rather than antibody. Preliminary results indicate an increase in lysozyme in the blood of restim-treated mice.

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Chlortetracycline and Bone Demineralization in the Rachitic Rat.* (28338)

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Certain antibiotics have been found to produce unexpected growth responses in experimental animals. Attempts have been made to explain these unusual responses in a number of ways. One of the more common theories suggests that the antibiotic stimulates an increased synthesis of essential nutrients in the digestive tract of the test animal. Another theory suggests that the antibiotic favorably affects absorption of essential nutrients from the digestive tract. However, there is evidence that neither of these theories adequately explain some of the observations made in this area of investigation. It appears that studies in the realm of mineral metabolism offer possibilities of throwing further light on this phenomenon. This is because mineral elements are not expected to be synthesized in the digestive tract but possibly they could be rendered more available by the presence of certain antibiotics in the diet. The differences in metabolic availability of some minerals in the presence or absence of antibiotics may be rather easily measured.

Migicovsky *et al.*(4) reported that an increase in calcium absorption resulted when penicillin was incorporated into a low-calcium diet fed to growing chicks. Ross *et al.*(6) reported that inclusion of penicillin in the diets of growing chicks resulted also in a higher bone-ash value. Rusoff *et al.*(7) reported that administering Aureomycin to young calves (either orally or parenterally) resulted in greater skeletal growth and an increase in bone size. Murray *et al.*(5) found that incorporation of Aureomycin into rachitogenic diet resulted in higher "line test" values for bones from rachitic rats receiving sub-optimal doses of Vit. D. On the other hand, Hartsook(3) reported that incorporation of Aureomycin into purified diets adequate in all known dietary essentials except calcium resulted in no increase in body-calcium retention by growing rats. It is to be noted that the diets employed by this in-

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TABLE I. Effect of Chlortetracycline on Bone Demineralization in the Rachitic Rat.

Days subsisting on rachitogenic diet	14	21	28	35
Rats per experimental group				
Rachitogenic diet	14	22	18	12
" " + chlortetracycline*	12	22	18	12
" " + vit D_3†	8	8	8	7
Mean initial wt of rats in groups (g)				
Rachitogenic diet	45.4	44.9	45.5	45.3
" " + chlortetracycline	43.4	44.6	44.8	44.0
" " + vit D_3	45.1	43.7	44.2	44.9
Mean increase in wt of rats (g)				
Rachitogenic diet	17.7	33.2	35.9	45.3
" " + chlortetracycline	17.1	34.7	37.9	50.2
" " + vit D_3	18.5	37.1	46.3	59.6
Mean diet consumed per rat during period (g)				
Rachitogenic diet	91.5	152.6	187.4	255.3
" " + chlortetracycline	96.0	149.8	201.3	287.9
" " + vit D_3	95.1	157.6	214.8	296.5
Mean bone ash of femurs (%)‡				
Rachitogenic diet	41.2 ± 2.1	37.9 ± 2.9	34.8 ± 3.4	33.9 ± 2.7
" " + chlortetracycline	43.9 ± 1.9	43.3 ± 2.8	42.2 ± 2.6	41.6 ± 3.5
" " + vit D_3	46.8 ± 2.6	47.4 ± 1.3	48.9 ± 3.1	52.2 ± 2.8

* 1.0 g chlortetracycline per kg. † 400 I.U. vit D_3 per kg. ‡ Mean and standard error.

vestigator were at least adequate (2.83 I.U. per g) with respect to Vit. D.

Since Guerrant(1,2) and others have reported data which show that certain antibiotics have a supplementary effect on the growth-promoting capacity of sub-optimal doses of Vit. A or of the pro-vitamin A, beta carotene, and since the findings of Murray *et al.*(5) suggest that Aureomycin has a "sparing" or supplementary effect on Vit. D, it appeared likely that the biological effect of the antibiotics extend widely into the realm of the fat-soluble vitamins. Further studies in this area seemed worthwhile.

The present report concerns a study of the effect of dietary chlortetracycline on bone demineralization in growing rats subsisting on a rachitogenic diet.

Experimental. Weanling male rats from the same genetic source, 21 to 23 days of age and ranging in weight from 42 to 46 g, were arranged into 3 experimental groups and placed in individual all-metal cages having one-half inch raised screen floors. The rats of one group received the U.S.P. ('55) rachitogenic diet. The rats of the second group received the rachitogenic diet after incorporating into it 1 g of chlortetracycline (Aureomycin) per kg and those of the third group received the rachitogenic diet after incorporating into it 400 I.U. of Vit. D_3 per kg. All rats received the diet and distilled water *ad lib.* Food intake and body-weight changes were recorded weekly. At intervals during the experimental period rats, representative with respect to weight and litter origin, were removed from each group and sacrificed. The femurs were removed, labeled and stored in 95% ethyl alcohol until extracted. The bones were first extracted in a soxhlet apparatus with 95% ethyl alcohol for 8 hours and then for a like period with diethyl ether. The extracted bones were freed of flesh and connective tissue, placed in weighed crucibles, dried to constant weight in an oven set at 100°C, incinerated for 4 hours at 600°C and the amount of bone ash determined.

The feeding experiment was continued over a period of 35 days. The results were summarized and are presented in Table I.

Results and discussion. Femurs taken from 8 representative weanling male rats (21-23 days of age) at the beginning of the experiment were found to have a mean ash value of 44.6%. This value was in the normal

range for rats of this age weaned from mothers subsisting on the particular breeding-colony diet.

The rats comprising the 3 dietary groups showed very little difference in food consumption or in increase in body weight during the first 2 weeks of the experiment. However, as the experiment progressed, the difference in these respects became more pronounced. The same was true for the mean bone ash values of the 3 groups of rats. After having reached the respective diets for 14 days, the femurs from the rats that received the rachitogenic diet were found to have decreased in bone ash, those from rats that received the clortetracycline-supplemented diet showed little change in ash value while femurs from rats that received the Vit. D_3-supplemented diet increased in ash content. These changes also became more pronounced as the experiment progressed. In the course of 35 days, the femurs from rats subsisting on the rachitogenic diet decreased in ash to the extent of 24% and femurs from rats that received the antibiotic-containing diet decreased 7%, whereas femurs from rats that subsisted on the Vit. D_3-enriched diet increased in ash content to the extent of 18%. The differences in ash content of femurs from rats that received the rachitogenic diet and those from rats subsisting on the chlortetracycline-containing diet were found to be significant ($P<0.01$) at both the 28th and the 35th day sampling period.

These results further emphasize the diversity of the biological effects that certain antibiotics exert on animal life.

To provide comparative data, the third group of rats was included. The rats of this group received the rachitogenic diet after it had been supplemented with 400 I.U. of Vit. D_3 per kg. This amount of vitamin had been calculated to provide a daily intake ranging from 2.5 to 3.5 I.U., depending on food intake, an amount previously found to promote appreciable recalcification in rachitic rats under similar experimental conditions. However, when this dosage was made available to undepleted rats, the response was greater than anticipated. The mean ash value increased percentage-wise with each sampling period and by the 35th day had attained a value approximately normal for rats of that age subsisting on a well-balanced diet.

Summary. Weanling male rats were maintained on the U.S.P. rachitogenic diet, with and without chlortetracycline supplementation, for a period of 35 days. Representative animals were sacrificed at intervals and the femurs removed, extracted and ashed under comparable conditions. The resulting data show that incorporation of 1 g chlortetracycline per kg in the rachitogenic diet slowed the rate of mineral depletion from the bones (femurs) as measured by the conventional bone-ash procedure.

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