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PASSAGE AND DISAPPEARANCE OF LABELLED SPERMATOZOA IN THE GENITAL TRACT OF THE MALE JAPANESE QUAIL IN SEGREGATION OR COHABITATION ⁽¹⁾

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SUMMARY

Male quails kept in segregation from or in cohabitation with females were injected with ³H-thymidine and the passage of labelled spermatozoa through the epididymis and the vas deferens was followed by autoradiography.

Labelled spermatozoa appeared in the epididymis on the 11th day after injection and reached the distal part of the vas deferens within 24 hours both in segregated males and in those kept with females.

Three sperm populations with decreasing intensity of labelling were identified in the epididymis on days 11, 14 and 15, and in the distal part of the vas deferens on days 12, 15 and 16 after injection.

From the distribution of labelled spermatozoa along the vas deferens of the males of the two groups, it was concluded that some slowing down in the transit of the main bulk of the spermatozoa had occurred in the males kept isolated from females. This fact indicated the role of the vas deferens as a transitory storage place of the segregated males, unejaculated spermatozoa which were eliminated about 24 h later than those of the males kept in cohabitation with females.

INTRODUCTION

MUNRO (1938) showed that cock spermatozoa acquire motility and fertilizing capacity during their passage through the epididymis and the proximal part of the vas deferens. Using indirect methods, he concluded that the duration of sperm transit through the excretory ducts is 24 hours in sexually active males but the transit « may require 72 to 96 hours » in non-active males. Using autoradiography, de RE-

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VIERS (1968) found that in regularly « collected » cocks the spermatozoa passed through the vas deferens within 1 to 3 days, according to season.

MUNRO (1938) attributed no storing function to the vas deferens, but LAKE (1957) and GLOVER and NICANDER (1971), on the grounds of anatomical and histological considerations, came to the conclusion that in the domestic cock the vas deferens does serve as a transitory storage place for spermatozoa. Also de REVIERS (1972), by counting the sperm reserves in the vas deferens of « collected » and « non-collected » cocks, concluded that the distal part of the vas deferens serves as a storage organ for unejaculated spermatozoa.

In view of the functions of the excurrent ducts of the domestic fowl as organs for the maturation and storage of spermatozoa, the present study was undertaken in order to study both the duration of sperm transit under different sexual regimes and the role of the vas deferens as a sperm storage organ in the Japanese quail. This was done by following the transit and the rate of disappearance of spermatozoa which had been labelled with ^3H -thymidine. This method, used previously by de REVIERS (1968) in cocks, enables us to distinguish between and to follow different sperm populations under normal physiological conditions. These advantages cannot be achieved by the use of India ink, or by the production of spermatozoal abnormalities with X-rays, methods employed by MUNRO (1938).

MATERIALS AND METHODS

Male Japanese quails which had been kept from puberty until the start of the experiment in isolation from females, were used. During rearing as well as during the experiment they were maintained under continuous light by supplying artificial illumination during the hours of darkness. The birds were injected intraperitoneally with 100 μCi of ^3H -thymidine (1.26 Ci/mM , C.E.A., France) in 0.5 ml 9 p. 100 NaCl sterile solution.

In the first series of experiments 30 males, three per cage, were used. Beginning 1 h after injection, two males were killed each day until the 14th day, by intracardial injection of 0.5 ml Nembutal (Abbott Laboratories, Ltd.). One testis, with the adjacent epididymis and vas deferens, was removed immediately. The vas deferens was divided by ligatures into the proximal, middle and distal parts. These segments, after having been stretched on cardboard, as well as the epididymis and a portion of the testis, were fixed in a 95% ethanol-formol-acetic acid (75-20-5) fixative for 24 h, embedded in paraffin, and cut in 4 μ sections. The parts of the vas deferens were cut longitudinally.

