strated greater Survival Time 50% values in every instance when administered at equal, at one-half, and at one-quarter the dosage used for the other tetracyclines. The persistence time of DOOTC at antibacterial concentrations within the host, and its chemotherapeutic efficacy in protecting mice from established infections, are advantageous properties and suggest possible advantages in clinical situations.

We are pleased to acknowledge the very capable technical assistance of Suzanne Little and Karin Carlsson in these studies.

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Received August 30, 1966. P.S.E.B.M., 1967, v124.

Effect of Estrone and Estriol on Salivary Glands and Dental Caries In Female Rats.* (31799)

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Reports that administration of estradiol or diethylstilbestrol causes a decrease in number and diameter of the granular tubules of submandibular salivary glands(1) and an increase in incidence of dental caries(2,3) in the female rat have been confirmed by this laboratory. Estradiol, estrone and estriol are found in low concentrations in the blood and urine of women having normal menstrual cycles. The blood levels and daily urinary excretion of the 3 hormones rise markedly with increased duration of pregnancy(4,5). It stimulated the interest to determine whether estrone or estriol would affect the dental caries incidence (DCI) and the submandibular granular tubules (SGT) as those caused by exogenous estradiol or diethylstilbestrol in female rats. This investigation might shed light on the relationship between the hormones and DCI in pregnant women.

Materials and methods. Weanling female rats of the Sprague-Dawley strain weighing between 40 and 45 g were randomly divided into 4 groups. Each animal was housed in an individual raised wire screen cage. All of the animals were kept under the same environmental conditions, supplied with distilled water and fed, *ad libitum*, with cariogenic diet.† Beginning at 23 days of age, each animal of the 2 treated groups received daily subcutaneous injections of 0.2 mg estrone,‡ or 0.5 mg estriol§ in 0.05 ml of oil solution for 6 weeks. For comparison, a third treated group of rats was injected similarly with 0.02 mg estradiol benzoate.‖ A fourth group of rats was injected with oil alone and served as control.

At 65 days of age, the animals were sacrificed by chloroform inhalation. Submandibular, parotid, thyroid, and adrenal glands and ovaries were immediately removed, weighed, fixed in 10% formalin solution, and sectioned at 5 μ. Submandibular sections were stained by Lillie's Azure A-Eosin B method for the granules of the granular tubular cells. The number and diameter of the SGT were measured by the aid of square and linear micrometers. Parotid, thyroid, adrenal and ovarian sections were stained with hematoxylin and eosin. Heads were boiled in a pressure-

* This investigation was supported by USPHS research grant DE-01621 from Nat. Inst. of Dental Research, Nat. Inst. Health, Bethesda, Md.

† The composition of the diet was as follows: ground rice, 100 lb.; powdered whole milk, 45 lb.; powdered alfalfa, 4.5 lb.; sodium chloride, 1.5 lb.

‡ Theelin, Parke, Davis and Co., Detroit, Mich.

§ Estriol, Delta Chemical Works, New York.

‖ Estradiol benzoate, Schering Corp., New Jersey.

TABLE I. Effect of Estrogenic Hormones on Salivary Glands and Dental Caries.

