NUTRITIONAL EFFECTS OF ANTIBIOTICS

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It is logical to expect that the rate of growth of an animal will be increased when a pathogenic or debilitating infection is eliminated. This, in a nutshell, is the reason for feeding antibiotics. It was found that adding small quantities of antibiotics to the diets of young animals not only served to control certain obvious disturbances such as diarrhea and mild respiratory infections but also produced unexpected increases in the growth rate even when the animals were asymptomatic. It appeared that subclinical intestinal infections of an unsuspected and undefined nature were commonly present; perhaps the infecting organisms merely displaced "beneficial" bacteria. In any event, improvements in growth were noted in apparently healthy animals, and popular interest was aroused in the feeding of antibiotics. Before such a practice could become widely useful, the following conditions were necessary:

1) Microorganisms which slow down the rate of growth must occur commonly in the digestive tracts of animals.

2) These microorganisms must be readily eliminated, or their harmful nature must be changed, by the addition of a small amount of a suitable antibiotic to the diet. This produces the "antibiotic growth effect."

3) The harmful microorganism must not become easily reestablished in a form which is resistant to antibiotics.

These criteria have evidently been met and the addition of small quantities of antibiotics to animal feeds is now a common practice.

Attention was drawn to the specific nutritional functions of the intestinal bacteria in the early 1940's by the deficiencies produced in rats which were fed purified diets to which a sulfonamide had been added (18, 134, 67, 19, 199, 122). The deficiencies reflected the production of certain vitamins, including biotin, folic acid and vitamin K, by the intestinal bacteria in the absence of sulfonamides. These vitamins had been omitted from the basal diet because they were unknown or were thought not to be needed by rats. The observations may have led to an attitude among students of nutrition that the intestinal flora had a predominantly beneficial effect on the nourishment of "normal" healthy animals.

There were, however, a few reports that antibacterial substances had a favorable effect on growth. Martin (136) reported that rats died in 6 or 7 weeks after attaining weights of 50 to 70 gms. on a purified diet containing seven B-complex vitamins. When 1 mg. of sulfanilamide was fed daily the rats varied in weight from 90 to 160 gms. at 6 weeks. Ascorbic acid, p-amino-benzoic acid and sulfasuxidine were all found to produce slight growth increases in an experiment with chicks on a purified diet in which six chicks were used per group (27). Moore and co-workers (144) in a single experiment with chicks on a basal purified diet noted that sulfasuxidine and streptomycin singly or in combination led to increased growth responses. The authors stated that the observation suggested the inhibition of intestinal bacteria that are either producing toxic materials or are rendering certain dietary vitamins unavailable to the animal, but that the possibility that the agents were acting systemically could not be overlooked. An observation by Morehouse and Mayfield (145) that 3-nitro-4-hydroxyphenylarsonic acid, which is not an antibiotic but rather a chemical with antibacterial activity, produced increased growth in chickens and turkeys when added to natural diets was made at about the same time. The finding described by Moore *et al.* appeared not to have been pursued and no further reports of growth-promoting effects of antibiotics were made until 1948 when Harned *et al.* (91) in an experiment with 12 chicks reported that the addition of aureomycin[1] increased the growth rate and attributed the effect to the elimination of some infection. The present interest in antibiotics as additives to animal feeds stems from investigations of *Streptomyces aureofaciens* as a source of vitamin B_{12} by Stokstad and co-workers (83). They found that a response was produced in chicks by adding crude preparations of *S. aureofaciens* culture materials to diets of natural foods even when vitamin B_{12} was present in adequate amounts in the basal diet. Brewers' dried yeast and other materials failed to produce such a response, despite the fact that these materials have been stated to contain unidentified growth factors for chicks. The crude preparations containing the "new growth factor" were examined by other investigators who confirmed the findings and extended them to other species. The response was later shown to be due to the presence of surprisingly small amounts of aureomycin, and other antibiotics were active; the effect was observed in chicks, turkeys and pigs (180, 109, 131, 200, 35, 179). It could be concluded that there existed endemically in the intestinal tract of animals an infective process, hitherto unsuspected, which interfered with growth. The infective process could be removed by adding an appropriate antibiotic to the diet. Antibiotics thereupon became an ingredient used routinely in feeding the young of several species of the common domestic animals. The new practice soon spread to various parts of the world, and its economic importance may be gauged by the following comment (135):

> "Used as feed supplements, antibiotics have created a near-revolution in poultry and livestock raising. . . . In Missouri, young pigs on supplemented feed were raised for market with 7 % less feed and a 28 % greater rate of gain than an identical group on the same ration without the added trace of antibiotic. A further dividend conferred by antibiotic-supplemented feeds is a reduction in the number of runts or slow-growing pigs. In Iowa alone, a million and a half runts are born annually. Antibiotics promise to reduce this number 60 to 75 %."

