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THE MOVEMENT OF GOLDEN HAMSTER SPERMATOZOA BEFORE AND AFTER CAPACITATION

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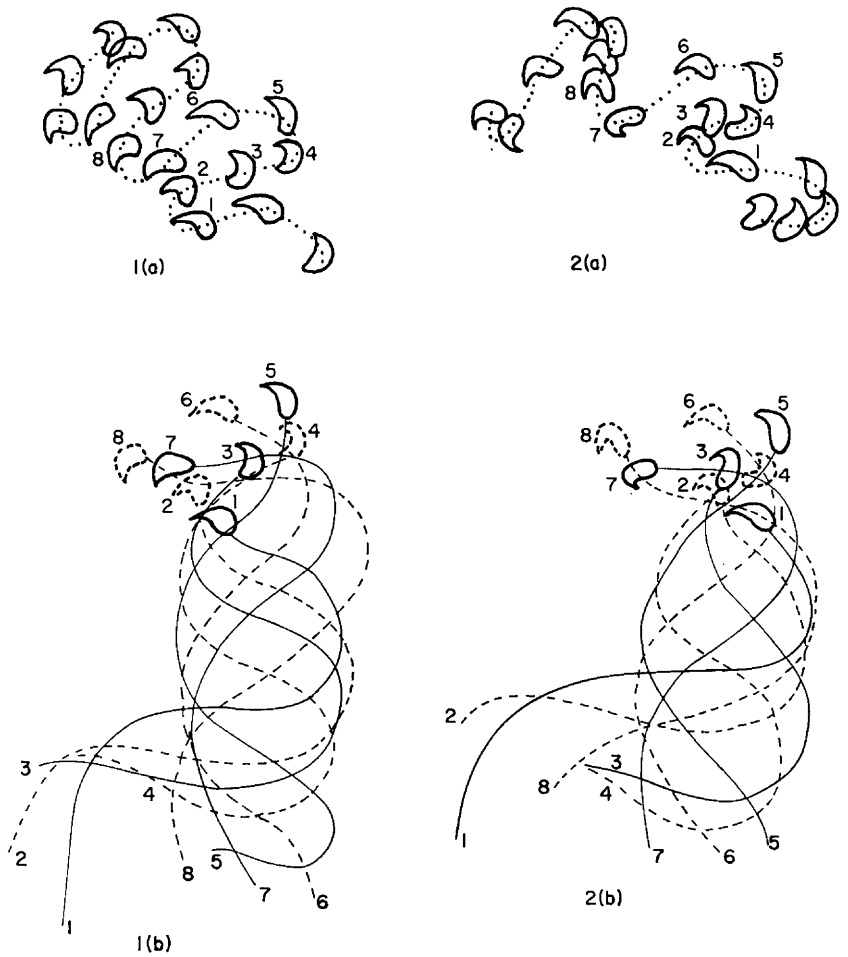
Epididymal spermatozoa of the golden hamster can be capacitated *in vitro* in the presence of oviduct fluid from the oestrous female hamster (Yanagimachi & Chang, 1964; Yanagimachi, 1966; Barros & Austin, 1967), follicular fluid from mature ovarian follicles of the hamster (Barros & Austin, 1967; Yanagimachi, 1969a), detoxified bovine follicular fluid (Gwatkin & Andersen, 1969; Yanagimachi, 1969b), and detoxified blood sera of the hamster and some other species (Yanagimachi, 1970). When incubated in media containing these biological fluids, the spermatozoa agglutinate head to head within ½ hr. About 2½ to 3 hr later, agglutinated spermatozoa disperse spontaneously and free spermatozoa show an extraordinarily active movement. In the majority (sometimes, 100%) of these spermatozoa, the acrosomal reaction is in progress or has been completed and the spermatozoa are ready to penetrate the eggs (Yanagimachi, 1969a, b, 1970). This communication reports that the movement of these capacitated spermatozoa is quite different from that of fresh epididymal (uncapacitated) spermatozoa.