	Treatment	No hormone	Estradiol benzoate	Estrone	Estriol
	No. of rats	17	17	18	14
Final body wt (g)		183.8 ± 2.4*	137.6 ± 2.4 (e)	124.7 ± 3.1 (e)	103.5 ± 3.6 (e)
Submandibular gland	Absolute wt (mg)	406.6 ± 8.9	330.7 ± 7.4 (e)	299.5 ± 8.2 (e)	273.4 ±10.1 (e)
	Wt (mg)/100 g body wt	221.5 ± 4.8	256.4 ± 6.6 (e)	240.9 ± 5.3 (b)	267.9 ±13.5 (d)
	Granular tubules No.†	161.1 ± 2.3	137.6 ± 2.0 (e)	137.1 ± 2.0 (e)	138.9 ± 1.5 (e)
	Diameter‡	39.1 ± .3	33.0 ± .1 (e)	34.2 ± .1 (e)	31.6 ± .4 (e)
Parotid gland	Absolute wt (mg)	367.0 ±14.6	346.6 ± 9.4 (NS)	311.2 ±10.7 (d)	304.9 ±11.3 (d)
	Wt (mg)/100 g body wt	199.0 ± 7.0	269.2 ± 9.1 (e)	245.5 ± 8.6 (e)	300.9 ±17.3 (e)
Dental caries	No./rat	.71± .2	1.71± .3 (b)	1.67± .3 (a)	1.86± .3 (c)
	Score/lesion	1.08± .08	1.17± .07 (NS)	1.23± .08 (NS)	1.58± .13 (d)
	% of molars affected	5.9 ± 1.9	14.2 ± 2.6	13.9 ± 2.9	15.5 ± 2.6
	% of rats affected	47.1	82.4	72.2	85.7

* Mean ± S.E.
† Per relative square area.
‡ Relative units.

Letters in parentheses indicate significance of differences from control group: (a) = $p < .050$, b = $p < .025$, (c) = $p < .010$, (d) = $p < .005$, (e) = $p < .001$, (NS) = not significant.

cooker for 15 minutes to remove soft tissue. Dental caries (DC) of dry molars were examined under a dissecting microscope at 30× magnification. The number of carious lesions of all molars and the number of carious molars of each rat, as well as the number of rats affected with DC in each group, were recorded. Severity of carious lesions was arbitrarily graded as 1, 2, 3, or 4: 1 being an initial carious lesion involving the fissure or exposed dentin on the occlusal surface with less than ½ of the surrounding cusp(s) destroyed, but not extending into the pulp chamber; 2 being the carious lesion involving more than one-half of the cusp(s) and/or penetrating into the pulp chamber, but less than one-fourth of the molar destroyed; 3 being one-fourth to one-half of the molar destroyed; 4 being more than one-half of the molar destroyed. All parameters of the DC, salivary glands, and other organs of the 3 estrogen-treated groups of rats were statistically compared with those of the appropriate controls by Student's t test.

Results. As shown in Table I, body growth of the 3 estrogen-treated groups of rats was significantly retarded. Mean absolute wet weights of submandibular and parotid glands of each treated group of rats were decreased, but the mean relative (organ wt in mg/100 g body wt) wet weights of these glands were increased in comparison with those of the controls. Mean number and diameter of the SGT of each treated group of rats were highly significantly decreased in comparison with those of the controls. No histologic alterations were observed on parotid glands. Administration of estradiol benzoate, estrone or estriol resulted in a significant increase in the mean number of the DC. Mean severity of carious lesions of estriol-treated rats was significantly increased in comparison with that of the controls; estradiol benzoate or estrone treatment caused a slight but not significant increase in mean severity. Mean percentage of molars affected with DC per rat and the percentage of rats affected with DC of each treated group of rats were twice as much as those of the controls.

No significant change in mean absolute wet

TABLE II . Effect of Estrogenic Hormones on Thyroid, Adrenal, and Ovarian Weights.*

	Treatment No. of rats	Control 17	Estradiol benzoate 17	Estrone 18	Estriol 14
Final body wt (g)		183.8 ± 2.4†	137.6 ± 2.4 (e)	124.7 ± 3.1 (e)	103.5 ± 3.6 (e)
Thyroid	Absolute wt (mg)	12.3 ± .5	11.3 ± .2 (NS)	11.4 ± .4 (NS)	11.3 ± .6 (NS)
	Wt (mg)/100 g body wt	6.8 ± .3	8.8 ± .2 (e)	9.2 ± .4 (e)	11.0 ± .7 (e)
Adrenal	Absolute wt (mg)	50.5 ± 1.3	46.5 ± 1.1 (a)	44.0 ± 1.4 (e)	36.3 ± 1.2 (e)
	Wt (mg)/100 g body wt	27.6 ± .8	36.0 ± 1.1 (e)	35.5 ± 1.3 (e)	35.6 ± 1.7 (e)
Ovary	Absolute wt (mg)	63.1 ± 2.0	13.1 ± 5.4 (e)	12.6 ± .7 (e)	10.6 ± .7 (e)
	Wt (mg)/100 g body wt	34.4 ± 1.1	8.8 ± .2 (e)	10.2 ± .6 (e)	10.3 ± .7 (e)

