corticosterone accumulation in tumor tissue may be explained by a combination of reduced synthesis and increased adrenal metabolism of corticosterone to other steroids. The finding of Δ^4-3-ketosteroid with 21-desoxy or 20,21-dihydroxy side chains, and of 11-dehydrocorticosterone lend support to this hypothesis.

The absence of any detectable amounts of 17α-hydroxylated corticosteroids in the mouse adrenal tumor or normal adrenal tissues confirms the *in vivo* study of Southcott(10) and the *in vitro* study of Hoffman(11).

Summary. 1) Slices from transplants of 2 secretory adrenocortical tumors were incubated with and without ACTH. Incubation mixtures were extracted and the total Δ^4-3-ketosteroids quantitatively determined from peak optical densities at 242 mμ. Basal steroid secretion of the mouse and rat tumor slices averaged 2.6 and 1.9 μg equiv. cortisol/100 mg/2 hours respectively; with maximum stimulating amounts of ACTH, the average rates were 13.6 and 17.4 μg, respectively. Both tumors responded maximally to 2-3 mU of the hormone. 2) Under ACTH stimulation, corticosterone and 11β-hydroxy-Δ^4-androstene-3,17-dione were produced in approximately equal amounts by the mouse adrenal tumors. However, 25-65% of the total Δ^4-3-ketosteroids consisted of compounds still to be identified. The secretion of the rat adrenal tumor slices consisted mainly of corticosterone. 3) Glucose in the buffer stimulated the total steroid secretion of the mouse adrenal tumor slices about 40%. 4) An adrenal tumor that secreted gonadal hormones was incubated with and without ACTH. No measurable Δ^4-3-ketosteroids were detected in the incubates.

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Effect of Varying Levels of DL-Tryptophan in Diet on Dental Caries in Rats.* (23203)

WOLFGANG BUTTNER† AND JOSEPH C. MUHLER

Indiana University, Department of Chemistry and School of Dentistry, Bloomington.

The chemistry of the salivary glands of the rat has recently received new interest due to experimental evidence indicating a correlation between activity of the thyroid gland, histologic picture of the salivary glands, and incidence of dental caries(1,2). Moreover, protein metabolism in the salivary glands as it relates to caries experience deserves special consideration, since proteolytic activity of rat salivary glands has been shown to be dependent upon normal thyroid function(3). Tryptophan is of particular interest in this relationship since "significantly greater amounts of tryptophan or tryptophan-like compounds in caries-free (human) salivas compared to caries-susceptible" has been reported(4).

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† Post-Doctorate Fellow. On leave of absence from University of Mainz, Germany.

TABLE I. DL-Tryptophan Contents of Various Diets Used, Salivary Tryptophan Levels and Incidence of Dental Caries in the Rat.

Group	DL-Tryptophan added to diet (g/kg diet)	Salivary tryptophan level			Dental caries experience		
		No. of samples	Conc. (mg %)	P	No. of animals	Mean No. of lesions	P
I	.6	24	32 ± 1.4*	.01	24	8.1 ± .5*	.3
II	1.8	28	42 ± 2.8	.001	28	8.0 ± .6	.4
III	5.4	25	48 ± 2.1	"	25	9.1 ± .5	.04
IV		27	25 ± 1.1		27	7.4 ± .5	

* Stand. dev.

Therefore, it is of interest to determine experimentally if DL-tryptophan possesses any anticariogenic properties if fed to rats receiving a cariogenic diet.

