test appears to reflect the clinical severity of the disease.

4. These data partially confirm the observations of Rose and his associates but indicate that the differential sheep cell agglutination test is of limited value as an aid in the diagnosis of active rheumatoid arthritis of mild severity.

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IV. Ineffectiveness of Certain Essential Nutrients in Prevention of Tooth Decay in Cotton Rat Molars.

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The molar teeth of cotton rats (*Sigmondon hispidus hispidus*) have been found to be susceptible to the initiation and development of carious lesions when the rats were maintained on purified rations for 10 weeks or more after weaning.[1,2] The basal rations used in these studies were believed to be adequate in known essential nutrients in so far as the growth and maintenance of rodents were concerned. When the fat, or the protein content, or both, were increased isocalorically at the expense of sucrose, weanling cotton rats fed the resulting rations developed a much lower average number and average extent of carious lesions than the littermates fed the basal ration for the same period.[3,4] Before extensive use of this ration and its various modifications in the investigation of the effect of diet during or after tooth development on the susceptibility to tooth decay, it seemed mandatory to determine whether superabundant amounts of known essential nutrients, or the same nutrients from different sources, would alter the initiation and development of carious lesions in cotton rats when the molar teeth were fully formed and were known to be highly susceptible to dental caries at the beginning of the experimental period.

Experimental. The purified ration (100) used in most of these studies has been described in detail.[5] In a few of these studies, a slight modification (130) of the basal ration was used which was made by the isocaloric replacement of 18 of each 67 g of sucrose with 8 g of lard. Both diets were nutritionally adequate for the production of normal growth and development of the cotton rat.

The cotton rats used in these investigations were raised in our stock colony and weaned at about 21 days of age. At this time, the first and second molars of the cotton rat are fully erupted and the crowns of the third molars are largely formed but have not erupted into the oral cavity. Experimental procedure was identical with that described for previous experiments.[5]

In the first experiment, a series of 7 groups of cotton rats was fed various combinations of water-soluble and fat-soluble vitamins in superabundant amounts as described in Table I. Parallel to each experimental group, a comparable group of control littermates was maintained on ration 100, except in the seventh group where ration 100 without liver concentrates was used.

1 Shaw, J. H., Schweigert, B. S., McIntire, J. M., Elvehjem, C. A., and Phillips, P. H., *J. Nutrition*, 1944, **28**, 333.

2 Shaw, J. H., Schweigert, B. S., Elvehjem, C. A., and Phillips, P. H., *J. Dental Research*, 1944, **23**, 47.

3 Schweigert, B. S., Shaw, J. H., Zepplin, M., and Elvehjem, C. A., *J. Nutrition*, 1946, **31**, 439.

4 Schweigert, B. S., Potts, E., Shaw, J. H., Zepplin, M., and Phillips, P. H., *J. Nutrition*, 1946, **32**, 405.

5 Shaw, J. H., *J. Dental Research*, 1947, **26**, 47.

TABLE I. Effect of Additions of Various Water-Soluble, Fat-Soluble Vitamins on Average Number, Average Extent Carious Lesions in Cotton Rat.

Ration No.	No. of cotton rats	Avg No. of carious lesions	S.E.M.*	C.R.†	Avg extent of carious lesions	S.E.M.*	C.R.†
100	7	29.6	2.1	.3	79+	10+	.4
100 + B-complex‡	7	28.9	1.8		86+	12+	
100	5	26.8	1.7	1.2	83+	7+	1.8
100 + 0.4% ascorbic acid	5	23.6	2.1		65+	7+	
100	5	24.8	4.2	.6	67+	18+	.2
100 + B-complex‡ + 0.4% ascorbic acid	7	27.6	2.7		71+	16+	
100	10	26.1	2.4	.3	73+	11+	.4
100 + ADEK§	11	27.1	2.6		78+	10+	
100	4	24.5	2.6	.9	74+	12+	.6
100 + 0.1% menadione	5	27.2	1.4		84+	9+	
100	9	33.0	1.0	1.2	110+	6+	.6
100 + 0.1% natural vitamin K_1	10	31.3	1.0		104+	8+	
100	12	26.7	1.1	.3	75+	6+	.4
100 + liver concentrates + 200 μg folic acid	11	27.2	1.0		79+	7+	

* Standard error of mean. † Critical ratio.

‡ To each kg of ration 100 were added an additional 10.5 mg of thiamine hydrochloride, 10.5 mg of riboflavin, 10.5 mg of pyridoxine hydrochloride, 75 mg of nicotinic acid, 60 mg of calcium pantothenate, 3.0 g of choline chloride, 3.0 g of inositol, and .9 g of para-aminobenzoic acid, *i.e.*, enough of each to make the final concentration 4 times the amounts normally supplied. In addition, 200 μg of biotin were added per kilo.

§ To each kg of ration 100 were added an additional 33 mg of beta-carotene, 9000 I.U. of irradiated ergosterol, 18 mg of 2-methyl, 1,4-naphthoquinone, and 150 mg of alpha-tocopherol, *i.e.*, enough of each to make the final concentration 4 times the amounts ordinarily supplied.