* These organs are from the same groups of rats as shown in Table I.
† Mean ± S.E.
Letters in parentheses indicate significance of differences from controls: (a) = $p < .050$, (e) = $p < .001$, (NS) = not significant.

weight of the thryroids of each treated group of rats was shown; the mean relative thyroid weight was significantly increased in comparison with that of the control (Table II). All treated groups of rats showed a significant decrease in mean absolute adrenal wet weights. However, their mean relative adrenal wet weights were increased in comparison with that of the controls. Mean absolute and relative ovarian wet weights of each treated group of rats were highly significantly decreased in comparison with those of the controls. Histologic examination of the thyroids, adrenals and ovaries of the 3 treated groups of rats revealed: 1) no detectable change in the epithelial cell height of and the colloid appearance in the thyroid follicles; 2) a slight increase in cell sizes of zonae fasciculata and reticularis of the adrenal cortices and 3) a marked reduction in the number of ovarian follicles and an atretic appearance in the existing follicles. Almost no corpus luteum was found in the ovarian sections.

Discussion. Present studies indicate that the effect of estrone and estriol is similar to that of estradiol benzoate in causing an increased DCI and a decrease in the number and diameter of the SGT.

A decrease in mean absolute wet weights but an increase in mean relative wet weights of the SG of each treated group of rats indicates that these estrogens are more deleterious to the general body growth than the growth of the SG. The mechanism of how these estrogens cause the histologic alteration of the SG and the increase in DC formation is not known. It has been reported that(6,7), in the rat, the estrogens inhibit the anterior pituitary from secreting growth hormone (GH). Money *et al*(8) reported that estradiol benzoate and estriol inhibited the thyroid activity causing decreased secretion of thyroid hormone (TH) which normally synergizes the GH action. Moreover, it has been reported that the deficiency of GH and possibly TH causes a decrease in intestinal calcium absorption and an increase in urinary calcium excretion(9). Evidences have been shown that normal secretion of TH and possibly GH and corticosteroids are important in the maintenance of normal structure and secretory function of the SG(10,11,12), and that TH is closely related to the DCI(13,14, 15). Therefore, the estrogen-induced histologic and possibly functional alterations of the SG, as well as the increased DCI, may both be mediated *via* the interference of the TH and GH. However, in the present experiment, it was found that only the mean relative

thyroid weight of each treated group of rats was increased, whereas there was no significant change in mean absolute thyroid weight, epithelial height and colloid content of the thyroid follicles, Therefore, further work, such as analysis of PBI, is necessary to clarify the functional state of thyroid glands in rats treated similarly with these estrogens.

Prolonged administration of corticosteroid caused histologic alterations of the SG and increased DCI(16,17). The data of the present experiments indicate that treatment by each of the 3 estrogens caused an increase in the mean relative adrenal weight which may have resulted in a hyperfunction of the adrenal cortex. However, the mean absolute adrenal weight of each treated group of rats was decreased as compared with that of the controls. Thus, the increased mean relative adrenal weight in treated rats was probably mainly due to the decrease in their body weights. Furthermore, Vogt(18) reported that prolonged administration of estrogens results in a nonfunctional adrenal hypertrophy. Therefore, the increased DCI and the histologic alteration of the SG of these estrogen-treated rats were probably not related to the hyperfunction of the adrenal cortex.

