to the outside of the body along the longitudinal groove of the erected phallus. The lymph in the phallus and lymph-fold runs off within a short time, e.g., a few seconds, through the same passage flowing in the opposite direction from the phallus and lymph-fold to vascular body and finally into the lymphatic vessels lying along the A. pudendalis interna; then, the phallus reverts to its original quiescent condition and the ejection of the transparent fluid ceases.

So far as the cock responds to sexual impulses, the erection of the phallus and the ejection of transparent fluid is caused every time regardless of whether the vas deferens semen is ejected or not, and in some birds the ejections of fluids are repeated four times or more.

Degeneration of the accessory organs of the cock is caused by early castration, but the organs may be recovered by androgen administration. The phallus, lymph-fold and vascular body recover their size and weight and histological appearances from the markedly degenerated state of capons to the state of normal cocks by androgen administration. And also, in the course of injection, the injected birds eject the transparent fluid as a result of recovering their function. From these evidences, the writer believes that the accessory reproductive organs of the cock are analogous in physiological points of view to the accessory reproductive glands of the mammal.

The pH value of the transparent fluid is observed to be 7.9 ± 0.13 and the pH value of an ejaculated semen is influenced by the mixture rate of the transparent fluid to the vas deferens semen, and as the rate of mixture increases, the pH value of the ejaculated semen shows a shift toward alkalinity. The protein content of the transparent fluid is observed to be less than 1% and the viscosity to be about 1.1. When small amount of this fluid is mixed with the vas deferens semen, the spermatozoa increase

their motility, but when considerable amount of it is mixed, the spermatozoa markedly decrease their motility. There occurs the formation of clumps made of spermatozoa in the collected semen short time after the collection, and the clumps are various in forms according to the activity and number of spermatozoa as well as the amount of the transparent fluid added to the vas deferens semen, and the intensity of the formation of clumps furthermore depends on the number of spermatozoa and on the amount of the transparent fluid, as well, and dilute semen which is faint white in color shows highest intensity to form clumps. Some forms of clump, i.e., large and irregular-shaped or cloudy mass, are considered to be caused by the adhesion of spermatozoa to an adhesive substance which seems to arise sporadically in the semen. The adhesive substance may be generated by partial coagulation of the transparent fluid, the cause of coagulation being similar to that of blood. Another form of clump, a minute and spherical shaped, is considered presumably to be formed by true sperm agglutination.