In the second series of experiments two groups of 30 males each were used: (a) three males per cage, and (b) one male with two females per cage. Two males from each group were killed each day from the 10th to 24th day after injection. Homogenates of the epididymis and the three segments of the vas deferens were prepared using a slow gear homogenator and 0.25 ml of an isotonic phosphate buffer (pH 7.2) containing 3 p. 100 PVP⁽¹⁾, to avoid swelling and disintegration of the cells (SCHINDLER and KEMPENICH-PINTO, personal communication). Smears were prepared from the homogenates as well as from homogenates from non injected birds. The sections and the smears were mounted on Ilford coated slides and dipped in K_2 and K_8 Ilford radioautographic emulsion, respectively. After 3 weeks of exposure of the sections and one month of exposure of the smears, the slides were developed in an Amidol developer (FERRAGI, 1952) and stained with Mayer's haemalum solution, except for the sections from the testes, which were stained with haematoxylin eosin.

On each smear the percentage of labelled spermatozoa was determined by a random count of at least 500 spermatozoa, using oil immersion ($\times 1500$). The intensity of labelling was determined by counting the number of silver grains above the head of each labelled spermatozoon. Observations of smears prepared from non-injected birds or from unlabelled parts of injected males revealed that fewer than 1 p. 100 of the spermatozoa were associated with background clusters of two or more grains in one lot of autoradiograms and three or more grains in a second

(¹) Polyvinylpyrrolidone (Sigma, U. S. A.).

lot. Thus, spermatozoa with three and four or more silver grains/nucleus for the two lots of autoradiograms, were considered as labelled. The labelled spermatozoa were divided into ten classes according to the number of grains above their heads, the last class being the one which included the spermatozoa having more than 29 or 30 grains. It was impossible to count with accuracy more than 30 grains over the strongly labelled spermatozoa.

Analysis of results

According to the labelling intensity of the spermatozoa, different sperm populations and their arrival in the epididymis and the distal part of the vas deferens at different intervals after the injection of the precursor, were presented graphically according to the method of LAZAR and CERARD-MARCHANT (1965). This method allows the linear representation of the cumulated frequencies of labelled spermatozoa, by plotting these frequencies, on a probit scale, against the logarithm of the maximum number of silver grains of each of the ten classes of labelled spermatozoa. All the spermatozoa which were found labelled among the 500 or more sperm cells counted in each bird, served for the calculation of each labelled sperm population (average of 240 labelled spermatozoa for each population).

RESULTS

The incorporation of the injected precursor into the germ cells, their advancement up to spermatozoa in the testis, and the passage of the labelled spermatozoa through the epididymis and the vas deferens of segregated males, were followed in autoradiograms of histological sections of these organs (transverse sections for the testis and the epididymis and longitudinal sections for the vas deferens).

Labelled primary spermatocytes found one hour after injection evolved to round spermatids on the 7th day and to elongated spermatids and spermatozoa on the 9th and 10th days. On the 11th day a great number of strongly labelled spermatozoa entered the epididymis and the proximal part of the vas deferens (Plate I, fig. 1, 2, 3), and some of them reached the upper portion of the middle part of the ductus. On the 12th day the middle and distal parts of the vas deferens contained many labelled spermatozoa (Plate I, fig. 4), whereas in the epididymis and the proximal part of the ductus, such spermatozoa were present only in small numbers.

On the 13th day only a few radioactive spermatozoa were found in the excurrent ducts, but on the 14th day many such spermatozoa entered the epididymis and the proximal part of the vas deferens again.

Having established the times of appearance of labelled spermatozoa in the different parts of the excurrent ducts in segregated males, the influence of copulation on the rate of passage of these spermatozoa through the epididymis and the vas deferens was studied in autoradiograms of smears prepared from the different parts of the genital tract in males kept in segregation or cohabitation. The percentages of labelled spermatozoa and the intensity of their labelling were scored. The examinations were carried out until no more labelled spermatozoa were found. The results are presented in table 1.

