TABLE I. Effects of Chorionic Gonadotropin on Testes and Secondary Sex Glands of Male Rats Deficient in Essential Fatty Acids. Six animals per group.

Dietary group	Final body wt, g	Testes wt, g	Prostate wt, mg avg	Prostate wt, mg range	Seminal vesicle wt, mg avg	Seminal vesicle wt, mg range
		Saline series				
Deficient rats	198.8	2.222	165	(122-215)	510	(256-873)
Linoleic supplemented controls	268.5	2.679	249	(208-294)	1154	(979-1419)
		Chorionic series				
Deficient rats	182.8	1.843	353	(243-466)	959	(452-1903)
Linoleic supplemented controls	273.2	2.573	378	(315-428)	1390	(1090-1869)

Results of the present experiment suggest that the reduction in prostate and seminal vesicle weights of rats fed a diet deficient in essential fatty acids was due to insufficiency of pituitary gonadotropin (interstitial cell stimulating hormone) secretion. This is indicated by the fact that gonadotropin stimulation restored the weight of these organs to normal. Available data, however, do not indicate whether this insufficiency was absolute or relative and to what extent, if any, the sensitivity of the interstitial cells of the testes or the target organs of its secretion may have been altered as a consequence of essential fatty acid deficiency. Caloric restriction *per se* does not appear to be the cause of the deficient gonadotropin secretion inasmuch as the food intake of rats fed the deficient diet was not less than that of animals in group I. It is possible, however, that the reduced efficiency of food utilization in the deficient rats resulted in an inanition effect despite the apparent adequacy of calories consumed.

Summary. Rats deficient in essential fatty acids exhibited a marked reduction in prostate and seminal vesicle weight. Administration of either methyl linoleate or chorionic gonadotropin restored these organs to normal weight.

The authors are indebted to Miss Lilla Anisfeld for her technical assistance.

Received October 8, 1951. P.S.E.B.M., 1951, v78.

Aureomycin and Terramycin in Human Saliva.* (19138)

FREDERICK W. KRAUS, DIANA W. CASEY, AND VILMA JOHNSON.
(Introduced by David Rapport.)
From Tufts College Dental School, Boston, Mass.

Many pathogens multiply in the oral cavity before spreading further. Antibiotics present in saliva might conceivably be effective in preventing the spread of microorganisms. However, little is known concerning the mean concentrations of antibiotics excreted in saliva of human subjects, and the changes in levels with time. Abraham *et al.*(1), Struble and Bellows(2), and Cutting *et al.*(3) detected penicillin in the stimulated saliva of medicated animals. Long(4) observed changes in the bacterial flora of the mouth and inferred from this that penicillin was excreted into human saliva. Direct measurements of such excretion have not been reported.

* This study was supported by a grant from the King Foundation. The aureomycin was kindly supplied by Dr. S. M. Hardy of Lederle Laboratories Division, American Cyanamid Co.; the terramycin through the courtesy of Dr. E. R. Weyer of Charles Pfizer and Co.

1. Abraham, E. P., *et al., Lancet,* 1941, v2, 177.
2. Struble, G. C. and Bellows, J. G., *J.A.M.A.,* 1944, v125, 685.
3. Cutting, W. C., *et al., J. Pharm.,* 1945, v85, 36.
4. Long, D. A., *British Med. J.,* 1947, v2, 819.

TABLE I. Saliva Levels of Antibiotic Following Administration of Aureomycin or Terramycin Capsules to 32 Subjects.

Total dose (g)	Time of assay after last dose (hr)	No. of subjects	Range (μg/ml)	Mean (μg/ml)	Stand. error of mean
.5	2.5	31	<.06- .5	.13	.012
3.0	2.5	32	<.13-2.0	.42	.052
3.0	26.5	25	<.06- .5	.11	.026

TABLE II. Saliva Levels of Antibiotics Following Administration of Aureomycin or Terramycin Troches to 10 Subjects.

	1 min.*	10 min.	30 min.	1 hr	3 hr	24 hr†	27 hr	28 hr‡	30 hr
Range, μg/ml	80-640	20-80	10-40	5-20	.5-10	0	1.0-12.5	0-1.0	0-.25
Mean, μg/ml	260	51.5	20.5	11.2	4.4	0	4.98	.5	.1
S.E.	52.3	7.0	2.73	1.27	1.0	0	1.32	.03	.003

S.E.—Stand. error of the mean.
*—After first troche.
†—Second troche administered following this sample.
‡—Test made after lunch.

