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Excretion of Exogenous and Endogenous Estrogen in Bile of Dogs and Humans.*

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Data reported previously[1,2] indicate that considerable quantities of exogenous estrogen are excreted in the bile, and the possibility of an enterohepatic circulation of estrogens was suggested. These observations are supplemented by the data presented in this communication.

Human Bile. Bile was obtained by duodenal intubation of 2 women at full-term pregnancy and 3 women 3, 4 and 7 days post-partum, respectively. The following values were obtained by assay[2] of the so-called "C" or liver-bile fraction. Full-term pregnancy: 990 and 600 I.U. free estrogen per 100 cc. Values for free estrogen in blood taken at the same time were 300 and 198 I.U., respectively. Post-partum: 3 days, 450 I.U. (blood 36 I.U.); 4 days, 360 I.U. (blood 18 I.U.); 7 days, 240 I.U. (blood, 9 I.U.).

In a menopausal woman, after withdrawal of control specimens, 1 mg of diethylstilbestrol (120,000 I.U. by the author's assay) was injected intramuscularly and "C" bile was collected in ½-hour fractions for 3½ hours. Controls: inactive; ½ hour, 330 I.U. (total excretion free estrogen); ½-1 hour, 440 I. U.; 1-1½ hours, 650 I.U.; 1½-2 hours, 8200 I.U.; 2-2½ hours, 5360 I.U.; 2½-3 hours, 470 I.U.; 3-3½ hours, 108 I.U. The total excretion in 3½ hours was 15,558 I.U. (13% of activity of administered diethylstilbestrol). Recovery of estrogenic activity in the bile of dogs is considerably lower following administration of diethylstilbestrol than after administration of estrone or estradiol.[2]

Bile Estrogen after Gonadotropins. Two bile-fistula dogs received 4000 units of chorionic gonadotropin (Korotrin)‡ and one 4000 units of pregnant mare serum gonadotropin (Anteron)§ intravenously. Bile was collected for 4 consecutive 24-hour periods. Control specimens were inactive. Total bile estrogen after chorionic gonadotropin: 24 hours, 26 and 80 I.U.; 48 hours, 272 and 105 I.U.; 72 hours, 376 and 132 I.U.; 96 hours, 462 I.U. After pregnant mare serum gonadotropin: 24 hours, 52 I.U.; 48 hours, 246 I.U.; 72 hours, 570 I.U.; 96 hours, 700 I.U.

Absorption of Alpha-Estradiol from Duodenum. 2.5 mg (250,000 I.U.) of alpha-estradiol in sesame oil were introduced into the duodenum (duodenal fistula) in a bile-fistula dog over a period of 8 hours. Bile was collected for four 24-hour periods. The recovery, in terms of percentage of the quantity administered, was approximately as follows: 24 hours: free, 3%; total, 10%. 48 hours: free, 1.3%; total, 10%. 72 hours: free, 0.0%; total, 0.4%. 96 hours: free, 0.0%; total, 0.13%.

Under sodium pentobarbital anesthesia, 3 mg of alpha-estradiol (300,000 I.U.) in 5 cc of bile were introduced into an isolated jejunal loop. After ¾ hour, blood was allowed to flow for ½ hour from the veins draining the loop. The free and total estrogen content of the blood was identical, 6000 I.U. per 100 cc.

Bile Estrogen after Splenic Implantation of Alpha-Estradiol. A 15 mg pellet of alpha-estradiol was implanted in the spleen of a bile-

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fistula dog. Bile and urine were collected for five 24-hour periods (beginning with the 2nd day) and on the 16th and 23rd days after the implantation. No estrogenic activity was exhibited by the urine at any time during this period. The free estrogen in the bile was as follows: 2nd day, 280 I.U.; 3rd day, 672 I.U.; 4th day, 476 I.U.; 5th day, 550 I.U.; 6th day, 420 I.U.; 16th day, 2025 I.U.; 23rd day, 1780 I.U.

Discussion and Conclusions. Previous observations[1,2] indicate that the bile is an important medium of excretion of exogenous estrogen (10,000-250,000 I.U. doses). The data reported here indicate that large amounts of *endogenous* as well as *exogenous* estrogen are excreted in the bile of human subjects and dogs, and may be present in the bile in comparatively high concentration at a time when the concentration in the peripheral blood and urine is very low. The same is shown to be true following splenic implantation of a pellet of alpha-estradiol. The possibility of the existence of an enterohepatic circulation of estrogen is supported further by the demonstration (1) of the presence of large amounts of estrogen in the mesenteric-vein blood (dog) after instillation of alpha-estradiol into an isolated intestinal loop and (2) of the excretion of estrogen in the bile of dogs following instillation of estrogen into the duodenum.

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Biotin Deficiency in Rats Fed Purified Diets Containing Succinylsulfathiazole and p-Aminobenzoic Acid.*

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Only a few reports have been made concerning the effects of succinylsulfathiazole (sulfasuxidine) on the nutrition of animals fed purified diets.[1–5] Welch[1] found no favorable effect on growth of rats when *p*-aminobenzoic acid was added to a purified diet containing sulfasuxidine. In none of the other studies has *p*-aminobenzoic acid (or inositol) been included in the basal diet in attempts to determine the effect of sulfasuxidine on the nutrition of animals.

The effects of sulfanilylguanidine (sulfaguanidine) have been given considerably more attention.[6–11] Through the use of sulfaguanidine or sulfasuxidine deficiencies of biotin,[3,10] folic acid,[2,10] and vitamin K[4] have been reported. The evidence strongly indicates that the vitamin deficiencies which result are due to a lowering by the drugs of microbial synthesis of these essential nutritional factors in the alimentary tract. Black *et al.*[4] found that *p*-aminobenzoic acid counteracts the hypoprothrombinemia and growth inhibition that occurs when sulfaguanidine is fed. Other

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