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Explanation of Plate 6

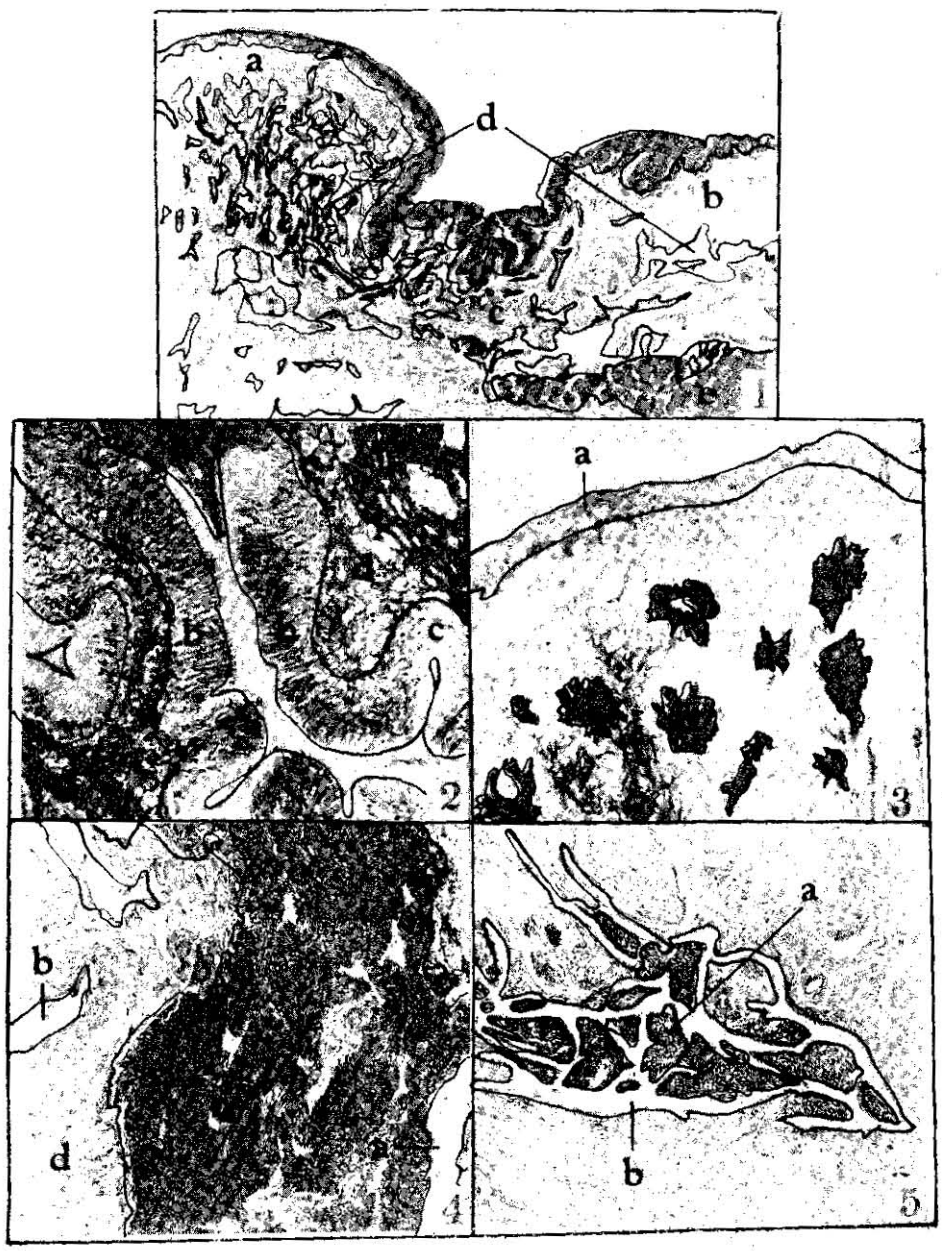
Fig. 1. Sagittal section of phallus, lymph-fold and the tissue joining phallus and lymph-fold of the cock. The lymph sinuses of the phallus and the lymph-fold connect with those of connecting portion (Cf. Fig. 5). The phallus is covered with stratified epithelium, but the lymph-fold is covered with columnar epithelium. ×20.
a, phallus; b, lymph-fold; c, the tissue joining the phallus and lymph-fold, it is covered with columnar epithelium; d, lymph sinuses; e, lymphoid tissues of the connecting portion.

Fig. 2. Sagittal section of lymph-fold, showing the extrusion of injected gelatin from the epithelium. ×220.
a, gelatin which flowed out of lymph-fold; b, columnar epithelium, the injected gelatin penetrates into the intercellular spaces of it; c, bottom of the crevice, the injected gelatin does not penetrate into the intercellular spaces of it; d, connective tissue lying beneath the columnar epithelium which is surrounded with the injected gelatin.

Fig. 3. Sagittal section of phallus, showing the stratified epithelium of it, in which the injected gelatin does not penetrate at all. ×52.
a, stratified epithelium; b, lymph sinuse of the phallus, which is filled up with the injected gelatin.

Fig. 4. Horizontal section of the vascular body of the cock. ×52.
a, lymph sinuse; b, blood vessel; c, lymphoid tissue of medulla; d, trabecula.

Fig. 5. Cross section of the connecting portion of the vascular body which connects itself to the phallus and lymph-fold. ×52.
a, lymphoid tissue; b, lymph sinuse.



Accessory reproductive organs in the cock