Spermatozoa were squeezed out of the cauda epididymidis of fertile males and suspended in Tyrode's solution (35° to 37° C), the final concentration of the spermatozoa in the solution being about 2×10^7/ml. A sample of the sperm suspension (0·05 to 0·4 ml) was immediately mixed with an equal volume of γ-globulin-free human serum (Hyland Laboratory, Los Angeles, Calif.) and incubated at 37° C under mineral oil in either a watch glass or a plastic organ culture dish (Falcon Plastics, Los Angeles, Calif.). Between 3 and 4 hr after the start of the incubation, by which time the spermatozoa are fully capacitated (Yanagimachi, 1970), one drop of the sperm suspension was gently placed in the centre of four wax dots on a clean slide and covered with a coverslip, both of which were previously coated with silicon. Slight pressure was applied to the coverslip so that the distance between the slide and coverslip became approximately 300 μ. The drop was sealed with mineral oil to prevent evaporation. The preparation was maintained at 37° C by means of an air-curtain incubator (Sega Instruments, White Plains, New York) and examined with dark field or dark medium-phase contrast objectives. Observations were recorded cinematographically on moving film taken at a speed of fifty frames/sec. Films obtained were projected frame by frame and consecutive positions

of individual spermatozoa were drawn on paper, neglecting motionless or moribund spermatozoa. The movement of uncapacitated spermatozoa (epididymal spermatozoa examined within 10 min of being suspended in Tyrode's solution) was recorded in the same way.

Observations showed that the pattern of the movement of capacitated sperma-

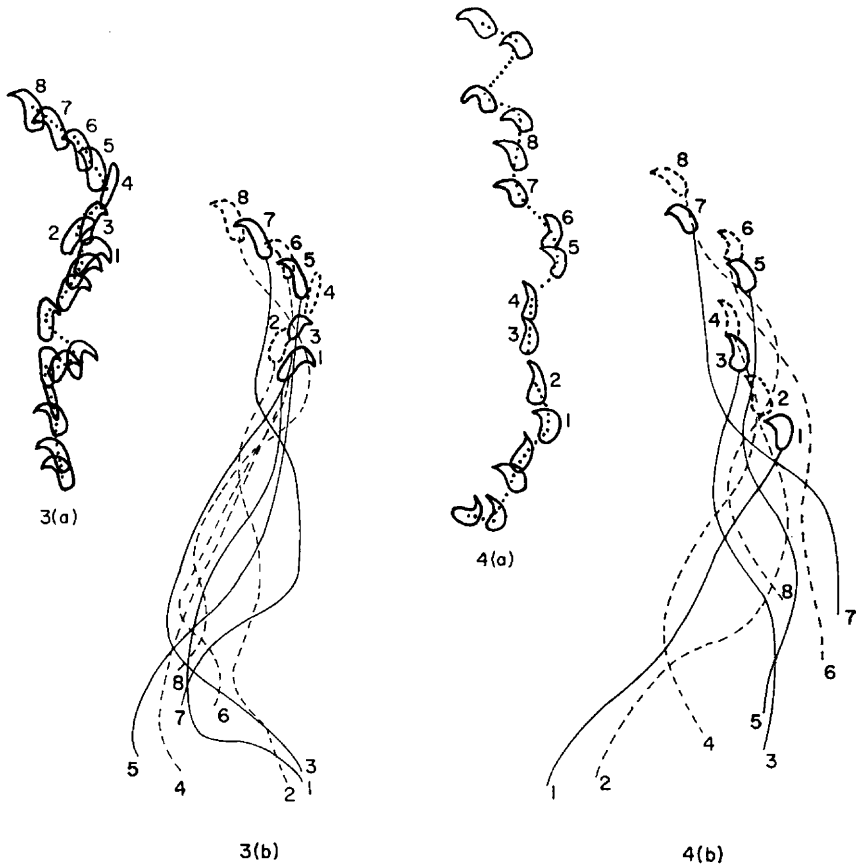


TEXT-FIGS. 1 and 2. Successive positions at 0·02-sec intervals of two capacitated spermatozoa. Figs. 1(a) and 2(a) show the paths of the heads of the spermatozoa. Figs. 1(b) and 2(b) include the tail positions at the eight selected positions.

tozoa (Text-figs. 1 and 2) was quite different from that of uncapacitated spermatozoa (Text-figs. 3 and 4). The beating amplitude of the flagellum was obviously far greater in the capacitated than in the uncapacitated spermatozoon.

