tend to relieve such inhibition. It would appear improbable, then, for the decrease of hydroxylase activity seen under conditions of experimental phenylketonuria, where excess substrate is administered, to be mainly accounted for in terms of catabolite inhibitions. The data of Udenfriend and Cooper(3) show a decrease of approximately 30% of hydroxylase activity when substrate in 10-fold excess of the optimum is added. This indicates that substrate choking of the hydroxylase's active site is of the same order of magnitude as the relatively weak inhibition shown herein for most catabolites. The value of Km reported for phenylalanine by these authors is in good agreement with that found in the present investigation. Also in phenylketonuria, whether of genetic or dietary origin, the fault is not with insufficient cofactor, but apparently enzyme *per se*. The greater inhibition observed with partially purified enzyme alone is somewhat overcome in those instances *in vitro* where protective cofactor is present, *i.e.*, crude hydroxylase and partially purified hydroxylase plus added cofactor. These latter are more representative in the state *in vivo*.

Summary. The activity of phenylalanine hydroxylase from rat liver can be inhibited *in vitro* by several organic acids including a few catabolites of phenylalanine, *e.g.*, phenylpyruvate and homogentisate. However, the concentrations of such compounds necessary to produce significant inhibition of a generally competitive nature is far in excess of physiological amounts, and the effects can be minimized when an adequate amount of pteridine cofactor is present. Thus, the lowered activity of hydroxylase measured in experimental phenylketonuria may be attributed to a real suppression of enzyme synthesis, rather than inhibition by catabolites.

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Effect of Estradiol Upon Oxytetracycline Utilization as Related to Blood Calcium.* (29777)

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Estradiol, an estrogenic hormone, has been shown to increase blood calcium in young chicks(1). It was later found that injecting chicks with estradiol increased the oxytetracycline (OTC) blood serum level(2). Elevated OTC blood serum levels were obtained in laying hens which were injected with 3 mg of estradiol for 3 consecutive days prior to taking blood(3). Hens which received estradiol injections were also found to have higher blood calcium levels than non-injected hens.

The purpose of this work was to examine further the possible activity of estradiol as an antibiotic potentiator and correlate its

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activity, if any, to blood calcium levels in the laying hen.

Experimental procedure and results. This study was made in a series of 4 experiments utilizing caged laying hens that were in 70% production. These hens were fed a commercial type 17% protein diet prior to and through the experimental periods. Three cc of blood were taken by heart puncture in each experiment and serum OTC levels were determined(4). Blood serum calcium levels were determined by procedures outlined by Hawk *et al*(5). Estradiol was administered by subcutaneous injection using a corn oil carrier.

Statements of probability and correlations are based on procedures outlined by Snedecor(6).

Experiment 1. This experiment was conducted to determine the effect of simultaneous injection of estradiol and OTC on the serum level of OTC in laying hens. Four groups of 10 hens each were injected with OTC 6 hours prior to the first bleeding time. Two groups received 6 and 12 mg estradiol, respectively, at the same time as the OTC injection. The third group was injected with 6 mg of estradiol 12 hours prior to the first bleeding time and another 6 mg 24 hours later. The fourth group did not receive estradiol and served as controls. Bleeding times were 6, 12, 24 and 48 hours following OTC injection.

Regardless of level or time, the injections of estradiol did not significantly affect the serum OTC levels. In seeking an explanation for this lack of serum OTC increase, another experiment was undertaken to study the response of serum calcium to estradiol injection.

Experiment 2. Fifteen laying hens were injected with 12 mg of estradiol and 15 non-injected hens were used as controls. Blood was taken at 3, 6, 9, 12, 15, 28, 34, and 58 hours after estradiol injections and serum calcium determinations were made.

