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).

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TABLE I.
Effect of Furacin on the Growth of H37Rv Tubercle Bacilli in Four Different Types of Fluid Medium.

A. Proskauer and Beck's Synthetic Medium.

Furacin Conc.	Amt of growth in days 8	12	16	21	28
Control	+	+	+	2+	2+
1:80×1000	+	+	+	2+	2+
1:40 "	0	±	+	+	2+
1:20 "	0	0	0	0	0
1:10 "	0	0	0	0	0
1: 5 "	0	0	0	0	0

B. Proskauer and Beck's with 10% Human Serum.

Furacin Conc.	Amt of growth in days 4	8	12	16	22
Control	+	2+	3+	4+	4+
1:80×1000	±	2+	3+	3+	3+
1:40 "	±	+	2+	2+	3+
1:20 "	0	±	±	±	±
1:10 "	0	±	±	±	±
1: 5 "	0	0	0	0	0

C. Kirschner's Synthetic Medium.

Furacin Conc.	Amt of growth in days 8	12	16	21	28
Control	3+	3+	4+	4+	4+
1:80×1000	2+	2+	3+	4+	4+
1:40 "	+	+	2+	2+	2+
1:20 "	±	±	±	±	±
1:10 "	0	±	±	±	±
1: 5 "	0	0	0	0	0

D. Tween-albumin Liquid Medium.

Furacin Conc.	Amt of growth in days 4	8	12	16	22
Control	2+	4+	4+	4+	4+
1:80×1000	+	3+	4+	4+	4+
1:40 "	±	2+	3+	4+	4+
1:20 "	0	0	0	±	±
1:10 "	0	0	0	0	0
1: 5 "	0	0	0	0	0

Using an inoculum of 0.2 cc (twice the original amount), it was found that a 1:20,000 concentration of Furacin still inhibited growth of the organisms. The H37 Rv culture which had been made resistant to streptomycin exhibited the same sensitivity to Furacin as the original streptomycin-sensitive strain.

Fig. 1 illustrates the effect of different concentrations of Furacin on the turbidity produced in Tween-albumin medium by the H37 Rv culture, as determined in the Klett-Summerson colorimeter. The technic was the same as that described previously for streptomycin.[8] Here again, it will be observed that Furacin in a 1:40,000 dilution produced slight inhibition of growth of the organisms, whereas the drug in a 1:20,000 dilution prevented the development of turbidity for 11 days.

Furacin exerts a bactericidal action on tubercle bacilli, as well as a growth-inhibiting action. This was illustrated by inoculating 0.5 cc of a culture of H37 Rv into 25 cc of Tween-albumin medium in a small flask, which was then incubated at 37°C. Similar flasks were set up, containing Furacin in dilutions of 1:40,000, 1:20,000, 1:10,000 and 1:5,000. At various intervals, samples were removed, appropriately diluted with Tween-albumin medium, and 0.1 cc amounts spread evenly over the surface of a solid egg-yolk, potato medium contained in flat glass tubes. Each dilution was planted in triplicate. Colony counts were made after incubation of the slants (in a horizontal position) for 30 days.

Fig. 2 illustrates the results of the experiment. Furacin in a concentration of 1:20,000 produced only a small decrease in the number of viable cells after 7 days, but in a concentration of 1:5,000 the drug had rendered non-viable the vast majority of tubercle bacilli by the 7th day.

In contrast to the bactericidal effect of Furacin on multiplying bacilli, there was no killing action whatsoever when the flasks were kept in the refrigerator at 5°C.

Tubercle bacilli of the H37 Rv strain could not be made resistant to the action of Furacin in the test tube. This culture was inoculated into a series of tubes of Tween-albumin medium containing the following concentrations of the drug: 1:80,000, 1:40,000, 1:20,000, 1:10,000, and 1:5000. After 10 to 12 days' incubation the medium in the tube containing the highest concentration of Furacin which allowed growth was used to inoculate a similar series of Furacin dilutions. At the end of

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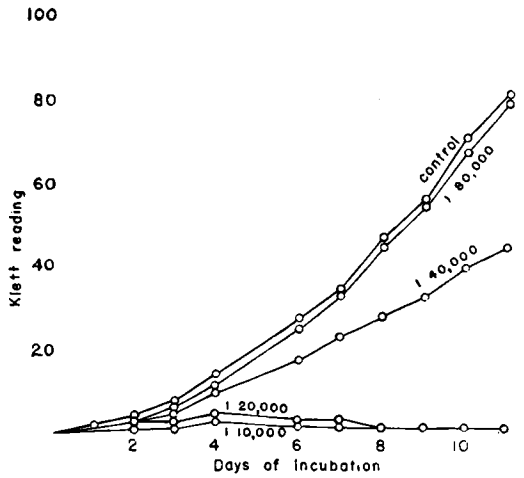

FIG. 1.