Many scientific publications dealing with the effects of antibiotic feeding have appeared since March 1950. This review will deal with a small proportion of them, but exigencies of space have prevented us from including many important references.

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[1] The names Aureomycin and Terramycin are now the trademarks for antibiotics which have recently been given the names chlortetracycline and oxytetracycline respectively (199a).

MECHANISM OF ACTION

It seemed from the first that the effect of the antibiotics on growth was due to their antibacterial effect on the intestinal flora. The following lines of evidence, which will be first listed and then discussed, point in this direction:

1) The antibiotic growth effect is shared by a group of substances of diverse chemical characteristics. The only known property which these substances have in common is their antibacterial potency.

2) Aureomycin, penicillin and streptomycin were ineffective in promoting the growth of chick embryos when injected under sterile conditions (111). "Germ-free" chicks did not show a growth response to antibiotics and neither did chicks which were kept under conditions which tended to reduce or eliminate contact with bacteria (129, 53, 56), thus indicating that the growth response is dependent upon an antibacterial effect.

3) Injected aureomycin and penicillin showed at the best a weak growth-promoting action as compared with their effect in the diet (72, 38). Any growth-promoting effects produced by injection could be explained by the excretion of a part of the injected dose into the intestine (205, 60).

4) Bacitracin and streptomycin, which are not readily taken up from the gut, have a growth-promoting effect when added to the diet of chicks, thus implying that the effect is due to their activity in the intestine.

5) Despite its wide antibacterial potency, chloramphenicol has only a weak antibiotic growth effect, which may be due to the fact that this antibiotic is absorbed through the gastric wall and hence does not tend to reach the intestinal tract when added in small quantities to the diet.

6) Hydrolysis with penicillinase led to a disappearance of the growth-promoting activity of penicillin in chicks. The antibacterial potency was also destroyed by this treatment (107).

It would appear unlikely that compounds varying markedly in chemical constitution would simultaneously be found to exert a vitamin-like action in promoting the growth of animals. The antibiotics differ from each other with respect to their known biological effects on living cells. Streptomycin is involved in the oxalacetate-pyruvate reaction (185, 186), penicillin prevents the uptake of glutamic acid by bacterial cells (82), and aureomycin uncouples phosphorylation from oxidation (125, 28). The only known common property of the antibiotics is their antibacterial action which leads to the conclusion that the antibiotics promote growth by acting upon the intestinal microorganisms. The differences in growth-promoting properties among the antibiotics support this conclusion and reflect the differences in antibacterial properties. Aureomycin inhibits a wide variety of microorganisms and promotes growth of a wide variety of animals, *e.g.*, poultry, hogs, calves, mink, rabbits and trout. Penicillin and bacitracin affect a more narrow array of bacterial types and have a smaller growth-promoting effect on hogs and calves than that of aureomycin. Chloramphenicol shows behavior which appears anomalous in that this substance fails to promote growth of animals to the extent expected from its wide antibacterial spectrum.

However, it is absorbed from the stomach so rapidly that it fails to reach the intestine, the site of action.

The possible ways in which an antibiotic may favorably affect the intestinal flora and hence promote growth have been frequently mentioned since the original listing by Moore *et al.* (144). These possibilities may be rephrased and expanded as follows:

1) The antibiotics may inhibit or destroy organisms which produce subclinical infections, that is, they suppress organisms which produce toxic reactions and cause a slowing of growth of the host animal.

2) Antibiotics may produce an increase in the number or activity of organisms which synthesize certain known or unknown vitamins or growth factors which are eventually made available to the host.

3) Antibiotics inhibit organisms which compete with the host for available nutrients.

