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DURATION OF TRANSIT OF SPERMATOZOA THROUGH THE HUMAN MALE DUCTULAR SYSTEM*

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The time of development of spermatogonia to mature spermatozoa within the Sertoli cell cytoplasm has been established for the human.[11] The time of transit from the release of the spermatozoa from the Sertoli cell cytoplasm to their appearance in the ejaculate has not been determined, with any precision, for the human. We therefore proposed to establish the most rapid, the average, and the maximal ductular transit time for human spermatozoa.

Table 1 summarizes ductular travel time as estimated and/or determined experimentally for several species of mammals.

MATERIALS AND METHODS

Adult normal male volunteers (age 26–40) were divided into two groups. Reproductive normalcy was determined by hormonal determinations, sperm counts, and testicular biopsy examination. A group of 5 subjects was given intratesticular injections of thymidine-H^3 at four sites in each testis for a total of 80 μc. The second group of 9 men received testicular X-ray irradiation of 100 or 300 r. Two of these subjects were first injected with thymidine and then immediately irradiated with 100 r. Each of the volunteers were candidates for vasectomy, which was performed at the termination of the investigation.

Thymidine-H^3. Seminal fluid was collected weekly, following 3 days of abstinence, for the first 30 days following injection of thymidine-H^3. From this time forward, every ejaculate was obtained and analyzed. Subjects were requested to submit samples every third day if possible. Each specimen was analyzed by 2 technicians using the hemocytometer method.

Radioautographs were made from smears of each seminal fluid specimen. The smears were coated with Diaphane, fixed in 95% alcohol, and stained with Casarett's stain.[3] Radioautography was done with Kodak NTB-3 Nuclear Track Emulsion using a "loop" method. Exposures were from 21–280 days with subsequent development in a two-stage developer.[20]

Counts of labeled spermatozoa were made on each slide and reported as cells labeled/1000 counted. Only those spermatozoa having 4 grains or more/nucleus were included. All slides were coded for counting.

X-ray Irradiation. X-ray irradiation was administered from a unit featuring twin X-ray tubes within a lead-lined box especially designed for this purpose. The patient lay prone over the unit with his testes suspended in a Plexiglas cup filled with water at scrotal temperature. The remainder of the body was fully protected by the lead shielding. Dosimetry revealed uniform delivery within ±0.5 r. at any point within the Plexiglas cup. Those subjects undergoing testicular X-ray irradiation collected seminal fluid once weekly throughout the experiment. Those given both X-ray irradiation and thymidine-H^3 followed the collection schedule given for thymidine-H^3.

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TABLE 1. *Ductular Travel Time*

Species	Median or Range of Transit Time	Authority	Method
	days		
Rat	19–22	Clubb[4]	India ink
	1–14	Jackson[15]	No supporting data
	15	MacMillan and Harrison[19]	Radiopaque
	13	Grant[9]	Trypan blue
Mouse	1–7	Jackson[15]	No supporting data
	8	Oakberg and DiMinno[21]	X-ray
	17	Sirlin and Edwards[20, 21]	Adenine 8-C^{14}
Guinea pig	14–19	Toothill and Young[24]	India ink
	22–25	Clubb[4]	India ink
Hamster	9–10	Clubb[4]	India ink
Rabbit	8–10	Swierstra and Foote[22]	Thymidine-H^3
	5–8	Hunt and Foote[14]	Thymidine-H^3
	4–6	Amann, Koefoed-Johnsen, and Levi[1]	Thymidine-H^3
	9–10	Orgebin-Crist[24]	Thymidine-H^3
	4–7	MacMillan and Harrison[19]	Radiopaque
Ram	12–15	Ortavant, Orgebin, and Singh[26]	P^{32}
	10–14	Dawson[6]	P^{32}
	13–15	Ortavant[25]	P^{32}
	7	Polovceva[28]	Epididymal ligature
	9	Phillips and McKenzie[27]	Appearance of abnormal sperm after heat
	11	Gunn[10]	India ink
Bull	8–11	Orgebin-Crist[22]	P^{32}
	8–11	Koefoed-Johnsen[16]	P^{32}
	8–10	Ortavant, Orgebin, and Singh[26]	P^{32}
	8–11	Orgebin[22]	P^{32}
Boar	9–12	Swierstra[22]	Thymidine-H^3
	14	Singh[29]	P^{32}
	13–14	Ortavant, Orgebin, and Singh[26]	P^{32}
Man	35	MacLeod and Gold[18]	Ductular depletion after high temperature
	14–21	MacLeod[17]	No supporting data
	5–12	Czerniak[5]	P^{32}
	19–23	Edwards[7]	Castration
	7+	Freund and Davis[8]	Vasectomy
	19–23	Brown[2]	Recovery time after illness

