composed of 12 % ethylene glycol–adipate polyester on Chromosorb-W coupled to a scintillation counter[4].

The following findings indicate that the enzyme responsible for norsynephrin formation is dopamine β-oxidase. First, adrenal-medulla particles prepared according to LEVIN *et al.*[5] were found to have similar requirements for norsynephrin formation as for norepinephrine formation. Ascorbate, fumarate and adenosine triphosphate all stimulated and KCN inhibited the hydroxylation. Secondly, dopamine was found to inhibit the oxidation of tyramine in a competitive manner. Finally, the localization of activity in adrenal medulla and brain-stem areas were also characteristic of dopamine β-oxidase[6].

Norsynephrin was first isolated from octopus salivary glands by ERSPAMER[7] who called it octopamine. Until its more recent finding in human and animal urine and in tissues, it was merely considered peculiar to the octopus. However, it is now apparent that a general aromatic L-amino acid decarboxylase in mammalian tissues[8] makes available tyramine (and other aromatic amines) for β-oxidation. The activity of the β-oxidase with a variety of amines is currently under investigation. The significance of synephrin and norsynephrin is not apparent, but it should be pointed out that they are fairly active pharmacologic agents[9]. Furthermore, as pointed out by ERSPAMER[7], they are readily oxidized by chemical means to epinephrine and norepinephrine respectively.

Laboratory of Clinical Biochemistry, National Heart Institute, National Institutes of Health, Public Health Service, U.S. Department of Health, Bethesda, Md. (U.S.A.)

JOHN J. PISANO
CYRUS R. CREVELING
SIDNEY UDENFRIEND

[1] Y. KAKIMOTO AND M. D. ARMSTRONG, *Federation Proc.*, 19 (1960) 295.
[2] J.J. PISANO, *Clin. Chim. Acta*, 5 (1960) 406.
[3] J. J. PISANO, J. OATES, A. KARMEN, A. SJOERDSMA AND S. UDENFRIEND, to be published.
[4] A. KARMEN AND H. R. TRITCH, *Nature*, 186 (1960) 150.
[5] E. R. LEVIN, B. LEVENBERG AND S. KAUFMAN, *J. Biol. Chem.*, 235 (1960) 2080.
[6] S. UDENFRIEND AND C. R. CREVELING, *J. Neurochem.*, 4 (1959) 350.
[7] V. ERSPAMER, *Nature*, 169 (1952) 375.
[8] S. UDENFRIEND, W. M. LOVENBERG AND H. WEISSBACH, *Federation Proc.*, 19 (1960) 7.
[9] A. M. LANDS AND J. I. GRANT, *J. Pharmacol. Exptl. Therap.*, 106 (1952) 341.

Received August 22nd, 1960

Biochim. Biophys. Acta, 43 (1960) 566–568

Biological activities of isomeric estriols

In a comprehensive study of the estrus-producing values of a large number of steroidal estrogens, we have employed in the St. Louis University laboratories both the Mather Modification[1] of the MARRIAN–PARKES[2] assay procedure using the adult castrate mouse and the CURTIS–DOISY[3] method using the 20-day-old intact female rat, as we believe that these two assay methods are suitable for the comparative study of estrogens of widely varying potencies. An average of about 50 animals was used for each dose level of each estrogen in obtaining dose-response curves.

As shown in Table I, the three isomeric estriols were surprisingly estrogenic. Especially noteworthy is the fact that 17-epiestriol in the CURTIS–DOISY assay is

substantially more active than estradiol-17β. PRELOG, RUZICKA AND WIELAND[4] reported the estrus-producing potency of 17-epiestriol as only 5–10 μg per rat unit in the adult castrate rat using the ALLEN–DOISY assay, and this isomeric estriol has never been considered as a very potent estrogen[5]. This is the first time, to our knowledge, that a naturally-occurring estrogen has been shown to be more estrogenic than estradiol-17β. This finding could conceivably account for the unexplainably high estrogenic titers found in human pregnancy urine at times[6]. BREUER[7] has reported that 17-epiestriol results from the incubation of 16α-hydroxyestrone with human-liver slices, from the incubation of 16-keto-estrone with human liver, ovary, and kidney slices, and more recently he has described its isolation from human pregnancy urine[8].

TABLE I

Estrogen	MARRIAN *Assay (μg giving 50 % response)*	CURTIS–DOISY *Assay (μg giving 50 % response)*	*% Variation from normal in 21-day-old male rats* (50 μg/day for 14 days)*		
			Body wt. gain	*Ventral prostate (wt.)*	*Testes (wt.)*
Estradiol-17β	0.02	0.09	— 38	— 53	— 50
Estriol	0.1	0.13	— 39	— 63	— 45
16-Epiestriol	0.2	0.2	— 32	— 72	— 37
17-Epiestriol	0.044	0.054	— 42	— 72	— 55

* Cf. ref. 10.

In Table I are also shown certain data extracted from our general screening assays employing young intact male rats, as performed by the Endocrine Laboratories of Madison, Wisc. under the direction of Dr. ELVA G. SHIPLEY. It is apparent that all of the estriols exert a very strong dampening effect on the growth of the ventral prostate. It appears that 16-epiestriol, which has been termed one of the weakest of all naturally-occurring estrogens[9], may produce in the young male rat a disproportionate suppression of pituitary gonadotrophin relative to estrus-producing potency.

Department of Biochemistry,
Saint Louis University School of Medicine,
Saint Louis, Missouri (U.S.A.)

PHILIP A. KATZMAN
JAMES A. MONTELEONE

Lasdon Foundation Research Institute of Chemotherapy,
Colorado Springs, Colorado (U.S.A.)

JAMES R. RHONE
MAX N. HUFFMAN

[1] A. MATHER, *J. Biol. Chem.*, 144 (1942) 617.
[2] G. F. MARRIAN AND A. S. PARKES, *J. Physiol.*, 67 (1929) 389.
[3] J. M. CURTIS AND E. A. DOISY, *J. Biol. Chem.*, 91 (1931) 647.
[4] V. PRELOG, L. RUZICKA AND P. WIELAND, *Helv. Chim. Acta*, 28 (1945) 250.
[5] M. N. HUFFMAN AND A. GROLLMAN, *Endocrinology*, 41 (1947) 12.
[6] O. W. SMITH AND G. V. SMITH, *Acta Endocrinol.*, 28 (1958) 479.
[7] H. BREUER, *Arzneimittel-Forsch.*, 9 (1959) 667.
[8] H. BREUER, *Nature*, 185 (1960) 613.
[9] J. T. VELARDO AND S. H. STURGIS, *Proc. Soc. Exptl. Biol. Med.*, 90 (1955) 181.
[10] W. W. SCOTT, *Trans. Am. Assoc. Genito-Urinary Surg.*, 158 (1956) 168.

Received August 15th, 1960