cellular proliferation, or d) a combination of these processes. However, the observed AIB distribution ratios paralleled an accepted physiologic response (increased parathyroid gland size) to parathyroid stimulus. Therefore it is concluded that the magnitude of active transport of AIB is an additional parameter of parathyroid function which should allow determination of relative changes in parathyroid activity under various physiologic conditions.

Summary. Active transport of α-aminoisobutyric acid by rat parathyroid tissue was much greater than by thyroid tissue under control conditions. Calcium deprivation doubled the parathyroid weight and tripled the α-aminoisobutyric acid transport into parathyroid cells, but had no effect on thyroid cells. The magnitude of this transport phenomenon is apparently a measure of physiologic activity of the parathyroid glands.

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Triphenyltetrazolium Reduction by Uterine Tissue of Rats.*† (29333)

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The reduction of 2-3-5 triphenyltetrazolium chloride (TTC) to formazan by the vaginal lining of the mouse has been used as a biological assay method for estrogens(1). Martin found that rate of reduction was proportional to degree of estrogenic stimulation. Progesterone, testosterone, and cortisol did not influence the reaction. Although estrogens are necessary for reproduction, excessive amounts can be harmful(2). Reproductive efficiency varies between females and within females from time to time. An index of the degree of estrogenic stimulation attained by the genitalia at time of breeding or during different stages of pregnancy should be useful in studying the reasons for some of these differences and lead to an increased understanding of the estrogen requirement for high reproductive efficiency. TTC reduction may serve as such an index.

The experiment reported here is an attempt to adapt the TTC reduction of uterine segments as an *in vitro* method of evaluating degree of estrogenic stimulation that the genitalia has attained. Changes in TTC reduction brought about in the uterus of spayed females and during different stages of the estrous cycle in intact females are reported.

Methods. A line-bred stock of female rats of Wistar origin 70-100 days of age was used. Immediately after killing, segments of the uterus (35-45-mg wet weight) were removed, blotted free of excess fluid and weighed to the nearest 0.1 mg. The segments were sliced

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into 4 or 5 pieces to expose surface for the reaction with TTC. (Homogenization of the uterine tissue before incubation with TTC decreased the amount of formazan produced compared to the sliced tissue.) The incubating medium was made up of 1 ml 0.1 M dibasic sodium phosphate; 2 ml of 0.05% water solution of TTC. This medium had a pH of 8.6 before incubation and 8.5 after one hour incubation. A number of buffered solutions were tried,‡ but the mixture just described gave a maximum rate of reduction when compared to the other solutions tested and with less formation of a cloudy precipitate. Incubation of the sliced uterine segments was carried out for one hour in a graduated 10 ml test tube placed in a constant temperature water bath at 42°C. One hour of incubation gave TTC reduction values 1.7 times the values of tissues incubated for one-half hour. After one hour of incubation the tubes were removed from the water bath and the reaction stopped by adding 3 ml acetone. To ensure extraction of all the formazan, the tissue while in the incubating medium was ground in a 10 ml capacity tissue grinder. Some acetone evaporated during this process and all volumes were brought to 5.5 ml. The ground tissue with the acetone solution of formazan was mixed by several shakings and centrifuged at 1500 rpm for 20 minutes. The supernatant formazan-acetone-water solution was decanted and color extinction read in a Coleman Junior Spectrophotometer at 475 mu. A tissue blank was used for the 100 transmission setting. A standard curve for formazan was prepared, using a formazan preparation obtained from Mann Research Laboratory. From the standard curve, the μg formazan per milligram of wet tissue weight was obtained. Per cent of dry matter of the uterine tissue was determined by heating the segment one hour at 105°C. Formazan produced by TTC reduction was calculated also as μg per mg dry weight of uterine tissue. The error of the method when duplicate determinations were made was of the order of 7.5%.

To relate degree of estrogenic stimulation of the rat uterus to TTC reduction rate, female rats 70 days of age were spayed and used after 2 weeks or when daily smears showed only scattered leucocytes with some cellular debris continuously. Estradiol in olive oil was injected subcutaneously daily for 2 days in amounts of 0.125, 0.250, 0.500, and 1.000 μg. Litter mates were assigned to different dosage levels. The character of the TTC reduction response to the log-dose of estradiol was determined according to a method described by Emmens(4) and Snedecor(5).