Effect of Furacin on growth of H37 "Rv" in Tween albumin medium.

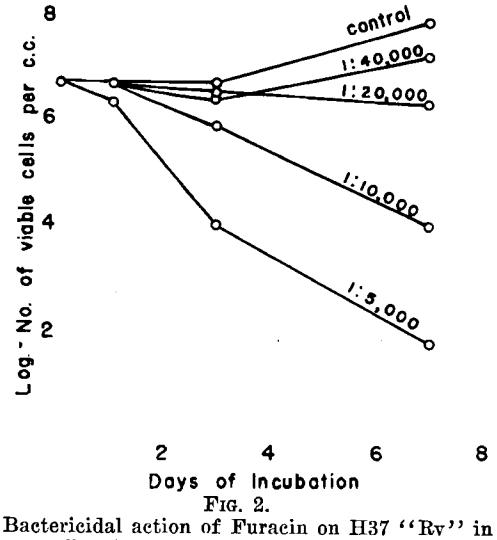

FIG. 2.

Bactericidal action of Furacin on H37 "Rv" in Tween albumin medium.

16 such transfers, growth of the culture was still inhibited by a 1:20,000 dilution of the drug.

The possible inhibitory effect of Furacin on the development of streptomycin resistance was tested *in vitro*. A sufficient amount of Furacin to make a final concentration of 1:50,000 was added to Tween-albumin liquid medium containing graded concentrations of streptomycin. Serial transfers of the H37 Rv culture were made to progressively higher concentrations of streptomycin in the usual manner, and the rate at which resistance developed was compared with a similarly handled culture in the absence of Furacin. It was found that the presence of Furacin in the medium delayed, but did not prevent, the development of streptomycin resistance by the tubercle bacilli.

In Vivo Tests in Guinea Pigs. Before initiating drug therapy, the toxicity of Furacin for guinea pigs was investigated. The drug was suspended in 10% gum acacia in a concentration of 150 mg per cc, and fed to normal pigs (average weight, 500 g) by means of a tuberculin syringe fitted with a blunt needle. It was found that 150 mg once a day, or 75 mg twice a day, killed all the animals used in the test within a period of 5 days. Guinea pigs fed 75 mg per day, either in 1 dose or 2, appeared very ill by the 10th day, and it was obvious that all the animals would have died shortly. The dosage was reduced to 37.5 mg once a day for 10 days. This treatment period was followed by a rest period of 10 days, after which time the drug was resumed. Three animals survived 28, 32, and 44 days, respectively, before succumbing to the toxic effects of the drug.

The major experiment was then begun. Thirty-five guinea pigs were infected with the human type H37 Rv culture. Each animal received 0.05 mg (dry weight) subcutaneously in the inguinal region. They were of mixed sexes and weighed between 600 and 900 g. Ten were kept as untreated controls, and 25 were started on Furacin treatment 6 days after infection.

The drug was suspended in 10% gum acacia in concentration of 75 mg per cc. Each treated animal was fed 18.75 mg at 12-hour intervals. After 11 days of treatment, several animals appeared listless and refused to eat. By the 13th day, 6 guinea pigs had died, and treatment was suspended for 17 days. Despite this rest period, another 5 animals died during the next 6 days, evidently of Furacin toxicity.

When Furacin was resumed 17 days later, the pigs had recovered sufficiently to appear healthy and to have good appetites again. The drug was then given in dosage of 18.75 mg once a day until the experiment was terminated by sacrificing all survivors 70

TABLE II.
Results in Guinea Pig Tuberculosis Treated with Furacin.