From the fact that labelled spermatozoa in both segregated and cohabiting males entered the epididymis on day 11 and reached the distal part of the vas deferens on day 12 (table 1), as was established also in the first experiment, it appears that the sperm transit through the excurrent ducts of the male quail can be accom-

plished within 24 h. But table 1 also shows that the distribution of labelled spermatozoa along the vas deferens was different in the two groups of males. On day 11 fewer labelled spermatozoa reached the proximal part of the vas deferens of segregated males (14 p. 100) than of those kept with females (41 p. 100). In accordance, more labelled spermatozoa remained in the middle and distal parts of the ductus on days 12 and 17 in segregated birds than in cohabiting males: on day 12: 53 p. 100 and 26 p. 100 vs. 7 p. 100 and 20 p. 100, and on day 17: 10 p. 100 and 8-33 p. 100 vs. 2 p. 100 and 3 p. 100. These facts show that in the segregated males the passage of the main bulk of spermatozoa was slowed down.

TABLE I

Percentage of labelled spermatozoa in the different parts of the genital tract of the male quail at different days after injection of ^3H -thymidine

(average of 2 males)

Part of the tract	Days after injection										
	10	11	12	13	14	15	16	17	18	19	20
<i>A. Males isolated from females</i>											
Epididymis ..	0	65*	10	4	3-20**	18	0-16**	2	1	1	1
Vas deferens :											
Proximal ..	0	14*	6	4	20-38**	18	8	0-15**	3	0	2
Middle	0	7*	53	7	9	41	7-24**	10	4	3	1
Distal	0	< 1* ←	26	4	1	25	12	8-33**	2-15**	5	2*
<i>B. Males kept with females</i>											
Epididymis ..	0	56	2	5	9	18	6	0	3	3	0
Vas deferens :											
Proximal ..	0	41	7	5	27	26	15	0	5	2	1
Middle	0	2	7	13	22	17	10	2	7	2	2
Distal	0	1	20	12	3	17	19	3	6	3	3*

* Data from one animal only.

** Data from both animals.

From Table 1 it can also be seen that waves of spermatozoa entered the epididymis on days 11, 14 and 15, whereas in the middle and distal parts of the vas deferens many labelled spermatozoa were found on days 12, 15 and 16. By plotting, for each of these days, the cumulated frequencies of labelled spermatozoa against the log of the number of grains of these spermatozoa, it was possible to distinguish three sperm populations at the two ends of the excretory duct in both the segregated and the cohabiting males (Text — fig. 1, 2). The mean radioactivity of these populations differed significantly from each other in all except one case.

In the cohabiting males the percentage of labelled spermatozoa in the vas

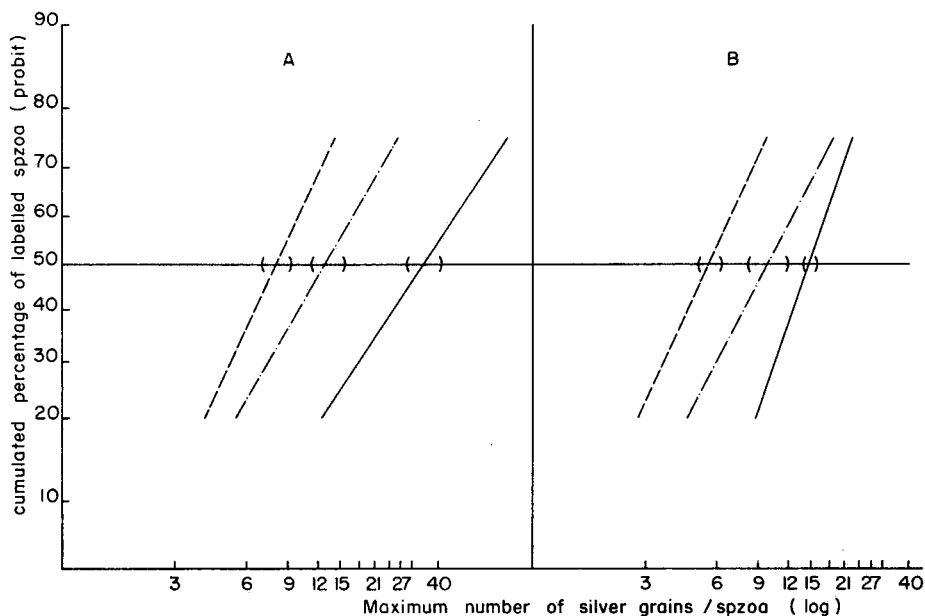


FIG. 1. — Labelling intensity of sperm populations which entered the epididymis of male quails kept in segregation (A) or cohabitation (B) at different days after ^3H -thymidine injection

Day no.

