by LPS; antibody was found in thymus when BSA was given with LPS.

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A Fluorometric Method for Measurement of Sperm Capacitation. (32290)

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The fact that rabbit sperm require several hours' residence in the female reproductive tract before acquiring the capacity to fertilize ova was reported independently by two investigators in 1951(1,2). Termed capacitation, this phenomenon has since been investigated in various species, in different anatomical structures of the female, and under the influence of several sex hormones (3,4,5). All work done to date has one thing in common, capacitation is measured by the ability of sperm to fertilize ova. Cleavage in recovered ova provides an accurate measure of capacitation, but this technique consumes time and, therefore, limits the number of animals and species which can be studied. Work in *in vitro* capacitation has been hampered for lack of a simpler method to measure capacitation.

In this paper, we describe a method to identify capacitated sperm fluorometrically.

Materials and methods. This technique is based on the observation that sperm bind with a fluorescent compound, tetracycline HCl (T-HCl). Steps involved in this procedure and the reaction of mammalian sperm to it have been reported(6). Sixteen experiments were performed with rabbits over a 15-month period. General procedures for these experiments were: 1) T-HCl in Tyrode's solution (3 mg/ml) mixed with semen at 6 $\mu g/10^6$ sperm for 10 minutes; 2) seminal fluid and excess T-HCl removed by centrifugation at 2900 rpm for 10 minutes; 3) sperm resuspended in Tyrode's solution for deposition in reproductive tract of estrous and pseudopregnant does. Twenty to fifty $\times$ 10^6 T-HCl-labeled sperm were injected through a 25-gauge needle into each uterine horn (.25 ml) and in some experiments 10 $\times$ 10^6 sperm in each oviduct (.10 ml). Laparotomy was performed under sodium pentobarbital and ether anesthesia. In one experiment 10% of the T-HCl bound to sperm was labeled with tritium. Sperm radioactivity was determined according to a procedure already described (7).

Sperm recovery took place 6-7 hours after incubation *in vivo*. Each uterine horn was clamped at both ends and 1.0 ml of Tyrode's solution injected into the lumen and after manual manipulation the fluid was recovered in a syringe; oviducts were flushed with 0.25 ml of Tyrode's solution. Recovered sperm cells were checked for fluorescence, motility and number. Sperm fluorescence was determined at 320 magnification with a Leitz Ortholux microscope equipped with a high pressure mercury vapor lamp. The microscope was also fitted with a BG 12 fluorescence filter for UV-blue light and a darkfield condensor.

Experiments consisted of: 1) comparison of

fluorescence of rabbit sperm recovered from uteri of estrous and pseudopregnant rabbits; 2) same experiment as (1) except human sperm were substituted in left uterine horn of each doe; 3) measurement of T-HCl removal from sperm placed in one or both end-ligated uterine horns; 4) comparison of T-HCl removal of sperm placed in uterus, oviduct, peritoneal cavity (in dialysis bag), eye chamber, ileum, appendix and blood serum *in vitro;* 5) determine if T-HCl is removed from sperm inside a macromolecular barrier by placing a one cm dialysis bag in each uterine horn—one with labeled sperm inside bag, the other with sperm outside.

To establish a relationship between loss of T-HCl from sperm and capacitation, the accepted method for determining capacitation was done. Sperm recovered from uteri of estrous and pseudopregnant does were introduced (.02 ml) into the oviducal ampulla of recipient does. The left oviduct received sperm recovered from estrous does and the right oviduct sperm from pseudopregnant animals. Recipient does were induced to ovulate by *i.v.* injection of 75 I.U. of HCG (Upjohn's Chorionic Gonadotropin) 12 hours before sperm deposit. Approximately 24 hours after oviducal insemination, the recipient does were killed and ova flushed from oviducts with Tyrode's solution. Ova were checked for stage of cleavage, number of polar bodies, and presence of sperm.

Young mature Dutch Belted does were used throughout. Semen samples collected with an artificial vagina from proven New Zealand and Dutch Belted males were pooled and checked for normal parameters(8). Estrous does were those which accepted a vasectomized buck; 2-3 matings with sterile males induced pseudopregnancy which was confirmed at autopsy by the presence of corpora lutea. Freshly collected human semen was treated like rabbit semen except it was not pooled.

