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Reduced Caries in Offspring of Rats Receiving Tetracycline* During Various Prenatal and Post-Partum Periods. (25764)

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It has been shown that aureomycin (chlortetracycline) inhibits caries in the rat when added to a caries-test diet after weaning(1,2). The present communication demonstrates the effectiveness of tetracycline in markedly reducing caries activity in offspring of mothers receiving the antibiotic during various prenatal and post-partum periods.

Methods. Sprague-Dawley rats were bred and insemination was diagnosed by vaginal smear. The animals were then divided into 4 groups. Groups A, B, and C received a solution containing 2.5 mg of tetracycline hydrochloride† per ml prepared fresh 3 times weekly, and Group D received distilled water. Group A received the antibiotic during gestation only (21 days); Group B, during gestation and 14 days of lactation; and Group C, during gestation, lactation and the remaining 7 days of the preweaning period. Group D received distilled water during gestation, lactation and until the offspring were weaned at 21 days of age. Purina laboratory chow was available during gestation and lactation and "non-cariogenic" Diet 550(3) was offered thereafter until the offspring were weaned. The offspring were then housed 2 per cage by sex and received distilled water and caries-test Diet 636(4) *ad libitum* for 60 days. Food and fluid intakes of mothers and offspring were recorded. The heads were cleaned of soft tissue using the dermestid beetle(5), and the teeth were scored for caries(6). The data are presented in Table I. A preliminary investigation which prompted the present extended study was comprised of groups similar to B and D, and the data are presented in Table II.

Results. Tetracycline did not affect fluid consumption of the mothers although the pH of the antibiotic solution was 3.0 (Tables I, II). In addition, tetracycline showed no adverse effect on litter production. Average number of offspring per litter for the 4 groups in Table I was 8.0, 10.6, 9.7 and 8.7 respectively. Because a portion of each litter was reserved for a separate study, only about half of each litter is represented. Tetracycline did not adversely affect weight gains or diet and fluid intakes of the offspring during the 60-day post-weaning experimental period. In the preliminary study (Table II), the control and experimental groups had 7.0 and 9.5 offspring per litter. Weight gains, diet and fluid intakes were somewhat less than in the expanded study. These findings are of interest because Keyes(7) found high mortality in hamsters born of mothers receiving penicillin during gestation and the first week of lactation. Rats appeared to show a better tolerance to penicillin(7).

In the present studies, the most striking effects of administration of tetracycline were

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† Stability of tetracycline hydrochloride as administered to the rat in drinking water was determined spectrophotometrically daily for 3 days. The characteristic spectrum(8) remained unchanged but optical density at the 2 maxima of 276 and 356 mu were depressed 9.7% and 9.4% respectively.

TABLE I. Weight Gain, Diet and Water Intake and Caries Evaluation of Rats Receiving Tetracycline in the Drinking Water during Various Prenatal and Post-partum Periods.

Group		A	B	C	D
Period of tetracycline intake*		G	G + L	G + L + PW	None
Mothers					
Fluid intake†	ml/day	53.1	50.2	49.0	47.3
Tetracycline intake	Total g	1.8	4.0	5.2	.0
Offspring‡					
Litters§	No.	9	11	11	22
Rats	No.	36	46	41	70
Wt gain	g/day	2.4	2.4	2.4	2.1
Diet intake	g/day	12.8	12.9	13.9	12.6
Fluid "	ml/day	17.1	15.4	20.3	15.6
Caries evaluation					
Rats with caries	%	50.0	71.7	48.8	95.7
Carious teeth/rat	No.	1.4	2.9	1.2	7.2
" areas/rat	No.	1.9	4.4	1.7	15.9
Caries score		2.3	4.5	1.8	20.3

* G = During gestation; G + L = During gestation and 14 days of lactation; G + L + PW = During gestation, lactation and the remaining 7 days prior to weaning. Group D consisted of controls and received distilled water for periods G + L + PW.

† Based on 42 days (21 days gestation + 21 days post-partum).

‡ During post weaning period of 60 days.

§ Only about half of the offspring/litter is represented in this study since the remainder was reserved for another study. Mean No. of offspring/litter was 10.6, 9.7, 8.7 and 8.0, respectively.

marked reduction in caries activity under all experimental regimens, and the high degree of fluorescence in the teeth. Mothers receiving the antibiotic during gestation and lactation showed a marked fluorescence of the incisors under ultraviolet light while almost none could be seen in the molars. However, both molars and incisors of the progeny at weaning showed the typical intense golden-yellow fluorescence previously observed in bone(8), thus again indicating that the antibiotic is localized in newly-calcifying tissues.