There were no responses in serum calcium levels until approximately 28 hours after injection with estradiol (Fig. 1). After this time the serum calcium level of the estradiol-injected hens increased significantly. These

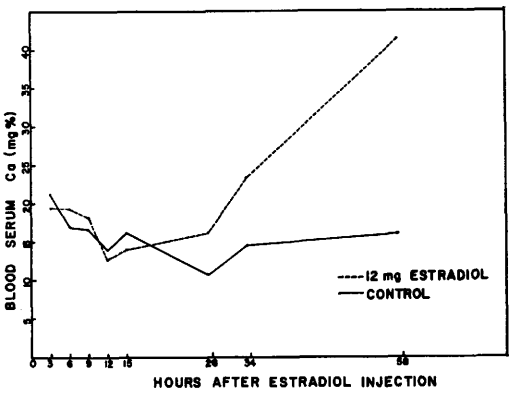

FIG. 1. Serum Ca levels following estradiol injection.

results offered a possible explanation for the lack of increased serum OTC levels with simultaneous injections of estradiol and OTC in Experiment 1, since estradiol did not increase serum calcium until after the majority of the OTC was cleared from the blood.

Experiment 3. Results of the 2 previous tests indicated that the lack of response to simultaneous injection of estradiol and OTC was due to failure of the estradiol to increase blood calcium during the period of time that high levels of OTC were present in the blood. To verify this 36 hens were injected with 12 mg and 36 received no estradiol. These injections were given simultaneously with 25 mg of OTC.

These results (Table I) confirmed the findings in Exp. 1 and 2 that simultaneous injections of estradiol and OTC gave no serum antibiotic increase and that serum calcium levels were not increased by estradiol injections until after 28 hours. Therefore, if estradiol acts indirectly on blood OTC levels through its effect on blood calcium, it would be necessary to give the estradiol injections several hours prior to OTC injections.

Experiment 4. It was postulated from the previous data that injections of estradiol 45 hours before injections of OTC would result in an increased serum OTC level. To check this hypothesis 20 hens were injected with 12 mg of estradiol and 20 non-injected hens were used for controls. Forty-five hours after the estradiol injections the hens were injected with 25 mg of OTC. Three hours after the OTC injections blood samples were

TABLE I. Serum Ca and OTC Levels of Hens Simultaneously Injected with Estradiol and OTC (Exp 2).

Estradiol, mg	OTC	Time after estradiol injection 6 hr	12 hr	24 hr	48 hr
		μg of OTC per ml of serum			
12	+*	3.29 (32.1)†	2.30 (29.0)	.86 (27.8)	.15 (30.7)
12	—	0 (29.5)	0 (26.8)	0 (29.5)	0 (36.4)
0	+	3.47 (29.4)	2.64 (29.7)	1.07 (26.2)	.22 (30.2)
0	—	0 (28.5)	0 (28.5)	0 (26.8)	0 (24.7)

* + Indicates 25 mg injections of OTC.
† Data in parentheses indicate mg % of serum calcium.

taken and calcium and OTC serum levels were determined.

Injection of estradiol 45 hours prior to OTC injections resulted in significantly elevated OTC serum levels (Table II). A positive correlation of 0.82 ($P<.01$) was obtained between OTC and serum calcium levels. This demonstrated that serum calcium levels were closely related to serum OTC levels.

TABLE II. Serum Ca and OTC Levels of Hens Injected with OTC 45 Hours After Estradiol Injections (Exp 4).

Estradiol, mg	Blood levels OTC, μg/ml	Ca, mg %
12	2.23*	40.2
0	1.74	27.7

* $P<.01$

Discussion. It is postulated that the action of estradiol is through an OTC-Calcium complex in the blood resulting in elevated OTC levels. This would explain the lack of response when estradiol and OTC were injected simultaneously. In this instance the OTC had been eliminated from the blood (Table I) before the blood calcium was elevated. It is suggested that when estradiol is used for OTC potentiation it is necessary that it be injected at least 45 hours before OTC administration.

Summary. Estradiol injections in layer hens caused increased serum calcium 28 hours after injection, but had no effect on serum OTC levels when OTC was injected simultaneously with estradiol. Estradiol administered 45 hours prior to OTC injections produced a significant increase in serum OTC levels corresponding to the increase in serum calcium levels. A positive correlation of 0.82 between serum calcium and OTC indicated that increased serum calcium was involved in OTC potentiation.

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