Good evidence is available to support the first possibility, however, some findings also support possibilities 2 and 3. Chick embryos treated under aseptic techniques with aureomycin, penicillin or streptomycin do not show a growth response (111). The chick embryo may be viewed as an animal without an intestinal flora. Chicks reared under aseptic conditions were found to approach the growth rate of antibiotic-supplemented animals and no further increase in growth rate was produced by the addition of antibiotics to the diet (129). Some investigators have attempted broad studies of the intestinal flora of chicks grown with and without antibiotics. Changes in certain groups of microorganisms were found, but there is little agreement among the various reports. Kratzer *et al.* (119) reported that poults fed streptomycin showed a five- to ten-fold increase in the number of yeasts in the excreta, whereas Williams *et al.* (203) found little or no change in the number of yeasts. Johansson *et al.* (103) reported an increase in intestinal enterococci from feeding aureomycin while Sieburth *et al.* (172) noted a decrease in coliforms and enterococci. Anderson *et al.* (1) and Moore *et al.* (144) found increases in lactobacilli following penicillin and streptomycin feeding, while Johansson *et al.* (102) noted a decrease in lactobacilli accompanying the feeding of aureomycin. Anderson *et al.* (103) and Moore *et al.* (144) differ as to the effect on the coliform group of organisms. Anderson *et al.* (1) reported an increase while Moore *et al.* (144) found a decrease in coliforms. An increase in coliforms, a decrease in anaerobes and no change in enterococci and lactobacilli as a result of penicillin feeding were the observations of Guzman-Garcia *et al.* (87). Johansson *et al.* (102) have made the most complete study on effects of antibiotics and diet on intestinal flora. It is interesting that some of the antibiotic effects which these authors noted earlier were changed by varying the carbohydrate or vitamin B_{12} level in the diet which may help to explain the many conflicting observations on changes in the intestinal flora.

Table I is an example of the differences which may be expected in broad groups of microorganisms as a result of feeding aureomycin (201). Many experiments showed that a consistent change in the coliform bacteria did not take place; however, the decrease in anaerobic bacteria appeared to be reproducible.

The effect on the intestinal flora reported most consistently was a decrease in the number of the anaerobic clostridia. This was first described by Sieburth *et al.* (171). An example of the decreases observed in the clostridia-type anaerobes is shown in Table II.

Since it is known that several species of this group produce toxins and can be counted on blood agar, an attempt to investigate the organisms responsible was made with the results shown in Table III. It was found that aureomycin completely eliminated the hemolytic clostridia; however, it is further apparent that the number of hemolytic clostridia was quite small. The feeding of high levels of live cells of these hemolytic toxin-producing clostridia did not reduce growth in chicks not receiving antibiotics. Cell-free toxins obtained from hemolytic clostridia failed to depress growth of unsupplemented chicks or chicks fed antibiotics. The toxin preparations, in fact, were slightly stimulatory. Thus, it appears that the observed decrease in numbers of the toxin-producing clostridia is merely a coincidental effect of antibiotic feeding and is not involved in the mechanism of the growth response.

TABLE I

Effect of ingested aureomycin on intestinal flora of chicks

MICROORGANISM	SUPPLEMENT	
	None	Aureomycin
Coliform bacteria	57,000*	14,000
Lactic acid bacteria	93,000	72,000
"Yeasts"	9,800	6,700
Anaerobic bacteria	15,000,000	100,000

* Number of colonies developing on plates per mg. wet feces.

TABLE II

Effect of dietary aureomycin and penicillin on Clostridia-type anaerobes in chick excreta

NO ANTIBIOTIC	PLUS AUREOMYCIN	PLUS PENICILLIN
15,000*	1,000	100
26,000	4,300	100
450,000	None	100
7,000	700	100
15,000	4,500	100
89,000	600	100
Avg. weight of chicks 273 gm.	320 gm.	315 gm.

* Number of colonies developing on plates per mg. of wet feces.

Other observations on general groups of bacteria were reported by Couch (62) who found that penicillin increased total bacterial count, the enterococcil yeasts and the penicillin-resistant bacteria in the feces. In general, the bacteria, counts of Romoser *et al.* (160) agree with those reported by Couch. These workers studied the cecal flora of chicks fed penicillin. The counts of *A. aerogenes* and

E. coli were markedly increased. However, it was shown that chicks in which the ceca had been ligated still showed the antibiotic growth response indicating that the cecal flora are not essential to this response (68). Baby pigs which gave a very marked weight increase as a result of feeding aureomycin, streptomycin and penicillin were studied by Wahlstrom *et al.* (192). No change was observed in the coliform, lactic acid bacteria or in the yeasts in the feces from these animals.

