compared to survival times of 10 to 12 days in the present experiment where the dose was 350 r. The rate in our preliminary work in this study was approximately 6 r per mm in air. Work at the Univ. of Tennessee(5) using gamma irradiation from Co^{60} and Zr^{95}/Nb^{95} sources tends to rule out the possibility that lethal dose or survival time is influenced by body size. These workers, however, were reporting on dosage levels of 455 to 1,250 r, which are above the 350 r level used in this study.

The lack of visual symptoms in the pigs fed the Vit. A low ration may have been due to several factors. First, the level of carotene in the basal ration was higher than that used by other investigators, thus, a longer time was required to develop a low level of Vit. A in the blood and liver. Secondly, the pigs were not carried for as long a period, once the blood levels in the Vit. A low pigs had dropped below 7 μg of Vit. A per 100 ml of plasma.

The maintenance of appetite and fairly good rate of gain demonstrated by the Vit. A low pigs, however, agrees with the results of Frape *et al.*(10) and Hentges *et al.*(9).

Summary. 1. Pigs irradiated with 350 r bilaterally at the rate of 11 r per minute 9 feet from source survived an average of 254 hours. 2. A moderate Vit. A deficiency had no effect on irradiation damage; in fact, the Vit. A low pigs lived an average of 2 days longer. 3. Pigs fed low levels of Vit. A and with very low liver and blood Vit. A levels showed no gross manifestations of a deficiency of Vit. A. Feed intake and growth rate were fairly normal. 4. Irradiation lowered the total white blood cell count rapidly. 5. The major symptoms of irradiation damage were: (a) depression, (b) skin hemorrhages, (c) hemorrhaging from eyes and mouth, (d) increased respiration, (e) mild muscular spasms just prior to death, (f) extensive hemorrhage of lymph nodes plus varying amounts of hemorrhage in the kidneys, heart, urinary bladder, intestine and stomach.

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Efficiency of Antibiotics in Supplementing the Growth Effect of Beta Carotene in Vitamin A-Depleted Rats.* (27697)

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It has been shown(1) that chlortetracycline, incorporated into a Vit. A-deficient diet markedly influenced the growth stimulating effect of supplementary beta carotene or Vit. A in Vit. A-deficient rats, the magnitude of the influence depending on amount of antibiotic incorporated in the deficient diet and

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TABLE I. Comparative Influence of Some Antibiotics on Growth Effect of Beta Carotene in Vit. A-Depleted Rats. (1.0 μg of beta carotene fed daily.)

Antibiotics	Amt fed, g/kg diet	Rats/group ♂	Rats/group ♀	Mean depleted wt, g	Mean gain in wt, g*	Food per g/gain, g*	Approximate wt gain over control, %
Chlortetracycline[1] HCl	none	12		125.4	42.4 ± 4.51†	6.11	
"	1.0	14		124.9	58.7 ± 4.86	4.72	38
"	none		12	119.8	37.4 ± 2.89	6.58	
"	1.0		14	119.1	50.1 ± 4.16	5.27	34
Chloromycetin[2]	none	13		123.4	43.3 ± 3.93	6.50	
"	1.0	13		122.7	48.9 ± 2.61	5.05	13
"	none		14	119.5	36.8 ± 4.08	6.61	
"	1.0		13	119.1	43.5 ± 3.86	5.43	16
Demethylchlortetracycline[1] HCl	none	11	11	122.1	36.2 ± 4.71	6.87	
	1.0	11	12	121.6	48.1 ± 5.13	5.68	33
Procaine penicillin[3]	none	8		120.1	40.1 ± 3.06	6.43	
"	1.0	8		120.6	48.8 ± 1.76	5.67	22
"	none		13	119.4	37.5 ± 3.82	6.69	
"	1.0		15	118.5	46.8 ± 2.69	5.90	25
"Pro-Pen"[3]	none	18		122.4	42.9 ± 2.19	5.92	
"	1.0	17		122.9	47.1 ± 3.82	5.44	11
Sodium acrylate[1]	none	6	8	123.0	38.2 ± 4.11	6.57	
"	1.0	6	8	122.4	39.6 ± 3.29	6.39	4
Streptomycin[3]	none	6	6	126.4	39.4 ± 4.17	6.85	
"	1.0	7	7	123.0	40.9 ± 2.94	6.82	4
Terramycin HCl[4]	none	8	9	121.3	41.1 ± 3.97	6.50	
"	1.0	10	12	119.7	57.0 ± 4.89	5.32	36
Tetracycline HCl[5]	none	10	12	126.4	39.3 ± 3.84	5.98	
"	1.0	11	11	124.9	56.8 ± 2.96	4.61	44
Zinc bacitracin[6]	none	16		118.9	42.8 ± 5.01	5.93	
"	1.0	18		118.5	52.3 ± 4.62	5.09	22
"	none		18	115.6	36.4 ± 2.64	6.62	
"	1.0		20	114.8	44.8 ± 3.22	5.67	23

* During a 28-day test period. † Stand. error of mean.

