c. Vagal reflex. In 1857 Claude Bernard showed that the bradycardia or asystole produced by stimulation of the vagus nerve could no longer be obtained after a dog had been curarized.[4] Present clinical and experimental studies have corroborated this long neglected observation.

Faradic stimulation of the vagus nerve low in the neck (10 dogs) during general anesthesia with ether or pentothal sodium caused arterial hypotension as well as bradycardia. The same stimulation after the intravenous injection of d-tubocurarine (2 units per kg) did not cause hypotension or bradycardia.

Clinically, these reflexes of circulatory depression during intrathoracic surgery due to vagal stimulation have been corrected by administration of d-tubocurarine.

Conclusion. The intravenous administration of a dose of d-tubocurarine sufficient to produce intercostal paralysis may abolish or alleviate certain autonomic reflexes that may be encountered during stimulation of autonomic pathways. Cardiocirculatory disturbances incident to stimulation of the celiac plexus, the carotid sinus, and the vagus nerve, may be so treated.

[4] Bernard, Claude, Legons Sur les Effets des Substances Toxigues et Medicamenteures (May 30, 1856), J. B. Bailtiere et Fils, Paris, 1883, 2nd edition, p. 348.

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Influence of Estradiol on Alkaline Phosphatase Activity in the Genital Tract of the Rat.

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That alkaline phosphatase may play some integral part in the physiological changes occurring in the genital tract during the normal reproductive cycle and during pregnancy was first suggested by the work of Wislocki and Dempsey.[1] More recently the influence of steroids on alkaline phosphatase distribution in the genital tract of the mouse has been studied by Atkinson and Elftman,[2] and by Jenner,[3] who found that estradiol caused increased phosphatase activity in the circular musculature and in the epithelium of the glands and of the lining of the uterus and vagina of castrated animals.

The work reported here concerns the effect of estradiol on the alkaline phosphatase activity in the genital tract of the rat with special emphasis on its activity in the endometrium of the cervix.

Methods and results. Forty-two albino rats were used in these experiments. Estradiol dissolved in sesame oil, was injected subcutaneously in doses ranging from a single injection of 0.2 μg to 7 daily injections of 0.4 μg. At autopsy, the genital tract, including the vagina, cervix, and uterus, was removed and prepared after the method of Gomori[4] for histochemical determination of alkaline phosphatase. Table I summarizes experimental observations.

Notes on table. A series of 8 female rats, given 2 to 3 weeks rest after removal of ovaries, served as controls. The least amount of phosphatase activity found in any one place in the genital tract of these animals has been assigned a value of one. The amount of enzymic activity found in other locations in the same animals and in treated animals is expressed in multiple factors of this unit.

[1] Wislocki, G. B., and Dempsey, E. W., *Am. J. Anat.*, 1945, **42**, 23.

[2] Atkinson, W. B., and Elftman, H., PROC. SOC. EXP. BIOL. AND MED., 1946, **62**, 148.

[3] Jenner, R., *Nature*, 1947, **159**, 578.

[4] Gomori, G. J., *Cell. and Comp. Physiol.*, 1941, **17**, 71.

TABLE I.
Effect of Estradiol on Phosphatase Activity.

Group	No. rats	Treatment	Uterine horns			Cervix			Vagina	
			Epithelium	Stroma of endometrium	Muscular layer of myometrium	Epithelium	Stroma	Myometrium	Epithelium	Stroma
I	8	Non-injected castrated females	1	0	0	2	0	0	2	0
II	9	Castrated adult ♀, inj. with 0.4 μg estradiol daily, 4-7 days	2	1-2	1	20	2	1	10	1
III	8	Non-inj. normal adult ♀, various stages of cycle	(Only cervix studied)			Metestrous 10 Proestrous 15	2	1	—	—
IV	10	Castrates and				5	—	—	—	—
	7	Immature animals given single inj. 0.2 to 0.4 μg estradiol	"	"	"	(after 24 hrs)				

Phosphatase activity which was localized in capillary walls is not included in this report. The cervix, in this study, is considered to begin in that part of the genital tract in which there is a sudden transition from columnar epithelium to stratified epithelium lining the lumen.[5]

Group I. Non-injected castrated females. In the endometrial epithelium of the controls, the enzyme was spread evenly throughout the cytoplasm of the cell.