Virgin female rats 80-100 days of age were used to determine the TTC reduction rate of uterine tissue during stages of the estrous cycle. Vaginal smears were taken daily from this group and litter mates assigned for each of the 4 stages of the cycle. The stages for which the determinations were made were: (1) estrus—cornified cells, (2) one-day post-estrus—leucocytes with few epithelial cells, (3) two days post-estrus—leucocytes with a few epithelial cells, and (4) proestrus—predominantly nucleated epithelial cells. Data were analyzed statistically following methods described in Snedecor(5).

Results. Uterine tissue from spayed females receiving no estradoil showed either no measurable amount or a low level of TTC reduction. The mean value for these females was 0.156 μg formazan per mg wet tissue and 1.02 μg per mg dry tissue (Table I). One ug estradiol increased the TTC reduction rate to 1.037 up formazan per mg wet tissue and 5.59 ug formazan per mg dry tissue. Statistical analysis of the data summarized in Table I showed that the reduction of TTC

TABLE I. Dose-Response Relationship Between Estradiol and *in vitro* TTC Reduction of Uterine Tissue Segments.

Dosage, μg/day	No. animals	μg formazan produced/mg tissue	
		Wet wt basis	Dry matter basis
0	12	.156 ± .055	1.02 ± .28
.125	12	.550 ± .050	3.03 ± .24
.250	12	.758 ± .068	4.21 ± .35
.500	12	.879 ± .080	4.86 ± .37
1.000	12	1.037 ± .065	5.59 ± .31

‡ A citrate buffer pH 6.2, succinate buffer pH 7.4, Tris-maleate buffer pH 8.0, glycine-NaOH buffer pH 9.8, and a phosphate buffer pH 8.0 prepared according to Gomore(3).

TABLE II. Mean TTC Reduction of Uterine Tissue from Female Rats at Various Stages of Estrous Cycle (20 Females with Duplicate Determination for Each of 4 Stages).

Stage of cycle	Estrus	Day 1 post-estrus	Day 2 post-estrus	Pro-estrus
μg formazan per mg uterine tissue				
Wet	.99 ± .021	.83 ± .040	.88 ± .045	1.14 ± .035
Dry	6.00 ± .20	4.83 ± .26	5.29 ± .29	6.96 ± .24

by uterine tissue was linear to the log-dose of estradiol administered. Variance among litter mates was statistically significant.

In normal cycling female rats, the level of TTC reduction by uterine tissue was lowest on day 1 post-estrus; during pro-estrus it was highest (Table II). When day 1 post-estrus level is taken as 100%, day 2 post-estrus averages 6% higher, pro-estrus 37% higher and during estrus about 20% higher. These data indicate that levels of estrogen increase at pro-estrus, decline during estrus, and reach a low level during diestrus. Analysis of these data shows that differences in TTC reduction during pro-estrus and estrus are significantly different from the levels during diestrus in the rat. Also differences in response between litters is significant. Comparing the mean values of TTC reduction obtained during the estrous cycle to the values obtained with spayed females injected with estradiol, it appears that at one-day post-estrus, the uterus is under the influence of an amount of estrogen equivalent to 0.40 μg estradiol administered daily. During pro-estrus the amount is approximately 1.6 μg estradiol daily. It is probable that the rat ovary secretes estradiol(6) and thus comparisons of TTC reduction levels in normal and estradiol treated spayed females may be valid.

Apparently the reduction rate of TTC by the uterine tissue of the rat represents a metabolic status that is primarily associated with degree of estrogenic stimulation. Thus it may serve as an estrogen status index. An estrogen status index may be of value in determining the optimal levels of estrogenic stimulation that are necessary for maximal reproductive performance.

Summary. An *in vitro* use of TTC reduction by uterine tissue in the female rat has been studied. Estrogen administration to spayed females results in levels of TTC reduction proportionate to the log-dose of estradiol. The level of TTC reduction fluctuates during the estrous cycle, being highest at pro-estrus and lowest on day 1 post-estrus.

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Biopotency of *l*-α-Tocopheryl Acetate for the Rat.* (29334)

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The recent availability(1,2) of *l*-α-tocopherol† (2 *l*, 4′ *d*, 8′ *d*-α-tocopherol /Distillation Products Industries/) has made possible the direct determination of the biopotency of this synthetic epimer of vitamin E. A report based on fetal resorption(3) indicated that the *l*

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