Materials and methods. A total of 120 weanling Sprague-Dawley strain rats were divided equally into 4 groups according to sex and initial body weight. Groups 1 through 3 received a cariogenic corn diet to which were added varying amounts of DL-tryptophan (Table I). Group 4 received the same diet without added tryptophan and served as a control. Composition of the cariogenic diet in percentage was as follows: yellow corn grits, 52.7; ground yellow corn, 11.3; powdered whole milk, 30.0; alfalfa, 4; iodized sodium chloride, 1.0; and irradiated yeast, 0.2. The animals were housed in pairs in raised screen cages in an air-conditioned room, and all received fluorine-low drinking water (F = 0.05 μg/ml) *ad libitum*. After 110 experimental days, saliva of each rat from each group was collected and analysed for tryptophan. Sodium nembutal was used for anesthesia (0.53 ml of a 0.5% stock solution of sodium nembutal per 100 g body weight was injected intraperitoneally). Pilocarpine hydrochloride (10 mg/g body weight injected intraperitoneally) was used to increase salivary flow. By such procedures, approximately 2 ml of saliva could be collected conveniently within 10 minutes. The method of tryptophan analysis(5,6) was adapted to salivary tryptophan analysis. 7 ml of distilled water was added to 1.0 ml of saliva. Following thorough mixing, 2 ml of the solution were transferred to a test tube, and 0.5 ml of glyoxylic acid(7), and 0.5 ml of 0.01 M copper sulfate solution were added. The test tube was placed in an ice water bath and 8.0 ml of a sulfuric acid solution (60 parts H_2SO_4 to 40 parts distilled water) were slowly added. After 10 minutes, the sample was placed into a boiling water bath for 3 minutes, then cooled at room temperature for 1 hour. The amount of salivary tryptophan was determined with the aid of a Klett-Summerson photoelectric colorimeter by comparison to a standard curve obtained by running known amounts of DL-tryptophan through the procedure. A 540 mμ filter was used. After 120 days, the animals were sacrificed by ether and the heads were removed for dental caries evaluation as previously described(8).

Results. The effect of tryptophan on incidence of dental caries and on salivary tryptophan level is shown in Table I. The effect of adding 0.6 or 1.8 g/kilo DL-tryptophan to the stock corn cariogenic diet was associated with a small and non-significant increase in incidence of dental caries when compared to controls receiving the same diet without added tryptophan. When 5.4 g/kilo tryptophan was added to the diet, there was a significant increase in incidence of dental caries.

Determinations of salivary tryptophan levels indicate an increasing amount of tryptophan with increasing dietary tryptophan concentration. It is not possible by this method of analysis to distinguish between free tryptophan or tryptophan bound in some simple form, but since there seems to be no correlation to salivary tryptophan level and dental caries in the rat, it does not seem important to design experiments in order to clarify this point. The failure of DL-tryptophan to reduce incidence of dental caries in the rat even though there was a significantly greater salivary tryptophan level suggests

that the higher salivary tryptophan level observed in caries-immune humans(4) is not the only factor involved in caries immunity in humans and that a similar immunity to caries cannot be induced in rats by feeding tryptophan.

The daily weight gains in the animals receiving the tryptophan were somewhat less. However, these differences were insignificant in all groups except in the one receiving 5.4 g tryptophan per kg of diet. In this group the final weight of both the males and the females was about 20% less than the controls.

The role of protein degradation in the oral cavity or within the salivary glands themselves must bear some relationship to caries immunity in the rat, since the salivary gland-thyroid studies clearly indicate an increased proteolytic effect of the salivary gland homogenates following administration of desiccated thyroid, while a marked decrease resulted following administration of I^{131} or thiouracil. The latter substances are known to be related to caries immunity or susceptibility, respectively. The anatomical structures within the salivary gland affected by thyroid activity are the serous tubules, and Shafer‡ has noted that following thyroxine administration large numbers of the granules pass into the excretory ducts of the salivary glands. Since these granules are thought to be predominantly protein in nature, the significance of proteolytic enzymes, protein metabolism, and the role of different amino acids on caries resistance or susceptibility in the rat needs much further study.

Summary. Dental caries experience in rats was studied following feeding of varying levels of DL-tryptophan as a constituent of a cariogenic diet. When either 0.6 or 1.8 g/kilo tryptophan was added to the diet there was an increase in dental caries, although the differences were not significant. When the diet contained 5.4 g/kilo tryptophan there was a significant increase in incidence of dental caries. Concomitant with the increased dietary tryptophan level, there was an increased salivary tryptophan concentration.

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Status of Deoxycorticosterone as Intermediary in the Biosynthesis of Cortisol.* (23204)

JOHN EICHHORN† AND OSCAR HECHTER
The Worcester Foundation for Experimental Biology, Shrewsbury, Mass., and Department of Physiology, Boston University School of Medicine, Boston, Mass.

The conversion of cholesterol to 17α-hydroxycorticosterone (cortisol) and corticosterone, demonstrated in adrenal preparations (1,1a,2,3) has been postulated to proceed via the reaction sequence shown in Fig. 1(1,4). All of the individual reactions shown have been definitively demonstrated in cell-free systems, and the available *in vivo* data are consistent with this scheme(4). The postulation of an orderly series of hydroxylations of the progesterone molecule was derived in large part

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