RESULTS

Thymidine-H^3. Thymidine-H^3 is incorporated into the deoxyribonucleic acid of the nuclei of germinal cells. In man, the most advanced labeled cell in the maturation process is the preleptotene spermatocyte.[11] It takes 46 days from the time of labeling of the preleptotene cell until it becomes a spermatozoon ready for release. During this time, it undergoes de-

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FIG. 1. Labeled spermatozoa found in CA II 46 days after thymidine-H^3 injection. Note residual bodies along edge of lumen, × 1200 (from Heller and Clermont[11]).

velopment as a spermatocyte, maturation and division, and further development as a spermatid. Such labeled spermatozoa about to be released are seen 46 days after labeling in Fig. 1, along with the residual bodies of companion spermatozoa that have recently left the Sertoli cell cytoplasm. This, then, becomes Day 0 in the timing of the transit of the spermatozoa through the ductular system.

X-ray Irradiation. X-ray irradiation, which applies 100 to 300 r., causes discernible damage to the spermatogonia in man within 4–6 hr.[13] None of the spermatogonia undergo mitosis to become preleptotene spermatocytes. At this X-ray dose, none of the preleptotene spermatocytes or any of the cells more advanced in their maturation are affected. They proceed through the maturation process normally in both numbers and rate. Spermatozoa are seen as the last remaining germinal cells leaving the Sertoli cell cytoplasm 46 days following irradiation. Figure 2 reveals a few remaining spermatozoa on Day 46 just emerging from the cytoplasm along with the residual bodies of the companion spermatozoa, which have just preceded them into the tubular lumen. Day 46 following irradiation thus becomes Day 0 of the transit time consumed during residence in the tubular system.

Most Rapid Transit Time. Each of the men injected with tritiated thymidine revealed labeled spermatozoa by Day 1 or in the first ejaculated specimen collected after Day 1 (Fig. 3). No subject had labeled spermatozoa in the ejaculate prior to Day 0. In 1 subject, labeled spermatozoa appeared on Day 0. Of 4 collecting on Day 1, 3 revealed labeled spermatozoa and 1 failed to do so. In the latter, the first labeled spermatozoa were observed in the next specimen collected (Day 15). Thus, we observed labeled spermatozoa by Day 1 in 4 subjects. In the fifth subject, no ejaculate was obtained between Day 1 and Day 15.

Average Transit Time. Average transit time was determined from the time that the maximal number of labeled spermato-

FIG. 2. Spermatozoa and residual bodies found in CA II 46 days after X-ray irradiation with 100 r. The other cell present within the tubule is a Sertoli cell. × 1200.

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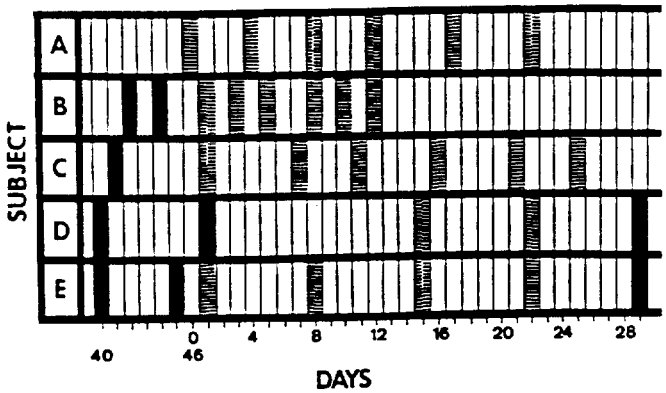

FIG. 3. The times of semen collection in 5 subjects who had received thymidine-H^3. Day 0 is Day 46 after thymidine-H^3 injection (Subjects *A*, *B*, and *C*) and thymidine-H^3 plus X-ray irradiation (Subjects *D* and *E*). The *black bars* indicate no labeled spermatozoa, *striped bars*, labeled spermatozoa, and *white bars* show the days that no collections were made. The specimens containing the largest number of labeled sperm/1000 for Subjects *A*, *B*, *C*, *D*, and *E* were collected on 12, 12, 11, 15 and 8 days respectively. Note absence of labeled spermatozoa in semen collected on Day 29 in Subjects *D* and *E*, who also received X-ray irradiation.