Results. T-HCl-labeled rabbit sperm placed in uteri of estrous does lost their fluorescence within the time required for capacitation. Bright fluorescence was retained on sperm recovered from uteri of pseudopregnant does with mature corpora lutea, but lost in pseudopregnant does with immature corpora lutea.

TABLE I. Capacitation of Rabbit and Human Sperm as Measured by Removal of Tetracycline HCl.

No. of does	Location	Sperm fluorescence
	Rabbit sperm	
25	Uterus (estrus)	None
2	" (day 3 pseudopregnant)	"
17	" (day 7-9 pseudopregnant)	Bright
6	Oviduct (estrus)	None
3	" (pseudopregnant)	"
	Human sperm	
3	Uterus (estrus)	None
2	" (day 8 pseudopregnant)	Weak

Labeled sperm lost their fluorescence in oviducts of both estrous and pseudopregnant females (Table I). That T-HCl molecules were indeed removed from nonfluorescent sperm was verified by labeling sperm with ^{3}H-tetracycline HCl. Results in Table II show unequivocally that T-HCl molecules were removed, and not simply quenched, from sperm placed in uteri capable of capacitating sperm. Human sperm reacted similarly to rabbit sperm when placed in uteri of estrous and pseudopregnant does except the fluorescence was not as bright on sperm recovered from pseudopregnant animals (Table I).

Labeled rabbit sperm placed in various regions of an estrous doe had on recovery these degrees of fluorescence—oviducts and uterine horns (none), eye chambers and appendix (weak), and sperm within a dialysis bag placed in peritoneal cavity (bright). Two does whose uterine horns had been ligated for 5 weeks lacked the ability to remove T-HCl from rabbit sperm; in 2 additional does, fluorescence was also retained on sperm placed in ligated ilea but removed from uterine sperm. Incubation *in vitro* of T-HCl rabbit sperm with blood serum or fluid from ligated uterine horns, for up to 22 hours, did not remove

TABLE II. Fluorescence and Radioactivity of ^{3}H-Tetracycline HCl-Labeled Rabbit Sperm Recovered from Estrous and Pseudopregnant Uteri.

Doe No.	Condition	Sperm fluorescence	DPM/10^6 sperm
1	Estrus	None	123
2	"	"	35
3	Day 8 pseudopregnant	Bright	20,595
4	" " "	"	7,966
	In vitro control	"	27,302

TABLE III. Capacitation Status of Sperm Recovered from Uteri of Estrous and Pseudopregnant Does and Inseminated in Oviducts of Recipient Does.

No of recipient oviducts	Sperm recovered from and (fluorescence)	Avg No. sperm inseminated	No. ova recovered	No. ova cleaved
	Trial I			
6	Estrous uterus (none)	31,000	16	5 (31.3%)
7	Pseudopregnant uterus (bright)	99,000	14	1 (7.1%)
	Trial II			
7	Estrous uterus (none)	47,000	20	14* (70.0%)
7	Pseudopregnant uterus (bright)	148,000	19	0† (0.0%)

* 4- and 8-cell stage of cleavage.
† Significantly ($P < .001$) less cleavage than ova fertilized with non-fluorescent sperm.

fluorescence. Finally, sperm inside a dialysis bag placed in a uterine hourn of an estrous doe did not lose any fluorescence, but sperm in the contralateral horn outside of the control dialysis bag lost all fluorescence.

A biological relationship between loss of fluorescence and capacitation was established when nonfluorescent sperm fertilized ova (Table III). Fluorescent sperm recovered from uteri of pseudopregnant does lacked the ability (noncapacitated) to fertilize ova, even though they had equal or better motility than nonfluorescent sperm. Although these ova did not cleave, numerous sperm were seen embedded in the mucin coat.