TABLE II. Weight Gain, Diet and Water Intake and Caries Evaluation of Rats Receiving Tetracycline in the Drinking Water during Gestation and 14 Days of Lactation.

		Control*	Exp.*
Mothers			
Fluid intake†	ml/day	37.3	42.9
Tetracycline intake	Total g	.0	3.9
Offspring‡			
Litters	No.	6	4
Rats	No.	42	38
Wt gain	g/day	1.4	1.5
Diet intake	g/day	9.9	9.8
Fluid "	ml/day	12.5	11.6
Caries evaluation			
Rats with caries	%	100.0	63.2
Carious teeth/rat	No.	9.4	1.7
" areas/rat	No.	30.3	2.7
Caries score/rat		44.7	3.1

* Control group received distilled water during gestation, lactation and 7 days prior to weaning. Experimental group received tetracycline during gestation and 14 days of lactation and then distilled water until weaned at 21 days of age.

† Based on 42 days (21 days gestation + 21 days post-partum).

‡ During post-weaning period of 60 days.

Detailed studies to be presented separately indicate that the extremely low level of caries activity in the tetracycline treated animals is probably not due to presence of the antibiotic in the teeth and lend support to the concept (7) that dental caries is an infectious process transmitted by the intestinal flora. The present studies suggest that tetracycline may be a valuable tool in depressing this flora and thus may provide a baseline for studying the effect of various imposed factors on experimental caries.

Summary. Offspring of mothers receiving tetracycline during various prenatal and post-partum periods showed markedly less caries activity during subsequent 60-day post-weaning assay period. Tetracycline administration did not adversely affect litter production, weight gains, diet intake or fluid consumption in either mother or offspring.

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Stoichiometric Relation between Liver-Receptor, Intrinsic Factor and Vitamin B_{12}.* (25765)

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Since the original report that hog intrinsic factor concentrate (HIFC) enhanced Co^{60}-labeled Vit. B_{12} uptake by rat liver slices(1), the mechanism of this phenomenon has been extensively studied. Data obtained confirm the hypothesis that receptors for intrinsic factor exist on rat liver slices and the low speed precipitate of the homogenate thereof. These receptors can "take up" either free intrinsic factor or intrinsic factor to which Co^{60}-B_{12} is attached(2). Identical receptors for intrinsic factor on small intestinal mucosa of the rat have also been demonstrated(3). The present report extends these studies and confirms the stoichiometry implied previously (2,4).

Materials and methods. Liver homogenate was prepared by mincing fresh (or fresh frozen) liver with scissors, adding 40 ml of 0.9% NaCl per 10 g liver, and homogenizing for 1 minute at room temperature in a Waring blendor. The homogenate was centrifuged at 3000 rpm for 5 minutes, the supernate discarded, and the remaining homogenate washed twice in 0.9% NaCl (40 ml/10 g initial liver tissue). The washed homogenate of "liver debris" (mainly cell walls and nuclei) was suspended in Krebs-Ringer-Tris containing 10 mM $CaCl_2$ (KRT) or in 0.9% NaCl containing 10 mM $CaCl_2$(NaCl-$CaCl_2$); 10 ml of medium was used per 1 g of whole liver starting material. Although it was generally used immediately, washed homogenate prior to suspension in medium may be refrigerated for as long as 2 hours prior to use if necessary. Incubations were performed for one half hour, in air, at room temperature, with constant gentle shaking. Each incubation beaker contained the washed homogenate from 1 g of liver, or 100 mg of lyophilized liver homogenate, suspended in 10 ml of medium, plus 1 ml of the appropriate added agent. Each incubation was followed by centrifugation at 3000 rpm for 5 minutes, discarding the supernatant fluid by decantation, and 3 washes of the homogenate with 10 ml aliquots of NaCl-$CaCl_2$. Each incubation was preceded by suspending the washed homogenate in 10 ml incubation medium. Although subsequent incubations were usually performed immediately, the homogenate may be refrigerated for as long as 1 hour prior to subsequent incubation without loss of activity. One ml of each agent was added to the liver homogenate suspension. Solid materials were made up to 1 ml in 0.9% NaCl. The HIFC preparation used‡ was potent by Schilling-type testing(6) in a daily oral dose of 5 mg. Unless otherwise specified, 25 μg HIFC was used. The amount of Co^{60}-B_{12} added to each sample was 0.01 μg (10^{-8} g) (specific activ-

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