Removal of ^{3}H-T-HCl and Acrosome Loss from Spermatozoa of Different Species in the Presence of Reproductive Tract Secretions

More than 20 years ago it was established that sperm cells require a certain period of time to acquire their fertilizing ability[1,2], thereby undergoing physiological and morphological changes[3–5]. Since then, loss of the acrosome has served as one indicator for sperm capacitation and the antibiotic tetracycline-HCl, as fluorophor or labelled with tritium[6–9] has been used to assess the effect of hormonal controlled environments on sperm capacitation[9] and to show metabolic changes spermatozoa undergo as they acquire their capacitation[10].

However, VAIDYA et al.[11] established, that the fluorometric method is not likely to fulfill the function as suitable indicator for sperm capacitation. In the present experiments, tritiated tetracycline-HCl was used to assess the effect of reproductive tract protein secretions on sperm capacitating ability in vitro and a histological method was used as comparison.

Materials and methods. Epididymal spermatozoa of the hamster, and ejaculated rabbit and human spermatozoa were examined. The cauda epididymis of a fertile hamster was cut into a watch-glass, using a known volume of Tyrode's solution. The final concentration was about 9×10^7 spermatozoa/ml. Ejaculated rabbit spermatozoa were collected by means of an artificial vagina. The sperm concentration was approximately 4.7×10^6 to 7×10^7 spermatozoa/ml. Human ejaculates were obtained from volunteers and the sperm concentration was about 1.8×10^7 sperm cells/ml. Rabbit and human sperm samples were liquified at room temperature, centrifuged at approximately $2000 \times g$ and resuspended in Tyrode's solution. All samples were incubated for 10 min in ^{3}H-T-HCl (specific activity 150 mCi/mM) at a concentration of 4 µg/10^6 spermatozoa[10]. The sperm samples were recovered and centrifuged again.

Hamster reproductive tract secretions were obtained on days 1 (metoestrus) to 4 of the oestrous cycle. Uteri and oviducts were flushed with 1.0 ml sterile isotonic saline. The flushings were centrifuged at approximately $9000 \times g$ for 10 min and concentrated at 4 °C in a dialysis bag placed in Aquacide II (Calbiochem). The protein concentration in all samples was measured as described by LOWRY et al.[12]. Oestrus rabbit uteri and oviducts were flushed with 5.0 ml sterile saline and the fluids processed in the same manner as described for the hamster. Human uterine fluid was obtained from volunteers as described by DANIEL[13], and samples of day 14 and 16 during the menstrual cycle were used.

From the sperm suspensions, 200 µl were incubated in the reproductive tract secretions at 37 °C in a 5% CO_2 in air atmosphere. Tyrode's, serum and no treatment served as controls. The incubation time for human, hamster and rabbit spermatozoa was 12–13, 4–6 and 6–8 h, respectively. The radioactivity in the vials, containing 50 µl of the sperm fraction or 50 µl of the supernatant mixed with 10 ml of the scintillation fluid (8 g, Butyl PBD; 0.5 g PBBO and 5% Biosolv R/l.) was counted in a scintillation spectrophotometer (Packard Model 3375 Tri-Carb).

Another group of hamster and rabbit spermatozoa was stained with a mixture of Eosin B, Fast Green and 95% alcohol[14]. From air dried and thereafter sealed slides 600–700 spermatozoa were assessed for the acrosome reaction.

Results. Figure 1, a–c shows changes in the removal of ^{3}H-T-HCl from epididymal spermatozoa incubated in uterine and oviducal fluid proteins during the oestrous cycle. The removal rate in uterine fluid proteins is 43.0% on day 4, and is not significantly different from the removal rates on days 1, 2 and 3, where it was found to be 42.0, 42.0 and 40.0%, respectively. However, 24.0% removal of ^{3}H-T-HCl in Tyrode's solution is significantly lower ($P > 0.01$) from the protein containing fractions tested.

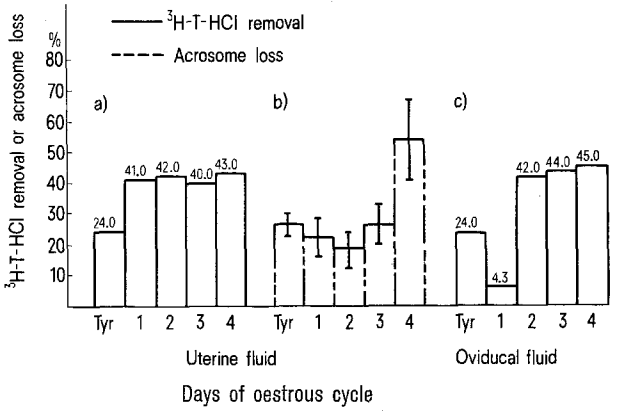

Fig. 1. a–c) Removal of ^{3}H-T-HCl and the acrosome reaction during the oestrous cycle in reproductive tract secretions of the golden hamster.

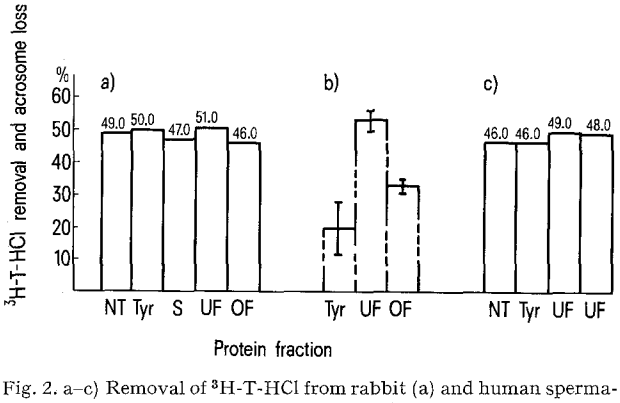

Fig. 2. a–c) Removal of ^{3}H-T-HCl from rabbit (a) and human spermatozoa (c) in vitro and the acrosome reaction (b) in rabbit spermatozoa, after incubation in oestrus genital tract secretions and human uterine fluid at the time of ovulation.