Group II. Injected castrated females. In the endometrial epithelium of the uterine horns, the enzyme was localized only at the free end of the cells. In the endometrial stroma, minor amounts were found in the glandular epithelium, and further deposits were noted in the connective tissue cells. Only the circular layer of the myometrium contained any of the phosphatase.

The most marked increase in alkaline phosphatase activity was found in the cervix. On a relative basis, due to the extremely dense precipitate found in the epithelial cells, the endometrial epithelium has been given a value of 20. This precipitate was found to be distributed throughout the cytoplasm but was negligible or lacking in the nuclei.

Group III. Non-injected normal adult females. Since small amounts of estrogen probably are produced continuously by the mature rat ovary, it is not surprising that alkaline phosphatase activity was found to be high in the genital tract at all stages of the estrous cycle in the normal rat. While the distribution of enzyme was similar to that of the estradiol-injected castrate, there seemed to be a definite cyclic change in the amount of enzyme present. The least activity was noted in metestrous, the most in proestrous.

Group IV. Castrates and immature animals. As a result of a single injection of relatively small amounts of estradiol, there was a detectable increase after 24 hours in alkaline phosphatase activity in the series of both castrate and immature animals. These changes, however, were difficult to determine

[5] Burack, E., Wolfe, J. M., Lansing, W., and Wright, A. M., *Cancer Research*, 1941, **1**, 227.

quantitatively and are a poor criterion for estrogen activity.

Discussion. These studies confirm previous observations that injections of estrogen stimulated increased alkaline phosphatase activity in certain tissues associated with reproduction in rodents.[1,3-6] They also call attention to one of the heaviest deposits of active phosphatase in the rat—that seen in the stratified epithelium lining the lumen of the cervix. In addition, the appearance of enzyme activity after injection of estradiol is noted in the endometrial stroma of the uterus and upper cervix, in contrast to that reported for the mouse.[2] Such activity has, however, been reported for the endometrial stroma of the pregnant sow.[1]

The significance of the relationship of estradiol to alkaline phosphatase in normal reproductive physiology is not known. The enzyme is known to be concerned with calcium metabolism as well as that of phosphorus due to the interrelationship of the two metals in their action in the body. Estradiol is also associated with calcium, causing its removal from some bones while laying it down in others.[7] It is highly possible that it may also have a bearing on the calcium metabolism of certain reproductive tissues. If this be so, it is then conceivable that some of the actions of estradiol may be due to the adjustments of tissue calcium through the relation of the hormone with the enzyme.

Summary. Estradiol causes a spectacular increase in the alkaline phosphatase activity of the genital tract of the rat. This increase is most marked in the stratified epithelium lining the lumen of the cervix, but it is also apparent in the connective tissue cells of the stroma of uterus and uterine cervix and in the circular musculature of the upper part of the genital tract.

In contrast to the stratified epithelium of the cervix and of the vagina, the columnar epithelium of the uterus shows very little phosphatase activity in the presence or absence of estradiol stimulation.

6 Talmage, R. V., *Anat. Rec.*, 1947, **99**, 15.

7 Gardner, W. U., and Pfeiffer, C. A., *Physiol. Rev.*, 1943, **23**, 139.

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Uterine Circulation Time in the Pregnant Primate, With the Uterus and Abdomen Intact.

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The determination of uterine circulation rate in the pregnant primate is of considerable importance. Recent investigation has led many authorities to believe that there is a relationship between abnormal uterine circulation and the late toxemic syndrome. The abnormality of circulation is considered to be uterine ischemia but this has never been demonstrated. Page[1] in discussing this theory of toxemia has stated that it is unfortunate that no one has been able to determine uterine circulation times throughout pregnancy. Moreover, Smith and Smith[2] consider that the hormonal changes they find in pre-eclampsia are the result of placental ischemia which is attributable, in turn, to uterine ischemia. The basic cause of this ischemia, which apparently develops late in pregnancy, is another matter. Page[1] lists a group of conditions such as hydromnios, multiple pregnancy, diabetes, etc., in which he feels uterine ischemia is most likely to occur and, indeed, toxemia is frequently found in such conditions

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1 Page, E. W., *Obs. and Gyn. Survey*, 1948, **3**, 517.

2 Smith, O. W., and Smith, G., *Am. J. Obs. and Gyn.*, 1949, **56**, 821.