We found that following the ingestion of aureomycin or terramycin in capsule form, the saliva becomes inhibitory to *B. cereus*(5), presumably due to the salivary excretion of the antibiotics. In order to ascertain the source of the antibiotics in saliva we catheterized the parotid and submaxillary ducts of 4 subjects who had been medicated with aureomycin capsules. Both parotid and submaxillary saliva gave positive assays. It appeared that the major salivary glands excrete the antibiotic. In the present study, systemic and topical administrations of both drugs were compared by evaluating their saliva concentrations and their inhibitory effect on salivary microorganisms.

Method. Dosage and sampling. A. *Capsules.* Each subject took two 0.25 g capsules of antibiotic every 4 hours until a total of 3 g was consumed. Samples of blood and unstimulated saliva were collected 2½ hours after the first dose, 2½ hours after the last dose, and 24 hours later. In some cases a control sample of unstimulated saliva was obtained before medication. The serum and saliva samples were assayed for contents of antibiotic and the number of bacteria present in saliva was determined. B. *Troches.* Each subject allowed one 15 mg troche to dissolve in his mouth on each of 2 successive mornings. On the first day, saliva samples were collected 1 minute, 5 minutes, 10 minutes, 30 minutes, 1 hour, and 3 hours after the troche was completely dissolved. On the second day, samples of saliva were obtained 3 hours, 4 hours, 5 hours, and 6 hours after dissolution of the troche. A control sample of unstimulated saliva was collected before each medication. The specimens were used for determinations of antibiotic concentration and for bacterial counts.

Assays. A tube dilution method(6) testing inhibition of *B. cereus No. 5 var. mycoides*† was employed and all specimens assayed at two different dilutions. Aureomycin standard endpoints were accepted only when they occurred in the fourth or fifth tubes. All samples were kept under refrigeration until ready for incubation except for the short time necessary for dilution and inoculation. The data for assay readings reported in Tables I and II are calculated from the observed values without any correction for the error of the method. The latter has been shown to range from zero to +400%(6). For statistical handling of data, values below the range of a particular assay were counted as zero, though they may have amounted to as much as .25 μg/ml.

5. Kraus, F. W., *J. D. R.*, 1951, v30, 495.

6. Kraus, F. W., 1951, in press

† The original strain was kindly supplied by Mr. A. C. Dornbush of Lederle Laboratories Division.

TABLE III. Colony Counts ($\times 10^7$) of Viable Bacteria in Saliva Before and After Administration of Aureomycin or Terramycin Capsules to 12 Subjects.

		.5 g		3.0 g		24 hr later	
	Before	Test	Control	Test	Control	Test	Control
Range	3.6-65	3.6-13.4	4.2-33	.2-7.2	2.3-100	1.6-16.9	2.3-100
Mean	15.40	7.77	12.70	3.16	31.10	9.37	31.10
S.E.	2.57	.85	1.91	.70	2.73	1.60	2.73
t_1*		2.82 (1% Sig.)		4.59 (1% Sig.)		2.02 (5% Sig.)	
t_2†			2.51 (5% Sig.)		9.70 (1% Sig.)		6.90 (1% Sig.)

* Comparing estimates following medication with those before medication (test vs. before).
† Comparing estimates at same hour of different days (test vs. control).

Bacterial counts. Saliva specimens were diluted serially by tenths in tryptic digest broth and duplicate 0.1 ml amounts of the third, fourth, fifth, and sixth dilutions were plated out with 5% horse blood agar. The poured plates were incubated at 37°C for 24 hours, and the colonies were counted with the aid of a Quebec colony counter according to standard methods(7). Two sets of controls were employed in the series testing the effect of capsules (Table III). The customary pre-experimental count served as one reference; the other consisted of colony counts repeated at the hours of the test on another day, with the same subjects, but without medication.

Results and discussion. Assays following administration of capsules. (Table I). Twenty subjects were studied with aureomycin and 12 with terramycin. Among 88 saliva assays, 29 were negative, *i.e.*, their concentrations were below the range of the particular assay. Among the remainder no statistical difference appeared that would justify separate tabulation of the aureomycin and terramycin series. Despite the failure in 29 assays to obtain demonstrable levels of antibiotic in saliva, the average effect was positive. The mean level was significantly different from zero at all 3 instances and, according to the Student's "t"-test, the probability of a difference was 99% or better. The antibiotic concentrations in plasma ranged from 4-10 times as great as in saliva. If the salivary glands were able to concentrate the antibiotic, we should have found levels higher in saliva than in blood. This happened only twice among 120 parallel determinations. The mode of excretion, therefore, is unlike the excretory mechanism of the kidney. The ratio of concentration in saliva to the concentration in plasma seems comparable with the proportion described for penicillin excretion in human milk(8).