() = confidence limits

— 11

- - - 14

--- 15

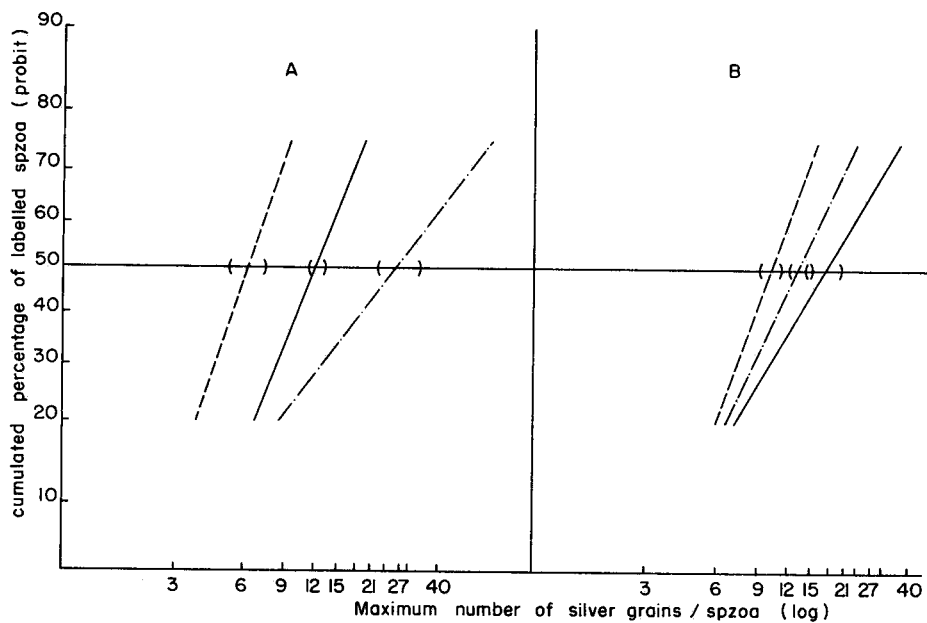


FIG. 2. — Labelling intensity of sperm populations which arrived in the distal part of the ductus deferens of male quails kept in segregation (A) or cohabitation (B) at different days after ^3H -thymidine injection

Day no.

() = confidence limits

— 12

- - - 15

--- 16

deferens decreased rapidly after the 16th day, whereas in the segregated males this decrease occurred one day later (table 1). After the 20th day fewer than 1 p. 100 of the weakly labelled spermatozoa (up to 6 grains/spermatozoon) were found in the different parts of the excurrent ducts in the males of the two groups.

DISCUSSION

The results of this work show that sperm transit in the male quail can be accomplished within 24 h. Therefore, the three sperm populations of declining labelling intensity which entered the epididymis on days 11, 14 and 15, and which were also found in the distal part of the vas deferens on days 12, 15 and 16, can be considered as corresponding ones passing through the ductus in about one day. The decline of the average intensity of labelling can be attributed to the additional divisions of the germ cells from which these sperm populations issued. The rate of this decline was indeed close to the theoretical relationship of 4 : 2 : 1. The actual deviations from this relationship may arise from (a) possible differences in the duration of DNA synthesis between the different types of germ cells — as reported for the bull (HOCHÉ-REAU, 1967), (b) the impossibility to count with accuracy more than 30 grains over the heads of the very strongly labelled spermatozoa and (c) the fact that each sperm population issued from a different pair of animals. In spite of an identical dose of injected precursor, different animals could have been labelled differently, as was observed in rams (AMIR and ORTAVANT, 1968).

