quired to prepare a CAM area free of traumatic lesions with this method.

The principal advantage of this new method is that it reduces nonspecific lesions due to trauma, thereby increasing the accuracy of titrations. The reduction of nonspecific lesions also facilitates detection of pock inducing viruses in diagnostic work which may be masked by nonspecific lesions.

Summary. A modified method of CAM inoculation is described which greatly reduces nonspecific lesions and increases the accuracy of titrations.

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Effect of Estradiol on Fibrinolytic Activity of Rat Uterus.* (23057)

OLE K. ALBRECHTSEN (Introduced by Tage Astrup)
Biological Institute, Carlsberg Foundation, Copenhagen, Denmark.

The rat uterus contains an activator of plasminogen(1). The present investigation demonstrates a correlation between concentration of the tissue activator in the rat uterus and the estrous cycle.

Methods. Concentration of the tissue activator of plasminogen in the uterus was estimated by the quantitative and selective method described previously(2). In principle the activator is extracted with potassium thiocyanate solution, precipitated at acid reaction, redissolved and the concentration estimated by fibrin plate method(3). The activator concentration is calculated in units of a standard preparation/gram fresh tissue. The estradiol dipropionate solution contained 20 μg/ml in olive oil. White rats weighing 200-300 g were used. The weight of animals, macroscopic appearance and weight of the uterus and microscopic appearance of the vaginal smear were recorded. Concentration and amount of tissue activator in 3 series of animals (total 32) are presented in Table I. Six animals were killed immediately with chloroform and tissue activator concentration in the uterus estimated as described. Twenty-six animals were ovariectomized under ether anesthesia. Eleven of these, after 4-7 weeks without further treatment, were killed with chloroform and the uterus immediately removed and investigated. Eight of the ovariectomized animals were given 1 ml estradiol dipropionate solution intramuscularly every third day (from 5-11 times). Animals were killed in early estrus some days after the last injection, after examination of the vaginal smear (Allen-Doisy). The uterus was then removed and investigated. Seven of the ovariectomized animals were given 1 ml estradiol dipropionate solution intramuscularly every third day (from 2 to 4 times). The animals were then kept until vaginal smear showed them to be in late estrus (after 17 to 56 days). They were then killed and the uterus analysed. Additional confirmative control experiments are not included in the Table.

Results. The present investigation concerns tissue activator concentration in the rat uterus during the estrous cycle in the hope of confirming experimentally previous results with samples of human endometrium (in different stages of menstrual cycle) and human

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TABLE I. Effect of Estradiol on Content of Tissue Activator of Plasminogen in the Rat Uterus.

Treatment	Uterus wt, mg	Units/g & (avg)	Units/whole uterus & (avg)
Untreated controls	427	18 (16)	8 (5)
	325	16	5
	555	6	3
		9	
		18	
		27	
Ovariectomized 4 to 7 wk (no epithelial cells in vaginal smear) (anestrus)	58	17 (18)	1 (1)
	59	12	1
	55	12	1
	53	12	1
	95	14	1
	60	21	1
	90	23	2
	80	23	2
	100	23	2
	75	10	1
	105	29	3
Ovariectomized; estradioldipropionate 5 to 11 times; killed some days after last inj. (early estrus)	280	30 (20)	8 (7)
	276	29	8
	310	30	9
	400	30	12
	370	8	3
	380	15	6
	475	10	5
	475	8	4
Ovariectomized; estradioldipropionate 2 to 4 times; killed 17 to 56 days after last inj. (late estrus)	240	43 (53)	10 (15)
	250	56	14
	375	45	17
	280	56	16
	250	96	24
	260	34	9
	320	38	12

myometrium.

Fibrinolytic activity of human *endometrium* is caused by an activator of plasminogen(4,5) similar to the activator present in other human and animal tissues(6,7). Its concentration is increased in the secretory stage and decreases with age. High concentrations are also found in certain cases of pathological uterine bleedings. This suggests that the tissue activator in the endometrium is under hormonal influence and probably indicates a correlation with the occurrence of bleedings(4). Independent of age the human *myometrium* contains large amounts of an activator of plasminogen(8).