G. pig No.	Fate	Days of infection	Days of* treatment	Amt of tuberculosis grossly at autopsy
Controls				
1	Died	19	0	6+
2	"	63	0	9.5+
3	"	66	0	7.5+
4	"	67	0	14+
5	Killed	71	0	10+
6	"	"	0	9+
7	"	"	0	11+
8	"	"	0	8+
9	"	"	0	9+
10	"	"	0	7.5+
			Avg‡	9.1+
Treated				
1	Died	13	7	0
2	"	17	11	1+
3	"	18	12	4+
4	"	"	"	5.5+
5	"	19	13	3+
6	"	"	"	3+
7	"	21	15	4.5+
8	"	22	16	3+
9	"	23	17	3+
10	"	25	19	4+
11	"	"	"	4.5+
12	"	53	47	12+
13	"	56	50	6+
14	"	61	55	7+
15	"	67	61	7+
16	Killed	70	64	10+
17	"	"	"	6.5+
18	"	"	"	7+
19	"	"	"	12+
20	"	"	"	10.5+
21	"	"	"	11+
22	**	"	"	10+
23	"	"	"	10+
24	"	"	"	9.5+
25	"	"	"	9.5+
			Avg‡	9.6+

* Including rest period of 17 days without Furacin after the first 13 days of treatment.
‡ Average of those animals killed at the termination of the experiment.

days after infection, or 64 days after Furacin treatment was begun.

The results of this experiment are shown in Table II. The amount of tuberculous disease in the spleen, liver, lungs, and lymph nodes of each pig was graded from zero to 4+, according to the gross appearance. Thus, the maximum disease for an animal was 16+. The average degree of tuberculosis of the 6 controls which survived the full period of 70 days was 9.1+, whereas the corresponding figure for the 10 Furacin-treated animals which were killed at the termination of the experiment was 9.6+ It was thus demonstrated that Furacin, in the comparatively low dosage necessary because of the evident toxicity for guinea pigs, had no tuberculostatic effect on this infection produced by H37 Rv organisms.

Discussion. The experiments herein described reveal that Furacin is a compound which exerts definite bacteriostatic and bactericidal action on virulent human tubercle bacilli in the test tube; upon the progression of a tuberculous infection in guinea pigs, however, the drug appears to have no inhibitory effect.

There are some possible explanations for

this failure *in vivo*. Furacin is relatively insoluble and must be administered as a suspension of large particles which tend to settle out easily. It is not known how much is absorbed or what happens to the compound in the body. No Furacin can be demonstrated in the blood of animals to whom the drug has been given, and only a very small amount in the urine.[9] It is not known how much of the drug is converted to an inactive compound in the body of the guinea pig.

The best chemotherapeutic results in mice infected with Staphylococci, Streptococci, and Salmonella organisms were obtained by feeding 150 to 200 mg per kg daily for 3 days.[5] It was not possible to administer this amount of Furacin to guinea pigs without fatal results, and it was necessary to decrease the dose to about 35 mg per kg daily, which seemed to be the maximum tolerated dose for prolonged therapy. This relatively small amount of Furacin was probably not sufficient to produce a bacteriostatic or bactericidal level in the body of the guinea pig.

It was observed that the toxic effects of Furacin continued to be manifest for many days after the administration of the drug was stopped. On the regimen of 18.75 mg per day, the guinea pigs appeared healthy for 10 days, but then almost all began to show loss of appetite, listlessness, and loss of weight. Deaths started to occur on the 11th day. Even after the drug was withdrawn, some animals continued to decline and die for the next 5 or 6 days, before improvement in the appearance of the group was noted.

Conclusions. 1. Furacin exerts a bacteriostatic and bactericidal action on virulent human tubercle bacilli *in vitro*. In a concentration of 1:20,000 (5 mg %) it prevented growth for 22 days in 4 different types of fluid media. A concentration of 1:5000 (20 mg%) was required for bactericidal effect.

2. Resistance to Furacin was not evident after many transfers of the H37 Rv culture in drug-containing medium.

3. The combination of Furacin and streptomycin in Tween-albumin liquid medium delayed, but did not prevent, the development of streptomycin-resistance by the H37 Rv tubercle bacillus.

4. Furacin, in the maximum tolerated dose of about 35 mg per kg daily for 64 days, did not appreciably affect the course of tuberculosis produced in guinea pigs by the human type H37 Rv culture.

[9] Unpublished results, Eaton Research Laboratories.

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Effects of Adding Carbon Dioxide to Inspired Oxygen on Tolerance to High Altitudes.*

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When CO_2 is added to the inspired air at altitudes around 20,000 feet or to low oxygen mixtures, there is an improvement in the physiological condition which is due to an increase in the alveolar pO_2.[1-4] There is no general agreement, however, on the question

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