TABLE III

Effect of dietary aureomycin and penicillin on hemolytic anaerobes in chick excreta

NO ANTIBIOTIC	PLUS AUREOMYCIN	PLUS PENICILLIN
30*	None	20
7,100	None	None
5,900	None	70
15,000	None	None
15,000	None	10
700	None	10
Avg. weight of chicks 273 gm.	320 gm.	315 gm.

* Number of large areas of hemolysis developing on plates, per gram of wet feces.

Many workers have observed that chicks or pigs grown in new, uncontaminated quarters fail to show an antibiotic growth response. Unfortunately, much of these data have remained unpublished. However, the data of Coates *et al.* (52) are typical:

	WEIGHT OF CHICKS	
	Without Penicillin	With Penicillin
Uncontaminated quarters	181 gm.	183 gm.
Contaminated quarters	143 gm.	191 gm.

It is apparent that the lack of response to penicillin in uncontaminated quarters was because of the excellent growth of the unsupplemented animals. This lends strong support to the first possibility listed above, indicating that the presence of certain as yet unidentified organisms is necessary to produce a depression of growth in animals unsupplemented with antibiotics. This effect was also observed in experiments with pigs. Work in our laboratories showed that in the first experiment with baby pigs in new quarters both the control and aureomycin-fed pigs weighed approximately 21 kilos at 8 weeks of age. In the second and all subsequent experiments, the control pigs reached a weight of about 16 kilos at 8 weeks while the aureomycin-fed pigs averaged approximately 21 kilos (202). These findings recall the original observation by Speer and co-workers regarding the absence of an antibiotic growth effect in pigs in "sanitary" surroundings (178).

Coates *et al.* (51) studied the effect of penicillin on certain B-vitamin deficiencies. The antibiotics had no effect on deficiencies of thiamine, riboflavin, pyridoxine or pantothenate. Penicillin lessened the degree of deficiency of biotin and folic acid, whereas it intensified the symptoms of nicotinic acid deficiency. They also observed that vitamin B_{12} increased the severity of nicotinic acid deficiency. Linkswiler *et al.* (124) found that, with rats, aureomycin caused all three forms of vitamin B_6, pyridoxal, pyridoxamine and pyridoxine, to be equivalent in promoting growth on a deficient diet. Without aureomycin, pyridoxal and pyridoxamine were less effective. The effect of antibiotics on vitamin requirements will be discussed in greater detail in a later section. However, the data indicate that there is no clear-cut or consistent effect on the requirement for any of the known vitamins in any species that responds to antibiotics. Therefore, at the present time it does not appear that the mechanism of the antibiotic growth response is concerned with decreasing the amount of the known vitamins required or by promoting synthesis of them in the intestinal tract.

A suggestion that antibiotics promote growth by favoring the growth of certain organisms in the gut which in turn exert some favorable effect on the host is not without support (159).

Aerobacter aerogenes was found to increase in numbers in the ceca of chicks during the feeding of penicillin. This organism was isolated from the cecal contents and was grown on various media in large amounts and fed to chicks. A slight but consistent growth response was obtained on diets containing the viable organisms with or without the inclusion of an antibiotic in the diet. The increase of this organism in the cecal contents did not account for the antibiotic growth effect since in some experiments the antibiotic promoted further growth in the presence of supplements of the organism. Other workers (3, 2, 4) have observed that penicillin causes a shift in the cecal flora from normal *E. coli* strains to what they termed a mutant or atypical strain of *E. coli*. These atypical strains of *E. coli* were isolated and grown in large amounts and included in the chick diets. The feeding of these microorganisms, isolated from the ceca of penicillin-fed chicks caused an increase in chick weight. However, again a further increase in growth rate frequently followed the addition of peniclilin to the diet.

Although certain strains of yeasts as well as *E. coli* and *A. aerogenes* have been found in a few experiments to predominate in the gut during antibiotic feeding, the isolation of these organisms and subsequent feeding of large amounts of these organisms in the chick diet did not promote consistent nor great enough growth stimulation to support the theory that this shift in flora accounted for the growth-promoting effect of antibiotics.