Discussion. Binding sperm with T-HCl does not alter motility, morphology(6) or, as shown with this work, fertility. Retention or removal of T-HCl from rabbit sperm correlates with published work of others on where capacitation will occur. Sperm capacitate in the oviduct and uterus of an estrous doe and oviduct of a pseudopregnant one, but not in the uterus of the same pseudopregnant doe (5). Fluorescence was absent from sperm in all cases where capacitation is known to occur, but present on sperm form pseudopregnant uteri which cannot capacitate them. While sperm can capacitate in the uterus in 6 hours, it is thought 10 hours are required in the oviduct. The time differential shown in previous work(9) may be explained by the fact that sperm were placed only in the oviduct and not placed, as in this work, simultaneously in the uterine horn. This new method for measuring capacitation was further strengthened with the results that sperm which lost fluorescence fertilized ova and those which retained fluorescence did not.

Attempts to capacitate sperm in regions other than the reproductive tract(10), in a dialysis bag(4), or in ligated uterine horn (11), have met with failure. Labeled sperm placed in the same regions or under the same conditions to test the T-HCl method of measuring capacitation did not lose fluorescence. These data support the conclusion that labeled sperm placed *in vivo* and recovered with fluorescence are not capacitated while those recovered without fluorescence are capacitated. Preliminary work with human sperm placed in does gave results which mimic rabbit sperm—additional work in humans will be necessary for conclusive results.

It has been hypothesized that capacitation involves alteration or removal of a seminal plasma substance which coats sperm(12,13). Results reported here provide indirect evidence that capacitation is indeed dependent on a coating substance. We hypothesize that removal of the sperm coat by capacitation would liberate a sperm enzyme system for the penetration and fertilization of ova. Tetracycline HCl molecules are thought to bind with this coat and ultimately be removed with the coat as sperm undergo capacitation.

Summary. A fluorometric technique for measurement of sperm capacitation is described. Rabbit sperm were labeled with tetracycline HCl (T-HCl) and placed in uteri and oviducts of estrous and pseudopregnant does and in other regions of estrous females. Sperm recovered from uteri and oviducts of estrous does and oviducts of pseudopregnant animals lost fluorescence while sperm which resided in pseudopregnant uteri retained fluorescence.

Nonfluorescent sperm from estrous uteri fertilized ova (that is, were capacitated), whereas fluorescent sperm from pseudopregnant uteri did not (noncapacitated). T-HCl-labeled sperm placed in the eye chamber, appendix, ileum or peritoneal cavity did not lose fluorescence. Presence or absence of fluorescence of labeled sperm placed in various regions and under different hormonal conditions correlates with what is known about where sperm will capacitate. Limited evidence with T-HCl-labeled human sperm compares with rabbit.

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Effect of Renin and Plasma Protein Loading on Albumin Catabolism in the Isolated Perfused Kidney.* (32291)

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It is generally accepted that in the normal kidney, plasma proteins are filtered at the glomerulus and are reabsorbed by the cells of the proximal tubules. However, little is known about how the kidney handles the reabsorbed proteins. We have shown that bilateral nephrectomy of the otherwise normal dog(1) or rat(2) does not significantly affect the rate of plasma albumin breakdown, leading to the conclusion that the normal kidney does not break down an appreciable amount of this plasma protein. Katz, Sellers and Bonorris(3) have shown that removal of the kidneys of rats made nephrotic and proteinuric by injection of aminonucleoside of puromycin or by the injection of nephrotoxic serum, results in at least a 50% reduction in the rate of plasma albumin catabolism. They have concluded that there was a correlation between the amount of proteinuria and the extent of albumin breakdown by the kidney. Finally, they used renin to produce a massive transient proteinuria in the rat, and noted a marked increase in albumin catabolism(4).

The experiments cited suggest a close relationship between proteinuria and albumin catabolism. By using the isolated perfused kidney preparation this relationship between proteinuria and albumin catabolism could be studied directly in the absence of all tissues. The rate of breakdown of I^{131} screened rabbit albumin has been studied in both the normal and the proteinuric perfused kidney. Proteinuria has been produced in the normal perfused kidney by administration of renin or by the elevation of the plasma protein concentration of the perfusion media. It was found that albumin catabolism was markedly increased in the perfused kidney during renin and protein loading proteinuria.

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