An unusually stronger labelling of the 15th-day animals may have been one of the causes of the 15th-day sperm population being labelled more strongly than the 12th-day population in the distal part of the vas deferens of the segregated males. Another possible contribution to this deviation may have been a delay in the release of the strongly labelled spermatozoa from the testes of the 15th-day animals, as reported for rabbits (AMANN, 1972); however, in none of the six animals killed 11 days after the injection of the precursor was such a delay in the release of labelled spermatozoa found.

Although no quantitative measurements of the sexual activity of the cohabiting males were made, nor was the possibility of semen emission by the segregated males checked, some slowing down of the sperm transit had occurred in the males kept isolated from females. This is shown by the fact that in these males fewer labelled spermatozoa arrived at the proximal part of the vas deferens on day 11 and more labelled spermatozoa remained in the middle and distal parts of the vas deferens on days 12 and 17, than in the males kept with females. In bulls (ORGBIN-CRIST, 1962) and rams (AMIR and ORTAVANT, 1968) frequent sperm collections were found to hasten the epididymal transit by 10-30 p. 100. The slowing down of the rate of sperm transit in the segregated males suggests that the vas deferens can serve as a transitory storage place for unejaculated spermatozoa, as was pointed out by LAKE (1957), GLOVER and NICANDER (1971) and de REVIERS (1972) for the cock. The similarity of the distribution of labelled spermatozoa in the vas deferens of the segregated males on day 17 with that of the cohabiting males on day 16, indicates that the storage

period may be of about one day, as far as could be assessed from the 24 h intervals at which the observations were made.

After this additional day of remaining in the vas deferens of the segregated males, the spermatozoa were eliminated as in the males kept with females, but one day later.

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RÉSUMÉ

PASSAGE ET DISPARITION DES SPERMATOZOÏDES MARQUÉS DANS LE TRACTUS GÉNITAL DE LA CAILLE MÂLE SÉPARÉE OU NON DES FEMELLES

Des cailles mâles séparées ou non des femelles ont été injectées avec de la thymidine-³H ; le passage des spermatozoïdes marqués dans l'épididyme et le canal déférent fut suivi par autoradiographie.

Des spermatozoïdes marqués apparaissent dans l'épididyme le 11^e jour après l'injection et sont trouvés dans la partie distale du canal déférent le 12^e jour chez les mâles de deux groupes. Le transit des spermatozoïdes dans les voies déférentes de la caille peut donc être accompli dans un délai de 24 h.

Trois populations de spermatozoïdes, d'intensité de marquage décroissante, ont été identifiées dans l'épididyme les jours 11, 14 et 15, et dans la partie distale du canal déférent les jours 12, 15, 16. Il semble que ce sont les mêmes populations de spermatozoïdes qui proviennent des différentes générations successives des cellules germinales.

La différence entre les cailles de deux groupes dans la distribution des spermatozoïdes marqués dans les canaux déférents montre que chez les mâles séparés des femelles il y a un ralentissement du transit de la masse principale des spermatozoïdes. Ce fait indique que le canal déférent peut servir comme un lieu de stockage transitoire des spermatozoïdes non éjaculés. Pour autant que des observations espacées de 24 h permettent d'en juger, ces spermatozoïdes sont éliminés une journée plus tard que chez les mâles maintenus en présence des femelles.

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PLATE I

*Histological sections and sperm smear from the epididymis
and the vas deferens of segregated males after ³H-thymidine injection
(Haemalum stain, × 1 500)*

FIG. 1, 2

Histological sections of the epididymis 10 (fig. 1, no labelled spermatozoa) and 11 days (fig. 2, many labelled spermatozoa) after ³H-thymidine injection.

FIG. 3

Sperm smear from the epididymis of a segregated male 11 days after injection showing strongly labelled spermatozoa.

FIG. 4

Histological section of the distal part of the vas deferens 12 days after injection (many labelled spermatozoa).