At the present time, a consistent and widely observed phenomenon regarding the mechanism of antibiotic action is the observed depression in growth when animals under practical conditions are taken from an extremely clean environment to a contaminated environment. From the observations of Coates *et al.* (52), Bird *et al.* (17), Briggs (26) and Hill *et al.* (93) and as discussed above, it appears that antibiotics relieve a depression in growth which is caused by certain microorganisms contaminating the intestines and thus allow the animal to grow at what can be considered as a truly normal rate. However, it is yet to be ascertained whether this depression is a contamination with one or more specific organisms, or whether it is due to an increase in the number of organisms already present under clean conditions. The identification of the growth-depressing organisms will contribute to an understanding of the mechanism of the antibiotic growth effect.

An unusually high antibiotic growth response in pigs was associated with the presence of diarrhea in the unsupplemented controls (109). This leads to the conclusion that the reverse might be true, namely, that under completely "sanitary" conditions the response might disappear. Attention was drawn (178) to the relation of the "disease level" (41) to the response. Conversely it was found that healthy pigs placed in clean indoor pens which had not previously been used for pigs did not respond to aureomycin (178). Studies with chickens fed penicillin produced similar results (52), leading to the conclusion that, as discussed on page 383, the growth-stimulatory-effect of antibiotics results from their suppression of microorganisms detrimental to their host.

TABLE IV

Effect of antibiotics injected in eggs on 17-day weight of chick embryos

ANTIBIOTIC	AMOUNT INJECTED*	SURVIVAL RATIO	AVERAGE WEIGHT	
			Whole egg	Embryo
	mg.		*gm.*	*gm.*
Aureomycin	1.2	19/30	54.1	12.0
Penicillin	1.2	20/30	53.7	12.6
	6.0	22/30	53.6	10.4
Streptomycin	1.2	20/30	54.6	12.5
	6.0	23/30	54.2	12.2
None—distilled H_2O		26/36	53.7	13.3

* The antibiotic was divided into 3 equal doses which were injected on three successive days.

The results with chicks, turkeys and pigs indicated that the effect of antibiotics on growth was most marked during the first few weeks of life when the logarithmic rate of growth is greater than it is subsequently. If this effect was due to a direct action on the tissues, then the growth of the chick embryo should be accelerated by antibiotics because the logarithmic rate of growth of the chick is greater during embryonic life than after hatching. If, however, the effect was due entirely to antibacterial action in the intestine, then there should be no acceleration of embryonic growth. Accordingly, chicken eggs containing living embryos were injected with sterile solutions of antibiotics starting on the seventh day of incubation and the embryos were harvested, separated from membranes and weighed on the seventeenth day (111). Some typical results are shown in Table IV. The antibiotics tested were devoid of growth-promoting effect, leading to the conclusion that they had no effect on growth in the absence of intestinal bacteria.

It was noted by Gordon (84) that the weight of the small intestine of either "germ-free" or antibiotic-fed chicks expressed as a percentage of the body weight was markedly lower than that of controls. This interesting observation was confirmed by Coates (50) who compared chicks in cages especially designed to exclude bacteria with controls with and without antibiotic feeding. The rate of growth was increased and the intestinal weight was decreased by either feeding antibiotics or excluding bacteria. Pepper and co-workers (150a) found that dietary aureomycin reduced the weight of the small intestine of chicks as measured at 5 weeks of age by about 7 %. There was a slight but non-significant increase in cecal weight. However, raising the manganese level of the diet from 10 to 50 parts per million increased the weight of the small intestine by about 12 % without affecting the body weight, perhaps indicating that the intestinal tissue is readily affected by dietary changes.

THE ANTIBIOTIC GROWTH EFFECT IN CHICKS AND TURKEYS

The use of chickens as experimental animals is widespread and many fields of investigation in nutrition stem from exploratory work with chicks. The early experiments with antibacterial substances in chick diets were reviewed in the introduction. Our report of the growth-promoting effect of aureomycin fermentation materials in 1949 (106) was followed by investigations with these materials in a number of laboratories. The effect was confirmed by Couch and Reed, Singsen and Matterson and McGinnis in the summer of 1949 (63, 173, 138). Their investigations stressed the fact that the addition of the aureomycin-B_{12} "APF" supplement improved the growth of chicks on vitamin B_{12}-deficient diets of vegetable origin to a point beyond that obtained on diets containing animal products as a source of vitamin B_{12}; furthermore, the supplements produced a growth response when added to diets containing fish products which supplied sufficient vitamin B_{12}. In addition (173, 138, 140), it was found that young turkeys gave little or no response to potent sources of vitamin B_{12} added to all-vegetable diets but did respond markedly under these conditions to the crude aureomycin fermentation supplement. In one experiment the weight of turkeys receiving the supplement was 55 % greater at 4 weeks than that of controls receiving liver extract as a source of vitamin B_{12}. These effects were shown to be due to aureomycin, and other antibiotics produced similar growth responses (180, 200, 179, 86, 139, 68).