It was found that prolonged administration of progesterone caused histologic alteration of the SG(19). Since almost no corpus luteum was found in the ovaries of these estrogen-treated rats there would be no excessive secretion of progesterone which might have affected the SG. The food intake of the 3 estrogen-treated groups of rats was reduced, and consequently they were undernourished. However, it has been reported(20) that in rats the reduction in food intake resulted in a decrease in the DCI.

It is interesting to note that these 3 estrogens and some corticoids are cariogenic in the rat. The daily excretion of estriol at the late stage of pregnancy in women is about a thousand times higher than that excreted daily during the menstrual cycle; the urinary estrone and estradiol levels rise approximately 100 times(5), and the blood 17-hydroxycorticosteroids rise at least 4 times above the normal level(21) during this time. If the information in regard to the DC obtained from the experimental animals can be transferred to the human being, one would expect an increase in DCI in pregnant women shortly before or after parturition. However, conflicting results have been reported on the incidence of DC in pregnant women(22,23, 24), perhaps due to the fact that a great variation of blood and urinary estrogens in different individuals exists during pregnancy (4,5). Determination of blood and urinary estrogens in pregnant women is being carried out in this laboratory to see whether there is a positive correlation between the levels of these estrogens and the DCI. A report(25) that pregnancies in rats do not cause increased DCI as compared with their nonpregnant littermates has been confirmed by this laboratory. This is probably due to the fact that in the rat there is no estrogen elaborated by the placenta(26) as there is in the human placenta during pregnancy(27).

Summary. Female rats of the Sprague-Dawley strain were maintained on a cariogenic diet and were injected subcutaneously daily with 0.2 mg estrone or 0.5 mg estriol for 6 weeks. Either treatment caused a decrease in absolute wet weights of the submandibular and parotid salivary glands and a decrease in the number of diameter of submandibular granular tubules. The incidence of dental caries of estriol- or estrone-treated rats was significantly increased, and the severity of dental caries was slightly increased in comparison with those of the controls. The effect of estriol and estrone on the submandibular glands and dental caries is identical to those caused by estradiol benzoate in the female rat.

The author wishes to express appreciation to Dr. G. J. Cox for the formula of the cariogenic diet and to Miss Iris Hoots and Mr. Robert Lofgren for excellent technical assistance.

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Received September 12, 1966. P.S.E.B.M., 1967, v124.

Effect of Hydrazine on DNA Content of the Liver. (31800)

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A single sub-convulsive dose of hydrazine has been shown to produce hepatic lesions when introduced into laboratory animals by various routes of administration. Subcutaneous administration of the compound to dogs resulted in centrolobular fatty degeneration which progressed outwardly(1). Hydrazine exposure produced hypoglycemia and marked reduction of glycogen stores of liver and skeletal muscle(2). The depression of liver glycogen has been correlated biochemically and histologically with accumulation of liver lipid in rats(3). This hydrazine-induced lipid deposition was related to an elevation in plasma free fatty acids(4) and to increased rate of transport from the circulating fatty acid pool into liver(5). Moreover, hydrazine depressed conversion of glycine to liver glycogen(6) and CO_2(7). The incorporation of amino acids into protein of liver slices was enhanced by treatment of the animals with hydrazine(7). The increased incorporation of amino acids into protein was ascribed to an expanded amino acid pool size resulting from hydrazine-induced inhibition of conversion of amino acids to keto acids. For example, liver transaminases are inhibited by hydrazine *in vitro,* presumably *via* formation of pyridoxal hydrazone(8).

Increased protein biosynthesis might be expected to be accompanied by changes in ribonucleic acid (RNA) content of the liver. Hydrazine treatment increased total RNA and protein contents in the liver, but not in kidneys or gonads in rats(9). Since alterations in

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† This research was conducted by personnel of USAF School of Aerospace Med., Aerospace Medical Division, AFSC, U.S. Air Force, Brooks AFB, Texas. Further reproduction is authorized to satisfy the needs of the U.S. Government.