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[2] Parke, Davis and Co., Detroit, Mich.
[3] Merck and Co., Inc., Rahway, N. J.
[4] Chas. Pfizer Co., Inc., New York, N. Y.
[5] Bristol Laboratories, Inc., Syracuse, N. Y.
[6] Commercial Solvents Corp., New York, N. Y.

on dosage of vitamin or provitamin fed. In addition, under the conditions of the study, the presence of the antibiotic in the diet enhanced the efficiency of food utilization for growth (weight increase). The present report concerns results obtained through extending the studies to include other antibiotics (see reference 1 for citations to related studies). Because of its greater stability under the conditions of the test, the provitamin (beta carotene) was used as the vitamin supplement.

Methods. The experimental procedure employed was essentially that previously reported(1). Weanling rats weighing between 40 and 46 g were depleted of their body-stores of Vit. A by maintaining them on the U.S.P. Vit. A-deficient diet† and distilled water until the test animals ceased to gain weight for at least 4 consecutive days. The test animals were maintained at all times in all-metal cages having raised screen floors (2-mesh per inch). When depleted, the rats were arranged into 2 or more groups comparable as to sex, litter origin, body-weight and stage of depletion. The antibiotics were mixed in the diets for the treated groups at the levels indicated in Table I. Food and distilled water were fur-

† U. S. Pharmacopeia XIV, 788.

nished *ad libitum*.

All rats received 1.0 μg (1.7 I.U.) beta carotene (in cottonseed oil) daily as the Vit. A supplement. Body-weight changes and food consumption records were kept. Supplementary feeding of the pro-vitamin continued for 28 days or until death intervened. The antibiotics studied, amounts incorporated in the basal diet and the comparative effect of their presence in the diet on food consumption and on growth of Vit. A-depleted rats are shown in Table I. Only responses of rats that consumed all of the beta carotene supplements and survived the test period have been included. Inasmuch as it had been found (1) that chlortetracycline (Aureomycin) exerted its maximum effect on the growth-promoting capacity of beta carotene and Vit. A in Vit. A-depleted rats when the antibiotic was incorporated in the basal diet at the level of 1 g/kg, this level was employed as the basis of comparison. However, it is recognized that under the conditions of the experiment, antibiotics other than chlortetracycline may not exert their optimum influence on growth at the 1 g/kg level. Since "Pro-Pen" was labeled as containing only 50% of procaine penicillin, this product was fed at both 1 g/kg and 2 g/kg levels.

Results and discussion. The presence of 1 g/kg of chlortetracycline in the basal diet resulted in a growth rate which exceeded that of the control rats by more than 30% (38% for males—34% for females). This increment, while appreciable, is somewhat less than that obtained previously(1) with an earlier sample of this antibiotic or that obtained with a third sample more recently tested. The reason for this apparent variation is unknown. Chloromycetin proved less effective in promoting growth than did chlortetracycline when incorporated in the diet at the 1 g/kg level. However, it should be pointed out that, possibly owing to its extreme bitterness, this antibiotic exerted a depressing effect on food intake thereby complicating its comparison with other antibiotics under the conditions of the test. Demethylchlortetracycline (Delcomycin) proved only slightly less effective in stimulating additional growth than did chlortetracycline. Procaine penicillin yielded growth responses considerably less than those observed when the same levels of tetracyclines were fed, but in the same order as those produced when zinc bacitracin was incorporated in the diet. The feeding of an impure form of procaine penicillin, "Pro-Pen", resulted in about one-half the rate of weight increase observed through feeding the pure antibiotic. Subsequent feeding of "Pro-Pen" at 2 g/kg of diet level resulted in growth responses in line with those produced by feeding a diet containing one-half the weight of the purer antibiotic. Sodium acrylate and streptomycin proved to be of questionable effectiveness in promoting additional growth in Vit. A-depleted rats at the 1 g/kg level.