In the rat uterus the myometrium could not be separated from the epithelial layer. However, the epithelial layer in an uterus from a castrated animal is atrophic. Therefore all activity found in these uteri must be contained in the myometrium. The average concentration of the tissue activator/gram of uterine tissue in the ovariectomized animals (18 units), in uteri from the normal controls (16 units) and in animals in early estrus (20 units) is nearly equal. Similar results (not included in the Table) were obtained with 14 normal animals (not ovariectomized) which were killed from 1 to 6 days after receiving 5 to 7 injections of 1 ml estradiol dipropionate. Concentration in the uteri from these animals varied from 9 to 33 units/gram of fresh tissue (average 18). These results indicate that tissue activator concentration in the *myometrium* is independent of castration. Total amount of tissue activator in the uterus decreases after castration because the uterus decreases in weight. These results are in accordance with those previously obtained with the human myometrium(8).

The last 7 animals were killed when the number of epithelial cells in the vaginal smear began to decrease and were replaced by leucocytes (late estrus). Concentration of the tissue activator in this group was increased and ranged from 34 to 96 units/gram (average 53 units) corresponding to total amount ranging from 9 to 24 units (average 15). Although not proved it is most probable that increase in concentration from early to late estrus is caused by increase in concentration of the tissue activator in the epithelial layer. This increase in tissue activator concentration during late estrus, corresponds therefore to increased concentration in the human endometrium in the secretory stage.

The heart, brain and lung from 4 of animals in anestrus, 3 of those in early estrus and 2 in late estrus were also investigated to estimate concentration of tissue activator during different stages of the estrous cycle. Tissue activator concentration varied considerably from animal to animal, but no correlation to the estrous cycle was found.

Discussion. Page, Glendening and Parkinson(9) suggested a fibrinolytic enzyme to be present in the rabbit uterus. Our previous investigations have shown that fibrinolytic activity in uterus of rat and rabbit is caused by an activator of plasminogen and not by a fibrinolytic enzyme proper(1). The distinction between a fibrinolytic enzyme and

an activator of plasminogen is possible by comparing effects on normal bovine fibrin (which contains plasminogen) and heated bovine fibrin (with no plasminogen) (10). Extracts from the rat and rabbit uterus digested normal bovine fibrin whereas no digestion was obtained on heated fibrin.

The "enzyme" of Page *et al.* increased in concentration during estrogen withdrawal whereas castration caused a decrease. Contrary to these findings we found no decrease in tissue activator concentration after castration of rats. The discrepancy between our results on rats and those of Page *et al.* on rabbits could be caused by differences between the animal species.

Summary. 1. Concentration of tissue activator of plasminogen in the rat uterus has been estimated in different stages of the estrous cycle. 2. An increase in concentration was recorded during late estrus, whereas castration had no influence upon the concentration. 3. These results are in accordance with those found for human endometrium and myometrium.

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Metabolism of Esters by *Streptomyces nitrificans.** (23058)

ALBERT SCHATZ, GILBERT S. TRELAWNY, VIVIAN SCHATZ, AND R. RAM MOHAN
Research Laboratory, National Agricultural College, Doylestown, Pa.

Considerable attention has been devoted to the inhibitive effects of physostigmine (eserine), prostigmine, miotine and other carbamates on acetylcholine esterase and cholinesterase(1,2), which are representative of the so-called "azolesterases"(3). Much less work has been done on effects of urethans on ordinary esterases, *i.e.* aliesterases, and on the enzymatic hydrolysis of carbamic acid esters(1,4). For example, eserine but not urethan inhibited a mycobacterial esterase that hydrolyzed tributyrin, ethyl and amyl butyrates, butyl propionate and benzoate, and ethyl valerate(5). In another study, 9 different urethans were attacked by a pig liver esterase extremely slowly if at all, but interfered with hydrolysis of methyl butyrate and tributyrin(6).

This difference in susceptibility of carbamic acid esters to pig liver esterase motivated the present investigations on metabolism of esters by *Streptomyces nitrificans,* an actinomycete which hydrolyzes(7,8), respires (9,10), nitrifies(8,9,11) and grows(7,10,12) on urethan and other carbamates. In an extensive survey of soil microflora, *S. nitrificans* was the only organism capable of developing in a mineral solution with ethyl carbamate as the source of available nitrogen, carbon, and energy(7). This unique culture was therefore assumed *a priori* to possess esterase activity not inhibited by urethan. The results presented in this paper show that urethan-grown cell material of *S. nitrificans* metabolizes a wide range of esters.

Materials and methods. Mycelium of *S. nitrificans* was grown on urethan in static cultures, harvested, washed, and homogenized as reported previously(10,12). Respiro-

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