trolysis in a cell provided with an aluminum electrode and a platinum spiral, but their results could not be substantiated.

We have successfully applied the rotation aluminum electrode to the amperometric titration of fluoride in aqueous buffers with aluminum nitrate; no good end-point was observed with thorium nitrate as reagent. Hydroxyl ions are the only other ions which anodically depolarize the electrode. They yield anodic limiting currents proportional to concentration. Use of this can be made in the amperometric titration of acids with a strong base. At a *p*H greater than 8 the electrode acts as a *p*OH (*p*H) electrode, but not in acid medium. In acid medium it acts like a *p*F electrode, the potential being somewhat dependent on *p*H in the presence of fluoride. Details concerning this interesting electrode will be reported elsewhere.

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INHIBITION OF PYRIDOXAL PHOSPHATE-DEPENDENT ENZYMES BY THE SULFATE ESTERS OF ESTRADIOL, ESTRONE AND DIETHYLSTILBESTROL[1]

Sir:

Systematic studies of kynurenine transaminase in this laboratory have revealed that it is inhibited by extremely low concentrations of estradiol disulfate and diethylstilbestrol disulfate(Table I). Estrone sulfate and pregnanediol glucuronide also inhibited but only at considerably higher levels. Several bile acids inhibited slightly at high concentrations (10^{-3} to 10^{-4} *M*), probably by denaturation. Unconjugated steroids and related compounds, *e.g.*, estradiol, estrone, diethylstilbestrol, progesterone, pregnanediol, methyl bisdehydrodoisynolic acid and methallenestril were without effect even at saturation levels.

The degree of inhibition varied with the concentration of pyridoxal phosphate, indicating that the estrogen sulfates may compete with the coenzyme for the apotransaminase. Reversibility was shown also by complete disappearance of the inhibitory effect during dialysis. The possibility, suggested here, that other pyridoxal phosphate-dependent enzymes may be inhibited led to tests of the effects of the estrogen sulfates on the activity of muscle phosphorylase (a crystalline preparation) and to the discovery that they inhibit this enzyme also and at very low levels (Table I).

Kynurenine transaminase was purified and assayed as described earlier.[2] Twice crystallized muscle phosphorylase *a* was dissolved in cysteine-glycerophosphate buffer, *p*H 6.8. Aliquots were preincubated at 37° for 30 minutes with the various inhibitors or an equal volume of water, then assayed for activity by the method of Illingworth and Cori.[3]

TABLE I

INHIBITION OF KYNURENINE TRANSAMINASE AND MUSCLE PHOSPHORYLASE BY STEROID CONJUGATES

Inhibitor	Inhibitor concn., *M*	Per cent. inhibition: Kynurenine transaminase	Per cent. inhibition: Muscle phosphorylase *a*
Estradiol disulfate	2.5×10^{-5}	97	48
	5×10^{-6}	50	19
	5×10^{-7}	9	..
Diethylstilbestrol disulfate	2.5×10^{-5}	99	76
	5×10^{-6}	60	28
	5×10^{-7}	11	..
Estrone sulfate	1×10^{-4}	23	15
	2.5×10^{-5}	9	2
	5×10^{-6}	0	0
Pregnanediol glucuronide	1×10^{-4}	7	..
	2.5×10^{-5}	0	..

Muscle phosphorylase contains tightly-bound pyridoxal phosphate which can be removed to yield preparations that are enzymatically active only in the presence of added pyridoxal phosphate.[4] In the present studies, the reactivation of such preparations with pyridoxal phosphate was inhibited by low concentrations of the estrogen sulfate. The degree of inhibition again was dependent on the concentration of added pyridoxal phosphate.

We believe this to be a unique case of a highly-sensitive *in vitro* estrogen–enzyme interaction in which both diethylstilbestrol and the natural estrogens can participate. From a speculative viewpoint, many of the *in vivo* effects of the estrogens correspond rather well to what we might expect from the demonstrated effects on pyridoxal phosphate-dependent enzymes. As an example, the increased storage of glycogen and protein that occurs in some species under the influence of the estrogens may be regulated through the inhibition of phosphorylase and transaminase enzymes. Experiments designed to test further the relationship of these *in vitro* effects to the physiological actions of the steroid hormones are in progress.

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STEREOCHEMISTRY OF THE DECOMPOSITION OF OPTICALLY ACTIVE N-NITROSO AND N-AMINO α,α'-DIMETHYLIDIBENZYLAMINE

Sir:

Recently it has been shown that the reduction of *cis* or *trans* N-nitroso-2,6-diphenylpiperidine[1] and oxidation of the corresponding N-amino compounds[2] proceeded to give either the *cis* or *trans* diphenylcyclopentane and some olefin. The cyclic compounds were formed with retention of configuration. In order to determine whether the same retention of configuration was operative in linear homologs, optically pure N-nitroso-α,α'-

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(3) B. Illingworth, and G. T. Cori, "Biochemical Preparations," Vol. 3, John Wiley and Sons, New York, N. Y., 1953, p. 1.

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(2) C. G. Overberger, J. G. Lombardino and R. G. Hiskey, *ibid.*, **79**, 6430 (1957).