Assays following administration of troches. (Table II). Five test subjects were examined with each of the 2 antibiotics. Since no statistical difference between the series was observed, they were treated together. The concentration was very high originally, dropped steadily during the 3 test hours, and no antibiotic could be detected at the end of 24 hours. After another troche, the 3 hour levels were comparable to those of the first day, which fact reaffirms the absence of any traces of antibiotic after 24 hours. Following ingestion of food the antibiotics were still detectable in 9 out of 10 cases, while 2 hours later only 4 concentrations were assayable.

Inhibition following administration of capsules. (Table III). Six subjects were tested with each antibiotic and the colony counts tabulated together. Notwithstanding the wide variation in control counts, the reduction in viable salivary bacteria following medication appeared to be highly significant. It was noticeable 2½ hours after the first .5 g dose, it became very obvious after the full dosage, and in 7 out of 10 cases it persisted for 24 hours thereafter. Highest mean inhibition

7. American Public Health Association, *Standard Methods for the Examination of Dairy Products*, 1948, 9th edition.

8. Florey, H. W., *et al., Antibiotics*, Oxford Univ. Press, 1949, 291.

TABLE IV. Colony Counts ($\times 10^7$) of Viable Bacteria in Saliva Before and After Administration of Aureomycin or Terramycin Troches to 10 Subjects.

	Before	1 min.*	10 min.	30 min.	1 hr	3 hr	24 hr†	28 hr‡
Range	1.53-15.2	.21-4.8	.19-3.2	.04-2.3	.05-1.4	0-3.6	.13-8.8	0-1.1
Mean	5.19	1.44	1.17	1.01	.68	.97	2.28	.31
S.E.	1.43	.41	.34	.19	.08	.34	.86	1.29
% Red.	0	72.6	77.4	80.4	86.6	81.3	58.0	94.0
Signif. %		5	5	1	1	1	N.S.	5

S.E.—Stand. error of the mean.
*—After first troche.
†—Second troche administered following this sample.
‡—Test made after lunch.

amounted to 79.5% and 89.8% of the two mean base lines, respectively. The highest number of microorganisms per ml of saliva counted was 1×10^9, the lowest under medication was 2×10^6.

Inhibition following administration of troches (Table IV). Five subjects were tested with each antibiotic. Within the first minute after dissolution of an aureomycin or terramycin troche a reduction in colony counts became apparent. The difference between the mean test count and the mean pre-experimental control count was statistically significant. The individual deviations from the mean narrowed under medication. After some fluctuations, the estimates among 9 out of 10 subjects reached their lowest point 1 hour later. At this point the mean reduction amounted to 86.6%. Bacterial counts of saliva usually rise gradually between meals. The reductions observed in our tests occurred at periods when the bacterial population would normally be increasing. Feirer and Leonard(9) have shown that bacterial counts of saliva increase almost 300% over a fasting period of 7 hours and others(10-12) have found that the counts are lower after meals than before.

Inhibition following administration of two troches consecutively (Table V). One group of 8 subjects received one troche of aureomycin, and another group was given a troche of terramycin. One hour later the mean estimates of their salivary microorganisms were significantly reduced and comparable to those in the preceding series (Table IV). Immediately thereafter, all subjects received another like troche to test for "additive" action. Five minutes after dissolution of the second troche 15 of the 16 bacterial counts showed another significant decrease. The mean total reduction at this point was 97.1% and the mean number of organisms per ml of saliva was reduced from 67 million to fewer than 2 million. The count recorded as zero showed fewer than 10,000 microorganisms per ml. It is of practical interest that this high degree of inhibition persisted unchanged for the remaining hour of the test.

Summary. (1) Aureomycin and terramycin are excreted in saliva when administered by mouth in capsule form. (2) The salivary glands do not concentrate the material excreted from plasma. (3) On an average, salivary excretion lasts for 24 hours after capsule medication has been discontinued. (4) A 3 g dose reduces bacterial counts in saliva, and the inhibitory effect seems to last

TABLE V. Colony Counts ($\times 10^7$) of Viable Bacteria in Saliva Before and After Administration of Two Consecutive Troches of Aureomycin or Terramycin to 16 Subjects.

	Before	1 hr*	5 min.†	1 hr†
Range	.97-36.7	.12-1.75	0-1.39	.03-3.50
Mean	6.72	.824	.192	.184
S.E.	1.31	.158	.053	.032
% Red.	0	84.8	97.1	97.3
Signif.		1%	1%	N.S.