TABLE II. Effect of the Replacement of Purified Casein by Crude Casein, Blood Fibrin, and Lactalbumin and of Additions of *dl*-Tryptophane on the Average Number and Average Extent of Carious Lesions in the Cotton Rat.

Ration No.	No. of cotton rats	Avg No. of carious lesions	S.E.M.*	C.R.†	Avg extent of carious lesions	S.E.M.*	C.R.†
100	4	37.0	.9	.3	120+	6+	1.2
400 (crude casein)	4	35.8	3.0		104+	10+	
130	3	24.3	3.8	.7	57+	17+	.3
430 (crude casein)	3	21.0	2.9		51+	13+	
100	8	29.5	1.3	2.0	83+	7+	2.0
200 (blood fibrin)	8	32.5	.7		102+	6+	
130	8	18.0	2.5	.5	41+	10+	.3
230 (blood fibrin)	9	17.3	2.3		36+	8+	
100	10	32.2	1.2	.9	104+	7+	1.1
300 (lactalbumin)	10	30.1	1.9		90+	10+	
130	6	19.5	1.9	1.5	41+	9+	1.1
330 (lactalbumin)	6	24.5	2.7		57+	11+	
100	9	27.8	2.0	1.1	77+	9+	.6
100 + 0.5% *dl*-tryptophane	9	25.2	1.4		70+	8+	

* Standard error of mean. † Critical ratio.

TABLE III.
Effect of Mineral Alterations on the Average Number and Average Extent of Carious Lesions in the Cotton Rat.

Ration No.	No. of cotton rats	Avg No. of carious lesions	S.E.M.*	C.R.†	Avg extent of carious lesions	S.E.M.*	C.R.†
100	7	27.6	1.8	.9	85+	10+	.9
100 + 2% salt mixt.	7	25.7	1.1		75+	6+	
100	9	29.4	1.4	.6	96+	6+	1.1
1100 (sol. salt mixt.)‡	9	28.3	1.1		87+	6+	
100	6	28.7	1.9	1.2	86+	10+	1.2
1100 + 2% sol.‡ salt mixt.	5	24.4	3.1		69+	10+	
100	7	24.3	2.8	1.5	69+	11+	1.0
100 + 0.5% sodium oxalate	9	19.3	1.9		55+	8+	
100	5	23.8	1.9	1.9	64+	11+	1.0
100 + 1.0% sodium oxalate	6	18.2	2.2		48+	12+	

* Standard error of mean
† Critical ratio.
‡ Calcium lactate 490 g, anhydrous calcium chloride 710 g, monosodium phosphate 1050 g, anhydrous sodium carbonate 207 g, anhydrous potassium carbonate 480 g, anhydrous magnesium sulfate 100 g, ferric citrate 55 g. potassium iodide 1.6 g, manganese sulfate 9.0 g, anhydrous zinc chloride 4.0 g, anhydrous cupric sulfate 2.4 g, and cobaltous sulfate 0.2 g.

TABLE IV.
Average Number and Extent of Carious Lesions Observed in Cotton Rats Maintained for 14 Weeks on Ration 100, Keyes' Hamster Ration, and on Ration 100 Plus Yeast.

Ration No.	No. of cotton rats	Avg No. of carious lesions	S.E.M.*	C.R.†	Avg extent of carious lesions	S.E.M.*	C.R.†
100	4	33.5	1.5	5.3	106+	7+	7.0
Keyes'	5	16.8	2.6		36+	8+	
100-liver	7	33.7	1.4	3.0	110+	6+	2.1
100-liver + 8% brewer's yeast	8	28.0	1.5		89+	8+	

*Standard error of mean.
† Critical ratio.

In the second experiment, crude casein, blood fibrin, and lactalbumin were used as individual protein sources by the complete replacement of the "de-vitaminized" casein in ration 100, or in ration 130. In addition, the effect of a supplement of 0.5% *dl*-tryptophane to ration 100 was tested.

In the third experiment, a series of mineral alterations was made, as detailed in Table III.

In the fourth experiment, one group of experimental cotton rats was fed the ration* used by Keyes[6] for the production of carious lesions in the hamster. A second group of cotton rats was fed ration 100 with the liver concentrates replaced by 8% dried brewer's yeast.

Results. The results of the first experiment in which additional amounts of the water-soluble and fat-soluble vitamins were added to ration 100 throughout the 14-week experimental period are summarized in Table I. In all cases, the additional amounts of these nutrients tested, after development of the teeth was largely completed, did not significantly alter the average number nor the average extent of carious lesions. The rate of increase in body weight was not altered by any of these supplements.

The results of the second experiment, in which the "de-vitaminized" casein in rations 100 or 130 was completely replaced by crude casein, blood fibrin, or lactalbumin, and where ration 100 was supplemented by 0.5% *dl*-tryptophane, are presented in Table II. The use of crude casein, blood fibrin, or lactalbumin did not alter the susceptibility of these cotton rats to develop carious lesions under the circumstances in this experimental regimen. Likewise, a supplement of 0.5% *dl*-tryptophane did not alter the initiation and development of carious lesions when fed after tooth development was complete. Increases in body weight were not altered by these supplements, except in the case of those cotton rats fed blood fibrin. These animals gained slightly more rapidly during the early weeks of the experiment and attained slightly higher average final body weights.