Effect on protein requirement of poultry

Machlin and co-workers (132) found that the protein requirement of chickens for maximum growth during the first 6 weeks was 19 % when aureomycin was added to the diet and 21 % in the absence of the antibiotic. No such differences were found by Slinger *et al.* (175) but these authors commented that antibiotics enhanced the utilization of both protein and energy compounds rather than reducing the requirements for such compounds and a similar conclusion was reached by Biely and co-workers (16) who also noted that antibiotics reduced the mortality in tryptophane-deficient chicks. Jones and Combs concluded that aureomycin spared the requirement for tryptophane but not for lysine (104), while Slinger *et al.* (176) observed that feeding aureomycin or penicillin markedly increased the incidence of white feathers in turkeys, a sign of lysine deficiency, on diets containing 20% protein. White feathering did not occur when the protein level was 28%. These results indicated that the requirement for lysine was increased by feeding antibiotics on the lower protein diet, presumably because of the increased growth rate when antibiotics were fed.

An improvement in the utilization of protein was found to accompany the addition of a supplement containing aureomycin and vitamin B_{12} in studies with quail (5a). The improvement was greatest at the lowest level of protein studied, which was 20%.

Effect on vitamin requirement

Our early observations with chicks on the antibiotic growth effect, like those by Moore *et al.* (144), showed that the effect was produced even when the basal diet was amply supplemented with the available vitamins. The effect was, therefore, not due to an improvement in the utilization of a basal diet which was slightly deficient in a known vitamin. It remained possible that an unidentified nutrient, a deficiency of which existed in most or all natural foods, was being spared by the antibiotic, either by an improvement in uptake from the intestine or by the establishment of new intestinal microflora which produced increased amounts of the postulated factor. An attempt was made to see if the effect was due to remedying the deficiency of an "unidentified vitamin" by adding certain crude materials which are thought to contain unknown growth factors, including fish solubles, yeast, alfalfa meal and distillers' solubles. The growth effect produced by the aureomycin fermentation residues still persisted (183).

The separate possibility existed that antibiotics might have a "sparing effect" on the requirement for certain vitamins. This can be manifested in one of two ways: 1) the level of the vitamin needed for maximal growth is lower in the presence than in the absence of the antibiotic, 2) the antibiotic growth response at suboptimal levels of the vitamin is greater than the response when an optimal level of the vitamin is supplied. It was to be anticipated that variations in the intestinal flora might produce marked differences in the effect of antibiotics on the requirement for vitamins as measured at different times or in different laboratories. Investigations were made in our laboratory of the effect of aureomycin on the response of chicks to increasing levels of specific vitamins added to the appropriate deficient diet. The results, some of which were mentioned in an abstract (182), are presented in Table V. The basal diets consisted of sucrose, or more rarely glucose, "vitamin-free casein" (Labco) with the other customary supplements (108) and with the vitamin under investigation omitted. The data indicate that no clear-cut "sparing effects" on any of the vitamins studied were encountered with the possible exception of riboflavin. Vitamin B_{12} was studied separately; some experiments showed a definite sparing effect of aureomycin on the requirement, although in other experiments no such effect was found (181).

The addition of 30 mg. of penicillin per kg. of diet was found to produce an increased accumulation of carotenoids in the blood serum and of vitamin A in the liver of chicks (30). The results are shown in Table VII.