* After dissolution of first troche.
† After dissolution of second troche.

9. Feirer, W. A., and Leonard, V., *D. Cosmos*, 1931, v73, 338.
10. Crowley, M. C., and Rickert, U. G., *J. Bact.*, 1935, v30, 395.
11. Florestano, H. L., *et al., J. Bact.*, 1941, v41, 605.
12. Sandy, C. E., and Bulate, L., *Austral. J. Dent.*, 1950, v54, 18.

for 24 hours. (5) One 15 mg troche decreases bacterial counts significantly for at least one hour. (6) A second troche administered one hour after the first adds considerably to the antibacterial effect.

We wish to express our appreciation to Miss Constance M. Marginot for her technical assistance; and to Mrs. Joan Keyser for the statistical handling of our data.

Received July 30, 1951. P.S.E.B.M., 1951, v78.

Liver Storage of Vitamin B_{12} by the Rat.* (19139)

HAROLD E. SCHEID, MONA M. ANDREWS, AND B. S. SCHWEIGERT.

From the Division of Biochemistry and Nutrition, American Meat Institute Foundation, and Department of Biochemistry, University of Chicago, Ill.

Assays for vit. B_{12} by the use of rat and chick growth, or microbiological methods, are well known. Although available information (1,2) indicates that the amount of vit. B_{12} in the liver or other organs was lower for animals fed a vit. B_{12} deficient diet as compared to a supplemented diet, it was of interest to extend these studies and to determine the vit. B_{12} potency of the livers of rats fed graded amounts of vit. B_{12}. Further, the liver storage of the vitamin, when fed as the crystalline vitamin or as a food, could be evaluated as a method for determining the vit. B_{12} potency of foods and could be compared with data obtained by the microbiological and animal growth methods. These studies have been conducted, and data have also been obtained on the effect of the level of vit. B_{12} fed on the liver weight to body weight ratio.

Experimental and results. Weanling male rats of the Holtzman strain were fed a basal ration (corn-soybean oil meal-iodinated casein) without added vit. B_{12} as a depletion ration for a period of 2 weeks. The rats were then randomized into groups and fed either 0, 3, 6, 9, 15 or 24 μg of crystalline vit. B_{12} per kilo of ration or crude supplements of unknown vit. B_{12} potency for a period of 4 weeks. The ration and procedure used have been described previously(3). Seven rats were used in each group. After completion of the rat growth assay, all animals from selected groups were retained for studies on the storage of vit. B_{12} in the liver. The animals were sacrificed by decapitation, the livers removed and weighed. Two gram samples were homogenized in a Waring blendor with 50 ml of 0.1 M phosphate buffer, pH 6.8, for one minute, then autoclaved for 5 minutes at 15 lb pressure. After autoclaving, the samples were cooled, made to 100 ml volume, filtered and stored in the refrigerator. Aliquots of these samples were taken for microbiological assay according to the method previously described(4,5). The enzymatic digestion step was eliminated since investigations with short-term autoclaving of the samples as compared to subsequent enzyme

TABLE I. Vitamin B_{12} Content of Rat Livers As Assayed Using *Lactobacillus leichmannii.*

μg B_{12} added/kg ration	μg B_{12}/ g liver*	μg B_{12}/ liver	Liver wt (% of body wt)
0	.026 ± .005	.200	6.20
3	.030 ± .003	.306	6.49
6	.037 ± .011	.361	6.09
9	.047 ± .009	.474	5.77
15	.048 ± .006	.442	5.20
24	.066 ± .017	.621	5.00

* Mean and stand. dev.

* We are indebted to Merck and Co. for supplying the vit. B_{12} used in these studies. Journal Paper No. 45, American Meat Institute Foundation.

1. Lewis, U. J., Register, U. D., and Elvehjem, C. A., PROC. SOC. EXP. BIOL. AND MED., 1949, v71, 590.

2. Richardson, L. R., Witten, P. W., and Couch, J. R., PROC. SOC. EXP. BIOL. AND MED., 1951, v76, 265.

3. Scheid, H. E., McBride, B. H., and Schweigert, B. S., PROC. SOC. EXP. BIOL. AND MED., 1950, v75, 236.

4. Scheid, H. E., and Schweigert, B. S., *J. Biol. Chem.*, 1950, v185, 1.

5. Scheid, H. E., and Schweigert, B. S., *J. Biol. Chem.*, 1951, v193, 299.