We have confirmed the report (3) that this enzyme hydrolyzes emulsions of ovolecithin but not DML. However, the hydrolysis of DCL must alter the view (3) that only lecithins containing unsaturated fatty acids are substrates. It suggests that unsaturation may lead to a favorable spatial situation rather than be specifically involved.

The results with DCL indicate that substrates for lecithinases A and D need not have long-chain fatty acids and favors further investigations of water-soluble phospholipids.

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2-Methoxyestriol: A New Metabolite of Estradiol in Man[1]

The recent isolation and identification of 2-methoxyestrone (1) suggested that other similarly constituted compounds might be formed *in vivo* from the female sex hormone. Since 2-methoxyestriol appeared to be a particularly favorable possibility, this compound was synthesized from estriol by the method of Fishman (2) and showed the following physical characteristics: m.p. 215–218°C.; $[\alpha]_D^{25}$ + 83° (ethanol); $\lambda_{max}^{ethanol}$ 286 mμ, ε 3700; $\lambda_{min}^{ethanol}$ 253 mμ, ε 350. The analysis was correct for $C_{19}H_{26}O_4$.

It was intended to employ this synthetic steroid as carrier for the potential urinary metabolite after administration of estradiol-17β-16-C^{14}. The separation scheme used in these laboratories (3) depends on initial purification by countercurrent distribution, first between 70% methanol and carbon tetrachloride (System A) and subsequent fractionation of the more "polar" components between ethyl acetate–cyclohexane 1:1 and ethanol–water 1:1 (System B). Estriol and 2-methoxyestriol distribute identically in System B with a peak of concentration

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at tube 27; this mixture is well separated, however, from the other metabolites of estradiol-17β. It was necessary to demonstrate that 2-methoxyestriol could be cleanly separated from estriol, since the latter compound would contain a major share of radioactivity. Studies were therefore made of the separation of inactive, synthetic 2-methoxyestriol to which a small quantity of pure, highly radioactive estriol-16-C^{14} had been added.[2] Separation of this mixture by fractional crystallization from several solvents proved impossible. However, chromatography on silica gel employing 3% ethanol in petroleum ether as the developing solvent readily separated this mixture in that 2-methoxyestriol, pure and devoid of radioactivity, was eluted from the column before estriol. The two steroids could also be completely separated by counter-current distribution between benzene and water (System C) (4). After 99 transfers in System C, 2-methoxyestriol had a peak of concentration at tube 52 while estriol had a peak of concentration at tube 16. Inactive 2-methoxyestriol admixed with a small amount of estriol-16-C^{14} was devoid of radioactivity after this counter-current separation. With these facts established, the transformation of estradiol to 2-methoxyestriol *in vivo* was studied.

Estradiol-17β-16-C^{14} (2.15 mg.; 1,890,000 c.p.m. per mg.) was administered intravenously to a woman with metastatic cancer of the breast who had been oöphorectomized and adrenalectomized. The three day urine collection after the hormone was processed as previously described (3). A portion (58%) of the neutral plus phenolic fraction (1,190,000 c.p.m.) was distributed counter-currently through 99 tubes in System A. The material in tubes 70 through 99 which contained estradiol and "more polar" compounds was redistributed through 99 tubes in System B. The contents of tubes 15 through 39 ("estriol region;" 400,000 c.p.m.) were mixed with 20 mg. of synthetic 2-methoxyestriol. The mixture was chromatographed on silica gel into 530 fractions of 10 ml. each using 3% ethanol in petroleum ether as the eluant. Fractions 370 to 440 were identical in melting point and ultraviolet spectrum with 2-methoxyestriol and coincided with a small peak of radioactivity. Radioactive estriol was detected initially in fraction 500. The 2-methoxyestriol was combined and partitioned through 99 tubes in System C. Radioactivity was present only in tubes 40 through 70 with the peak of activity at tube 54. This coincided with the peak of concentration as measured by absorption at 286 mμ. The material in each tube from 48 to 58 had a constant specific activity of 600 c.p.m. per mg., using the weight calculated from the absorption at 286 mμ. Recrystallization from benzene–methanol yielded a product with unaltered specific activity.

In this experiment 2-methoxyestriol represented 3% of the radioactivity in the "estriol" region; this corresponds to 1% of the total urinary radioactivity in the first 3 days after the hormone administration. These results indicate that 2-methoxyestriol is formed *in vivo* from estradiol-17β by man. Investigation of other potential 2-methoxy metabolites is in progress; details of these studies, including synthesis of 2-methoxyestriol, will be published in the near future.

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[2] Synthesized by Dr. Mortimer Levitz, New York University, New York, N. Y.

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Kinetics of Homogentisic Acid Autoxidation

Although it has long since been established that homogentisic acid solutions, including the urine of alcaptonuric patients, tend to darken upon exposure to the atmosphere, little interest appears to have been directed either to the nature or to the kinetics of this process. In previous communications, however, it has been suggested that homogentisic acid is, in all probability, oxidized initially to the corresponding quinone, 1,4-benzoquinonacetic acid, and thence to a more complex polymer of as yet undefined properties (1, 2). It is the purpose of this note to present preliminary data on the kinetics of the autoxidation process.

Three homogentisic acid preparations were employed: one, was a recrystallized preparation obtained from the urines of two alcaptonuric patients; the other two were commercially available preparations obtained from different manufacturers. Each was assayed for homogeneity by melting point determination and ultraviolet absorption spectrophotometry. Samples of each were dissolved in water (20–200 mg./ml.) and tank oxygen bubbled continuously through each for periods up to 400 hr. Aliquots were removed at intervals, corrected for changes in volume and osmolality determined using a model B Fiske osmometer with a 6 volt Rubicon galvanometer. Readings were accurate to within 1% and all data reproducible to within 2%.

The three preparations behaved identically in all osmolality studies, with no detectable difference between the synthetic and the recrystallized preparations. Despite previous spectrophotometric evidence, suggesting that at least two distinct processes could be identified during the first 48 hr. of homogentisic acid autoxidation, the present data could be readily interpreted as a first-order reaction over a period of about 50 hr.; thereafter, from 50 to 400 hr., there was a linear relationship of osmolality with time and no significant alterations in osmolality could be identified. The instantaneous rate constant (k) of the exponential function characteristic of the first 50± hours of autoxidation was determined graphically and by both the least squares method and the method of the mean slope of adjacent points. Calculated k values were 2.15×10^{-3} in the first instance and 2.85×10^{-3} in the second, with a mean value, at the 0.01 level, of $2.50 \pm 0.05 \times 10^{-3}$.