TABLE V

*Growth responses of chicks to various vitamins with and without dietary aureomycin**

EXPERIMENT	VITAMIN AND LEVEL ADDED PER KG. OF DIET	WEIGHT AT 25 DAYS		RESPONSE TO AUREOMYCIN
		Without Aureomycin	With Aureomycin	
	mg.	*gm.*	*gm.*	%
1. PGA	0	199	233	17
PGA	0.1	234	327	40
PGA	0.2	243	316	30
PGA	1.0	255	323	27
2. PGA	0	148	177	19
PGA	0.1	170	258	52
PGA	0.2	199	250	26
PGA	1.0	197	290	47
3. PGA	0	120	123	3
PGA	0.1	153	192	25
PGA	0.2	202	272	35
PGA	1.0	255	300	17
4. Calcium pantothenate	0	100	125	25
Calcium pantothenate	3.	168	207	23
Calcium pantothenate	6.	229	327	43
Calcium pantothenate	25.	279	320	15
5. Calcium pantothenate	0	75	79	4
Calcium pantothenate	3.	89	112	27
Calcium pantothenate	6.	166	177	7
Calcium pantothenate	10.	220	256	16
Calcium pantothenate	25.	203	271	33
6. Niacinamide	0	73	92	26
Niacinamide	6.	125	152	22
Niacinamide	10.	148	184	24
Niacinamide	15.	180	217	21
Niacinamide	25.	218	292	34
Niacinamide	40.	232	317	37
7. Vitamin B_6	0.0	†	†	
Vitamin B_6	0.5	130	140	8
Vitamin B_6	1.0	211	246	17
Vitamin B_6	2.0	327	373	14
Vitamin B_6	5.0	343	372	8
8. Riboflavin	0	64	62	No effect
Riboflavin	1.	85	110	29
Riboflavin	2.	141	205	45
Riboflavin	4.	218	254	17
9. Riboflavin	0	57	54	No effect
Riboflavin	1.	87	89	No effect
Riboflavin	2.	126	152	21
Riboflavin	4.	170	199	17
Riboflavin	6.	172	187	9

* Each figure represents the average weight of duplicate groups of 12 chicks.

† All dead.

It was found by Coates and co-workers that not only was the liver storage of vitamin A improved by feeding penicillin (54, 55) or by excluding avenues of infection but the treatment with penicillin increased the efficiency of conversion of β carotene to vitamin A in the intestinal wall (50). It was noted that dietary aureomycin produced an augmentation of the effect of injected estrogen in increasing the serum calcium and riboflavin (61), perhaps indicating improved uptake of calcium and riboflavin from the digestive tract.

TABLE VI

Effects of antibiotics on the B-vitamin requirements of chicks

INVESTIGATOR	VITAMIN STUDIED	ANTIBIOTIC USED	SPARING EFFECT
Common *et al.* (61)	Riboflavin	Aureomycin	*
Biely & March (15)	Riboflavin	Aureomycin	Yes
	Niacin	Aureomycin	Yes
	Folic acid	Aureomycin	Yes
Coates *et al.* (51)	Thiamine	Penicillin	No
	Riboflavin	Penicillin	No
	Pyridoxine	Penicillin	No
	Pantothenic acid	Penicillin	No
	Biotin	Penicillin	Yes
	Folic acid	Penicillin	Yes
	Niacin	Penicillin *	Deficiency increased
Nelson & Scott (147)	Niacin	Aureomycin	No
Hsu, *et al.* (turkeys) (98)	Riboflavin	Penicillin	Yes
Waibel *et al.* (194)	Pyridoxine	Aureomycin	No
	Thiamine	Aureomycin	Yes
	Thiamine	Penicillin	Yes
Waibel *et al.* (195)	Biotin	Penicillin	†
	Folic acid	Penicillin	†

* Blood level of riboflavin increased when antibiotic was fed.

† Biotin and folic acid deposition in eggs increased when antibiotic was fed.

The hatchability of hens' eggs is depressed by a deficiency of vitamin B_{12}, and an increase in hatchability is readily obtained by adding small amounts of vitamin B_{12} to the diet under these conditions. The effects of various supplements on the hatchability of eggs laid by hens on a diet deficient in vitamin B_{12} were studied by Elam *et al.* (71) who made the interesting observation that dietary penicillin produced an improvement. When penicillin was injected into hens on the unsupplemented diet, no eggs were hatched. The per cent hatchability with the treatments was as follows: injected B_{12}, 72%; penicillin in diet, 39%; injected B_{12} *plus* penicillin in diet, 80%.