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Using the histological method, the percentage ± standard error of the acrosome loss was 22.0 ± 6.4, 18.0 ± 5.8, 27.0 ± 6.6 and 56.0 ± 13.5 on days 1, 2, 3 and 4, respectively. The acrosome loss in spermatozoa incubated in Tyrode's alone was 26.0 ± 3.8 (Figure 1b). The acrosome reaction on day 4 in the hamster is significantly higher ($P > 0.005$) from that observed on days 1, 2, 3 and Tyrode's, respectively.

In oestrus hamster oviducal fluid the removal rate is highest (45.0%) and declines to 4.3% in metoestrus. Thereafter tetracycline removal increases again to 42.0 and 44.0% on days 2 and 3, respectively.

Figure 2a shows ^{3}H-T-HCl removal in oestrus uterine fluid to be 51.0%. Oestrus rabbit oviducal fluid proteins and serum show 46.0 and 47.0% ^{3}H-T-HCl removal. The removal rate from spermatozoa in Tyrode's solution alone and those that were incubated without treatment, results in 50.0 and 49.0%. The staining technique reveals a remarkable difference between uterine and oviducal fluid in inducing the acrosome reaction. Oestrus rabbit uterine fluid induces an acrosome loss of 53.6 ± 3.1%, whereas the percentage for oviducal fluid is 33.3 ± 1.3%. Tyrode's solution shows an acrosome reaction of 20.5 ± 8.1% (Figure 2b). The removal of the tetracycline molecule from untreated and Tyrode's treated human spermatozoa is 46.0% (Figure 2c). The samples obtained close to the time of estimated ovulation slightly increase the removal rate of ^{3}H-T-HCl and show a radioactivity loss of 49.0% and 48.0% on day 14 and 16, respectively.

Discussion. Removal of the fluorescent label from spermatozoa has been stated to be an indication for the initial step in sperm capacitation[11]. The present results show that the rate of removal of the labelled tetracycline molecule depends on the different protein containing fluid samples (Figures 1 and 2). However, the level of significance increases highly, when a histological method is used (Figures 1b and 2b). This is due to a clear distinction of the presence or absence of the acrosome, whereas accurate determination of the number of molecules bound to a spermatozoon, is subject to estimation. The relatively high background in untreated spermatozoa and those incubated in Tyrode's may be explainable by ^{3}H-T-HCl molecules, that have not been bound, and permits the conclusion, that only about 50% of the labelled molecules will bind to the surface of spermatozoa in these species. The low level of tetracycline removal on day 1, may be due to changes in the uterine protein environment being unfavourable for spermatozoa at that time.

These data suggest, that the initial steps of capacitation can be achieved in oestrus uterine fluid proteins of the hamster in vitro, and are comparable to the acrosome reaction in hamster serum[15]. Also, higher tetracycline removal in oestrus rabbit uterine fluid may be due to either conditions being satisfactory in vitro, or the ^{3}H-T-HCl removal refers to a potential and not actual achievement of the fertilizing ability.

We conclude, that the loss of the acrosome is facilitated by exposure to female genital tract secretions. Tetracycline removal, however, is not a reliable indicator for sperm capacitation[16].

Zusammenfassung. Die Wirkung von Sekreten des Hamster- und Kaninchenreproduktionstrakts wurde auf die Akrosomreaktion in vitro getestet und festgestellt, dass sie am Tag 4 des Zyklus, sowie in uterinen Sekreten des Kaninchens höher ist als in Oviduktsekreten (wenn die Färbemethode mit der Entfernung von Tetrazyklinmolekülen verglichen wird).

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Deoxyguanosine, a potent Cytokinesis Inhibitor in Plant Cells

During our work on the mechanism of action of hydroxyurea[1,2], while trying to antagonize its action by addition of deoxy ribonucleosides, we have seen that deoxyguanosine induces the formation of a great number of binucleate cells, and we think it is interesting to describe this action which, as far as we know, had not yet been reported. Acetic orcein squash root meristems of *Allium sativum* L. have been prepared; 2,000 cells have been scored in each meristem and percentages of binucleate cells and mitosis given below are the mean value for 2 meristems. Late telophases devoid of phragmoplast have been set up by scoring all the telophases of 2 meristems:

Deoxyguanosine concentration (M/ml)

		Control	3.75×10^{-7}	7.5×10^{-7}	1.5×10^{-6}	2×10^{-6}	3×10^{-6}
Binucleate cells (%)	3 h	0	scarce	scarce	scarce	scarce	scarce
	6 h	0	5.3	4	4	4.2	1.8
	24 h	0	6.6	16.3	21.3	25.7	15.7
Late telophases without phragmoplast (%)	3 h	0	4	15	28.7	37.5	43.6
	6 h	0	26.8	37.8	46	65.6	73.6
	24 h	0	24.4	42.5	36.5	62	52.5
Mitotic index (%)	3 h	9.3	4.6	7.6	7.5	5.8	5.6
	6 h	11.5	5.3	12.8	6.8	7.2	7.4
	24 h	11.6	6.6	7.8	4.9	7.1	7.4