in respiratory volume, but no changes in respiratory rate, blood pressure, or heart rate. Similar effects were observed with the same doses of the natural vitamin. Neither substance produced congestion or necrosis in the rabbit's ear by subcutaneous injection in the dosage of 1 mg. dissolved in 0.1 cc. of saline. The minimal lethal dose (M.L.D.) in guinea pigs is the same with both the natural and the synthetic products, by intravenous injection, as shown in Table II. The vitamin solution employed for the toxicological study was 2% in each case, and the weight of the animals varied from 210 to 265 gm. Clonic convulsions occurred after doses of 150 mg. per kg., or more, had been administered. Those animals which survived the sublethal doses recovered completely within 1½ and 5 minutes, apparently without any after effects.

TABLE II.
Toxicity of Natural and Synthetic Vitamin B_1 Hydrochloride.

Vitamin B_1.HCl	Dose mg. per kg.	No. of Pigs Died Over No. Used	M. L. D. mg. per kg.
Synthetic	300	1/1	180
	200	1/1	
	180	2/3	
	160	0/2	
	150	0/1	
	100	0/1	
Natural	180	2/2	180
	160	1/3	

Summary. Results obtained in animals indicate that the natural crystalline vitamin B_1 and the synthetic product are identical.

9271 P

Increased Estrogenic Potency of Human Urine after Hydrogenation.

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A method described by us[1] for the hydrolysis and extraction of urine for estrogens involves boiling with 15 vol. % of HCl for 10

[1] Smith, G. V., and Smith, O. W., *Am. J. Physiol.*, 1935, **112**, 340.

minutes, followed by continuous extraction with benzene (Benzol, Merck's Reagent) for 24 hours. It has been shown that the benzene extraction recovers all estrogens present after hydrolysis. It has also been demonstrated that this short hydrolysis with a high concentration of acid gives values which check with those obtained after the Cohen and Marrian[2] technique,* and does not affect the potency of estrone or estriol added to urine of known "total" estrogenic content.

Although the application of this method of quantitation has been found to yield consistent physiological curves of estrogen excretion,[3, 4] there is no proof that all bound estrogens are freed by the acid hydrolysis or that destruction of combined estrogens may not occur. With the idea of possibly increasing hydrolysis as well as preventing any destruction of combined estrogens through oxidation,

TABLE I.
Comparative Estrogenic Potency of Human Urines Hydrolyzed With and Without Addition of Zinc.

Name	Date	Clinical Notes	Total Estrin T_o rat units–24 hr.	Total Estrin T_{zn} rat units–24 hr.	T_{zn}/T_o
Y.B.	8/ 1/36	5 mo. pregnant	*12000	*28000	2.33
"	9/23/36	6½ " "	*28000	*75000	2.67
"	11/26/36	8½ " "	*64000	*130000	2.05
L.	1/30/37	3 " "	1800	7250	4.0
	Results on Specimens Collected Throughout a Menstrual Cycle.				
		Menstruating days of cycle			
H.S.	1/12-14	1-3	20	134	6.6
	1/14-16	3-5	33	134	4.0
	1/16-18	5-7	55	110	2.0
	1/18-20	7-9	100	220	2.2
	1/20-22	9-11	270	890	3.3
	1/22-24	11-13	200	670	3.3
	1/24-26	13-15	100	333	3.3
	1/26-28	15-17	100	333	3.3
	1/28-30	17-19	134	450	3.3
	1/30-2/1	19-21	134	450	3.3
	2/1-3	21-23	134	450	3.3
	2/3-5	23-25	55	134	2.5
	2/5-7	1-3	33	220	6.6
	2/7-8	3-4	20	134	6.6

* Assays made on unextracted specimens diluted with water. All other specimens were extracted with benzene and assayed in olive oil solution.

[2] Cohen, S. L., and Marrian, G. F., *Biochem. J.*, 1934, **28**, 603.

* The Cohen and Marrian hydrolyses were performed in the Biological Laboratories of Harvard University through the courtesy of Dr. Gregory Pincus. Assays, by the Allen-Doisy method, were made in this laboratory.

[3] Smith, G. V., and Smith, O. W., *New Eng. J. Med.*, 1936, **215**, 908.

[4] Smith, G. V., and Smith, O. W., *Am. J. Obstet. and Gynec.*, 1937, **33**, 365.

zinc (Zinc Dust, Merck's Reagent) has been added to urines prior to the acid treatment. A marked rise in the estrogenic potency of urines from both pregnant and non-pregnant women has resulted (Table I).

"T_0" signifies the addition of 15 vol. % HCl and 10-minute boiling under a reflux condenser; T_{zn} the addition of 15 vol. % HCl and 4% Zn and 3-hour boiling under a reflux. Four percent zinc constitutes an excess with 15 vol. % HCl. Maximum increase in potency occurs after 2 hours and is not changed after 5 hours of boiling with acid and zinc. Evolution of hydrogen continues both during boiling and extraction. The titratable acidity is reduced from around 1.5 N to around 1.3 N in 3 hours of boiling. It is to be noted that hydrogenation does not affect a uniform increase in potency, since the ratios of T_{zn} to T_0, when assayed in olive oil, vary between 2.0 and 6.6.

The processes involved in this augmentation of urinary estrogen by zinc hydrolysis have not as yet been identified. The results thus far, however, are in accord with the hypothesis that the explanation lies in increased hydrolysis, and also conversion of estrone (but not estriol) into a reduced form of greater estrogenic activity, possibly dihydro-estrin (dihydro-theelin, estradiol). It is apparent for the present that hydrolysis with the addition of zinc may not be employed in physiological studies of estrogen excretion, although there is some indication that the ratio of T_{zn} to T_0 may provide an index of the relative estrone content of specimens analyzed.

9272 P

Changes of Hydrogen Ion Concentration of the Cerebral Cortex.

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Using a glass electrode (of the MacInnes type) with an active area of less than 0.5 mm.^{2}, in conjunction with the microvoltmeter recently described by Burr, Lane and Nims (1936), having a grid-leak of 100 megohms, it is possible to measure the hydrogen ion concentration in physico-chemical systems to $\pm$.002 pH. The same apparatus is applicable to biological systems *in vivo*. In the present instance it was used for a study of the pH of the cerebral cortex.