Table 1 shows that the vigorous, characteristic movement of the spermatozoa ('activation' of the spermatozoa) starts between 2 and 3 hr after incubation. In order to determine whether sperm activation is due to changes in hydrogen ion concentration in the medium, Tyrode's solutions with various pH values

(7·2 to 8·4, adjusted with N/7 HCl or NaOH) were prepared and fresh epididymal spermatozoa were suspended in these solutions. Regardless of the duration of the period of incubation (5 min to 5 hr), the spermatozoa were never activated in such media, suggesting that the changes in pH values of the medium are not the prime factor responsible for sperm activation.



TEXT-FIGS. 3 and 4. Successive positions at 0·02-sec intervals of two uncapacitated spermatozoa. Figs. 3(a) and 4(a) show the paths of the heads of the spermatozoa. Figs. 3(b) and 4(b) include the tail positions at the eight selected positions.

Hamster spermatozoa showing the vigorous movement characteristic of activation can be seen through the wall of the ampullary portion of the oviduct about the time of fertilization (Yanagimachi, unpublished observations). The possible significance of sperm activation at the time of normal fertilization is to provide more propelling power to spermatozoa approaching or at the site of fertilization, or passing through the cumulus oophorus and zona pellucida. Whether such activation of the spermatozoa is peculiar to the golden hamster or a general feature of sperm capacitation in all the mammalian species remains to be determined.

TABLE 1

ACTIVATION OF HAMSTER SPERMATOZOA IN MEDIA WITH γ-GLOBULIN-FREE HUMAN SERUM

	Incubation time (hr)					
	$\frac{1}{12}$	1	2	3	$3\frac{1}{2}$	4
Series 1						
Motile sperm. (%)[a]	70 to 90	60 to 80	60 to 80	60 to 70	60 to 70	60 to 70
Activation of sperm.[b]	—	—	—	+~+++	++~++++	+++~++++
pH of inc. medium[c]	7·24 0·07	7·42 0·07	7·44 0·07	7·49 0·07	7·54 0·08	7·55 0·07
Series 2						
Motile sperm. (%)[a]	70 to 90	60 to 80	60 to 70	60 to 70	50 to 60	50 to 60
Activation of sperm.[b]	—	—	—	+~+++	++~++++	+++~++++
pH of inc. medium[c]	7·50 0·05	7·55 0·07	7·63 0·07	7·72 0·06	7·80 0·06	7·80 0·06
Series 3						
Motile sperm. (%)[a]	70 to 90	60 to 80	60 to 70	60 to 70	50 to 60	50 to 60
Activation of sperm.[b]	—	—	−~+	+~+++	++~++++	+++~++++
pH of inc. medium[c]	7·70 0·06	7·70 0·06	7·78 0·04	7·84 0·04	7·90 0·03	7·90 0·03

Series 1. A suspension (0·4 ml) of epididymal spermatozoa (about $2{\cdot}5 \times 10^7$ spermatozoa/ml of Tyrode's solution) was mixed with an equal volume of γ-globulin-free human serum and incubated at 37° C under mineral oil in a plastic organ culture dish. *Series* 2 *and* 3. Immediately after the epididymal sperm suspension was mixed with γ-globulin-free human serum, the pH of the mixture was raised to 7·50 (*Series* 2) and 7·70 (*Series* 3) by adding a small amount of N/7 NaOH.

[a] The percentage of motile spermatozoa was determined by placing the dish on the stage of a microscope and examining through dark field objectives (×4 to 10), at 37° C.

[b] (−), None of the motile spermatozoa show vigorous, characteristic (activated) movement; (+), very few; (++), less than 50%; (+++), about 50%; or (++++), over 80% of the motile spermatozoa show activated movement.

[c] Mean and S.D. *Series* 1, fifteen to twenty-six determinations; *Series* 2 and 3, twelve determinations. The pH was measured at 37° C with micro-electrode.

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