Terramycin hydrochloride was found to be one of the more effective antibiotics in stimulating additional growth in rats receiving a limited intake of beta carotene, whereas tetracycline hydrochloride proved to be the most effective of the antibiotics thus far tested in stimulating additional growth. Zinc bacitracin was found to be about one-half as effective as the tetracyclines in stimulating additional growth at the level employed. More recent tests of magnesium bacitracin indicated that this form of the antibiotic exerted essentially the same effect on growth as did the zinc compound.

In all instances where the presence of the antibiotic in the diet enhanced the growth rate of the test animals, there was also an increase in the efficiency of food utilization for weight increase.

Summary. The capacity of some antibiotics to stimulate additional growth in rats receiving a sub-optimum intake of Vit. A (as beta carotene) has been determined. The additional growth response obtained under the conditions of these studies ranged from essentially no additional growth when sodium acrylate or streptomycin was used to a 44% increase when tetracycline HCl was incorporated into the Vit. A-deficient diet. While relationship of structure of the antibiotic to growth-stimulating capacity was not fully apparent, the tetracyclines as a group appeared to be the most effective in this respect. This may have been related to the specific level of antibiotic feeding in that the 1 g/kg level had

been found previously to be optimum when chlortetracycline was fed. This may not have been true for other antibiotics. The mode of action of certain antibiotics in the economy of Vit. A and beta carotene metabolism is being studied.

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A Study with Low and High Molecular Weights of Hexadimethrine Bromide—an Antiheparin Agent. (27698)

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Chemicals available for neutralization of heparin include toluidine blue, an organic dye; protamine sulfate, a polypeptide of marine origin; and, more recently, hexadimethrine bromide,* a synthetic polymer. Certain pharmacologic and toxicologic properties of hexadimethrine have been described(1-5). Experimentally, a basic question still remains as to the possible relationship between polymer size, toxicity, *in vitro* mast cell disruption and antiheparin potency.

This report is concerned with certain toxicologic and pharmacologic aspects of various polymer sizes of hexadimethrine. Our initial studies were carried out with polymers whose molecular weights were expressed as average values; subsequent studies were carried out with dialysate fractions of several of these polymers.

Methods and materials. Toxicity Studies. Acute toxicities were determined for each lot of hexadimethrine bromide in female Scientific strain mice weighing between 17-24 g, using 10 to 20 animals per dose level. Each lot was diluted in saline to 1 and 2 mg/ml concentration and injected at a rate of 1.2 to 2 ml/minute. Survivors were observed for 1 to 2 weeks. The LD_{50} and associated confidence limits were determined by the method of Litchfield and Wilcoxon(6).

Mast Cell Studies. Studies on the effect of hexadimethrine bromide on tissue mast cells were carried out *in vitro,* using mast cells from rat mesentery as test objects. This method, described previously by Norton(7), is an adaptation of the technic of Mota *et al.* (8). Female Holtzman rats, weighing between 150-250 g, were used in our study. Approximately 10 pieces of mesentery were obtained from each rat and immersed in various concentrations of hexadimethrine bromide for 30 ± 1 minutes. Pieces of mesentery from at least 3 different rats were used for each concentration. After fixing and mounting, 4 separate fields were selected under 125 × magnification. Each of these fields was re-observed under 250 × magnification for mast cell disruption, using the first 10 cells, reading clockwise from the upper left-hand corner. For each drug concentration, approximately 560-600 cells were examined.

Antiheparin Studies. The antiheparin potency of each lot of hexadimethrine was determined by using a modification of the U.S.P. XV assay method for heparin. Briefly, graded amounts of the polymer are added to a series of test tubes each containing fixed amounts of heparin and citrated sheep plasma. That tube forming a 50% clot is taken as the end point. Calculations with results obtained with a hexadimethrine standard run concurrently yielded antiheparin potencies expressed as per cent of the standard.

Molecular Weight Determinations. Light Scattering. Light scattering molecular weights were determined on a Brice-Speiser-Phoenix light scattering photometer, using light of 4358 A. The refractive index increment, dn/dc, at this wave length was 0.154 ml/g.

Sedimentation. Sedimentation data were obtained on a Spinco Model E Ultracentri-

* Polybrene®, Abbott Laboratories, North Chicago, Ill.