The results of the mineral alterations in the third experiment are presented in Table III. A supplement of an additional 2% of the regular salt mixture, and the replacement of the regular salt mixture by a more soluble salt mixture at 3 and 5% did not alter the susceptibility to tooth decay when fed after tooth development was largely finished. The supplements of both 0.5 and 1.0% sodium oxalate to ration 100 seemed to reduce the average number of carious lesions and the average extent slightly, but not sufficiently to be judged statistically significant by the test used. Both groups of cotton rats fed sodium oxalate had a severe and prolonged diarrhea and did not gain weight as rapidly as their controls nor attain as high final body weights. These factors may have been responsible to some degree for any tendency to reduce the development of carious lesions.

When cotton rats were fed a ration described by Keyes[6] for production of a high incidence of carious lesions in the hamster, the average number and average extent of carious lesions were significantly lower than for littermates fed ration 100 during the same period (Table IV). Those lesions which did develop in cotton rats fed Keyes' ration were characteristic in location and in type with those observed in cotton rats after only 6 to 10 weeks on experiment. Unlike the hamster, no lesions were produced on the smooth surfaces of the molar teeth in the cotton rat. One possible explanation for the reduced dental caries attack rate in cotton rats fed Keyes' ration may be based upon the low food intake and low rate of growth observed. Due to the apparent dislike of this ration by cotton rats, more ration was scattered and wasted than was eaten. A similar effect of enforced food restriction on the initiation and development of carious lesions has been described previously.[7]

The replacement of the liver concentrates in ration 100 by 8% of brewer's yeast resulted in a small reduction, bordering on statistical

* Whole wheat flour 20%, cornstarch 25%, confectionery sugar 20%, whole powdered milk 30%, and ground alfalfa 5%.

6 Keyes, P. H., *J. Dental Research*, 1946, **25**, 341.

7 Shaw, J. H., *J. Am. Diet. Assn.*, 1948, **24**, 181.

significance in the average number and average extent of the carious lesions.

Summary. A variety of tests were made to determine if superabundant amounts of essential nutrients or optimal amounts of these nutrients from different sources would alter the initiation and development of carious lesions in caries-susceptible cotton rats after tooth development was almost complete. Negative results were obtained in all cases indicating that initiation and development of carious lesions in teeth formed before the beginning of the experimental period were not influenced by additional amounts of the known nutrients tested.

The ration described by Keyes for production of carious lesions in hamsters resulted in very poor growth and a low rate of initiation and development of carious lesions in the cotton rat. A supplement of 8% dried brewer's yeast to the purified ration produced good growth and permitted a slight reduction in the dental caries experience of cotton rats.

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Tuberculostatic Effect of Furacin *in vitro* and *in vivo*.*

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Furacin is a nitrofuran compound (5-nitro-2-furaldehyde semicarbazone) which has definite chemotherapeutic effects against certain infections *in vivo*, as well as bacteriostatic and bactericidal properties against gram-positive and gram-negative organisms in the test tube.[1-7]

This report presents the results of experiments designed to show the effect of Furacin on the growth of tubercle bacilli in the test tube, and on a tuberculous infection in guinea pigs.

To determine the influence of Furacin on the growth of the human type H37 Rv microörganisms, 4 different fluid media were employed: Proskauer and Beck's synthetic medium, Proskauer and Beck's medium with 10% human serum, Kirschner's synthetic medium, and the Tween-albumin medium of Dubos. They were dispensed in 5 cc amounts in 18x150 mm test tubes. The inoculum into each tube was 0.1 cc of a 7-day culture of H37 Rv microörganisms (about 0.03 mg dry weight) in Tween-albumin liquid medium. All determinations were made in triplicate.

Table I reveals that Furacin in a concentration of 1:40,000 produced a slight inhibition of growth of the organisms, whereas in a concentration of 1:20,000 it prevented any appreciable growth for 28 days.

* The Furacin was supplied through the courtesy of Dr. L. Eugene Daily of the Eaton Laboratories, Norwich, N. Y. (Lot No. 1050).

1 Dodd, M. C., and Stillman, W. B., *J. Pharm. and Exp. Therap.*, 1944, **82**, 11.

2 Snyder, M. L., Kiehn, C. L., and Christopherson, J. W., *Mil. Surgeon*, 1945, **97**, 380.

3 Cramer, D. L., and Dodd, M. C., *J. Bact.*, 1946, **51**, 293.

4 Neter, E., and Lamberti, T. G., *Am. J. Surg.*, 1946, **72**, 246.

5 Dodd, M. C., *J. Pharm. and Exp. Therap.*, 1946, **86**, 311.

6 Shipley, E. R., and Dodd, M. C., *Surg., Gynec., and Obst.*, 1947, **84**, 366.

7 Green, M. N., and Mudd, Stuart, PROC. SOC. EXP. BIOL. AND MED., 1947, **64**, 57.