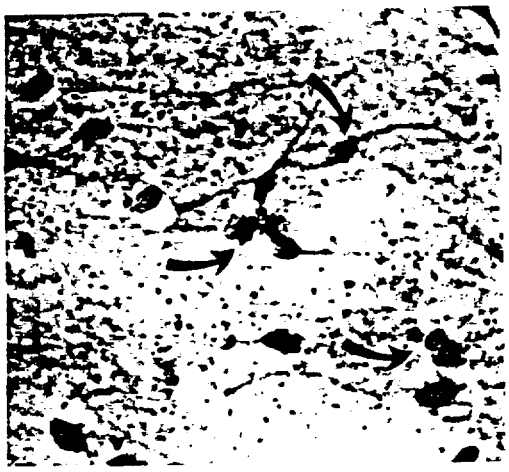

FIG. 4. Labeled spermatozoa from the ejaculate of a subject 58 days after the injection of thymidine-H^3. × 1200.

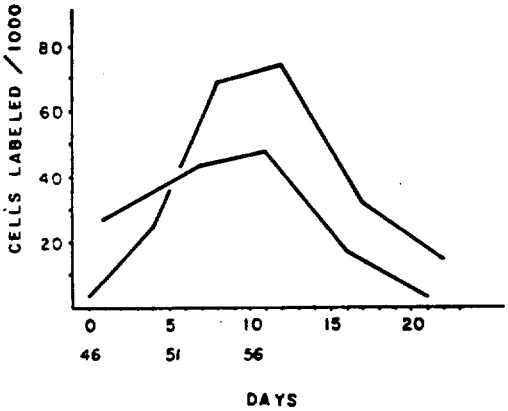

FIG. 5. The number of labeled spermatozoa/1000 in 2 subjects (*A* and *C*) injected with thymidine-H^3. Day 0 is Day 46 after thymidine-H^3 injection.

zoa (Fig. 4) appeared in the smears. The mode as well as the median were found by statistical analysis to be 12 days for each. The maximal numbers of labeled spermatozoa were found on Days 12, 12 and 11 for those receiving thymidine-H^3 alone. Figure 5 illustrates the first appearance of labeled spermatozoa and the greatest concentration of labeled spermatozoa for 2 typical subjects. The 2 subjects who received X-ray irradiation at the same time revealed the maximal number labeled at 8 and 15 days (the midpoint being 11 or 12 days), no samples having been collected between 8 and 15 days in either subject (Fig. 3, Subjects *D* and *E*).

Maximal Transit Time. The maximal transit time cannot be determined with the use of thymidine labeling alone because the spermatogonia as well as the preleptotene spermatocytes are labeled with the isotope. The resulting labeled spermatozoa continued to be excreted for a long period after the spermatozoa resulting from la-

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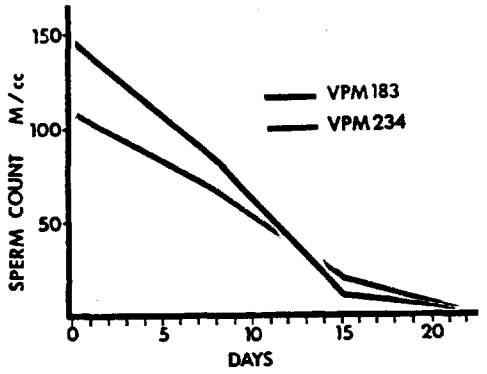

FIG. 6. The depletion of sperm count in 2 men irradiated with 100 r. (VPM 234) and 200 r (VPM 183) of X-rays. Day 0 is Day 46 after irradiation.

beled preleptotene spermatocytes were gone. Therefore, the irradiation study was necessary to establish the maximal travel time. The testicular irradiation of 9 men (including the 2 receiving thymidine) resulted in a maintenance of sperm count at normal preradiation levels for the first 46 days until Day 1 of transit time. At this time sperm count began to drop and continued to decrease until only an occasional spermatozoon was found in the ejaculate on Days 20–22 (Fig. 6). Allowances must be made for the fact that the regular day of collection after irradiation varied. Therefore, 1 subject may have collected on Days 0, 7, 14, 21, and 28, while another might collect on Days 1, 8, 15, 22, and 29. It should be noted that the last labeled spermatozoa in the seminal fluid in the 2 subjects injected with thymidine-H^3 and then irradiated were also observed in each on Day 22. Little individual variation was found. All injected with thymidine-H^3 showed labeling on Day 0 or 1 or the first day a sample was available (Fig. 3), and all irradiated with X-ray were depleted or sperm by 21 ± 1 days.

DISCUSSION

The precision of this method for determining the most rapid, the average, and the maximal transit time of spermatozoa traveling through the ductular system depends not only upon the data and their analysis as presented in the results but also upon the assumption that Day 46 after thymidine injection and/or X-ray irradiation (the time of preleptotene maturation into spermatozoa) is really Day 0 of transit time as well.

Examination of testicular biopsies (untreated controls, X-ray irradiated, and thymidine-H^3 labeled) reveals that no spermatozoa are ever found in cell associations (CA) I and III, which precede and follow CA II.[11] Thus, all spermatozoa are released from the Sertoli cell cytoplasm during CA II, which has a duration of 2.7 days.[12] It is not apparent from examining CA II at what exact time within the CA release occurs, but it is hypothesized that the majority are released on Day 46. Neither the beginning nor the end of release has been further determined.

It has been assumed in this paper, for the purpose of clarity and practicability, that the release occurs only during a 1-day period. We already know that release is not an instantaneous or simultaneous process. This is demonstrated in Figs. 1 and 2 in which spermatozoa still held within the Sertoli cell cytoplasm are associated with the residual bodies of spermatozoa already released. It may be that some spermatozoa are, in fact, released before Day 0. Since Day 46 is only 0.7 days into CA II, this cannot occur any sooner than 1 day before Day 0 and probably involves only a minority of the spermatozoa. Hence, the early release will have no bearing on the median or maximal transit time, but may cause an error in the most rapid transit time so that it becomes 1 day more than assumed in the results.

Czerniak[5] used tracer doses of radioactive phosphorus injected intravenously to try to determine the timing of human spermatogenesis and spermatozoan travel time. Unfortunately, his measurements

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were not accurate in regard to the timing of spermatogenesis. He has subsequently been proven incorrect by the thymidine-H^3 work reported by Heller and Clermont.[11] The combination of the times that he gives for spermatogenesis and transport (2 weeks) probably reflects transport time alone. Although it is known that P^{32} can label spermatogonia,[6] Czerniak did not continue his experiments long enough to observe ejaculated spermatozoa that had been labeled when they were in their spermatogonial or even spermatocyte state.

The use of tritiated thymidine as a tracer to measure transit time is a technic developed relatively recently (see Table 1). Only one study has been done utilizing X-ray irradiation to determine transit time. Oakberg and DiMinno[20] determined the average transport time in the mouse to be 8 days using this technic.

The combination of X-ray irradiation and thymidine-H^3 labeling to determine travel time has not been applied to this problem previously. Without this double determination, precision timing of either the most rapid or slowest transit of a group of spermatozoa released from the Sertoli cell cytoplasm at the same time cannot be made. Thymidine-H^3 labeling will not allow definition of the maximal transit time. Other investigators have been limited to determining only one or the other precisely. We feel that thymidine and X-ray are a particularly useful combination to bracket the travel time with precision.

SUMMARY AND CONCLUSION

The time of transit of human spermatozoa through the male ductular system, from release from the Sertoli cell cytoplasm to their appearance in the ejaculate, has been determined using two methods of dating. These two methods were mutually confirmatory. The shortest transit time was 1 day, the median transit time 12 days, and the maximal transit time 21 days.

Tritiated thymidine-labeled sperm are released from the Sertoli cell cytoplasm into the seminiferous tubule lumen 46 days after intratesticular injection of the isotope. A few labeled sperm appear in the ejaculate on Day 46. Maximal labeling is observed between Day 54 and Day 61 following isotope injection. Thus, the most rapid transit time is 1 day or less and the average transit time is 12 days, as determined by thymidine-H^3 labeling.

Applying 100 or 300 r. directly to the testes alone caused immediate spermatogonial degeneration, while allowing normal maturation of all more mature cells of the germinal series. The last of these normal cells were released from the Sertoli cell cytoplasm 46 days following irradiation. The ejaculated seminal fluid was essentially azoospermic by Day 67 after irradiation. Therefore, the maximal transit time is 21 days ± 1.

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