Hsu and co-workers (97) found that either terramycin, 15 mg. per kg. of diet, or vitamin B_{12} fed to hens on a diet deficient in vitamin B_{12} increased the hatchability of eggs. However, terramycin did not produce the increases in erythrocyte count and hemoglobin level in the newly hatched chicks which were observed in the case of vitamin B_{12} supplementation; perhaps the terramycin increased the B_{12} supply enough to produce an improvement in hatchability but not enough to affect the blood picture.

An investigation by Sizemore and co-workers (174) showed that hens which had received a supplement of aureomycin during their growing and developing period produced eggs with a higher rate of hatchability during a subsequent period when aureomycin was not fed than did unsupplemented controls (Table VIII). The authors concluded that the feeding of aureomycin to pullets during the growing period made them resistant to the vitamin B_{12} deficiency in the breeder diet and that this resistance persisted for periods up to 36 weeks after the cessation of antibiotic feeding. However, it was not established that the effect was specifically due to vitamin B_{12} because 1) no differences were noted among the different groups with respect to vitamin B_{12} content of the eggs, 2) the breeder diets contained either 4% or 8% of fishmeal which supplies varying amounts of vitamin B_{12}, and 3) although the hatchability responded to the addition of a crude supplement containing vitamin B_{12} and aureomycin, the possible presence of unknown factors in the supplement is not excluded.

TABLE VII

Effect of Dietary penicillin on vitamin A metabolism in chicks (from Burgess et al. (30))

	EXPERIMENT I		EXPERIMENT II	
	Controls	Penicillin	Controls	Penicillin
Body weight at 26 days, gm.	212	279	163	174
Liver weight/100 gm. body wt.	2.46	2.29	2.76	2.26
Feed consumption, gm./bird	420	540	245	266
Vitamin A, I.U./gm. fresh liver	82	118	125	176
Carotenoids, γ/100 ml. serum	261	402	295	718

Experiment I—New Hampshire chicks. Experiment II—White Leghorns.

TABLE VIII

Effect of aureomycin supplementation during the growth period of pullets upon their subsequent reproductive performance (from Sizemore et al. (174))

DIET DURING GROWTH	ADDITION TO BREEDING DIET	
	No supplement Hatchability*	Aureomycin 5 to 40 p.p.m Hatchability*
Diet 1	59.8	70.1
Diet 1 + B_{12}-aureomycin supplement	70.3	74.2
Diet 2	48.0	62.2
Diet 2 + B_{12}-aureomycin supplement	81.3	74.2

* Percent hatch of fertile eggs.

Effect on mineral requirement

An improvement in the utilization of calcium was reported to occur in chicks as a result of adding penicillin to a rachitogenic diet (143). The effect was shown by measuring the percentage of an oral dose of Ca^{45} which appeared in the tibia 48 hours after dosing. An average value of 1.14 was obtained on the basal diet and 1.92 with penicillin, 30 mg. per kg. of diet. An analogous conclusion was reached by Ross and Yacowitz (161) who found increased in the per cent bone ash of 3-week-old chicks which received levels of 8 and 30 I. U. of vitamin D per 100 gm. of diet containing penicillin as compared with controls without the antibiotic. The bone ash was not increased by penicillin at vitamin D levels of 4 and 600 I. U. per 100 gm. Somewhat similarly, Lindblad and co-workers (150) found that aureomycin lowered the phosphorus requirement of chicks; optimum growth and feed efficiency were obtained with 0.6% inorganic phosphorus in the absence of the antibiotic and with 0.4% in its presence. The requirement for manganese for growth and the prevention of bone deformities in chicks was found to be reduced by adding aureomycin (123). It was also noted that the dietary levels of sodium chloride and manganese influenced the response to aureomycin.

The findings on the effect of antibiotics on the mineral requirement of chicks indicate that antibiotics improve the uptake of calcium, phosphorus and manganese from the gut, perhaps by eliminating bacteria that interfere with the normal functioning of the intestinal wall.

Effect on reproduction

As noted above, antibiotics improve the hatchability of eggs laid by hens on diets deficient in vitamin B_{12}. No such effect was obtained when the supply of vitamin B_{12} was adequate (14). No increase in egg production or hatchability was found to be associated with the feeding of antibiotics in experiments by Sunde and co-workers (184) and by Berg and co-workers (14), by Lillie and Bird (122a), by Waibel and co-workers (195) and by Brown and co-workers (29a). The addition of penicillin and bacitracin to a purified diet was found to improve egg production by hens (73) but the increases appear to be of doubtful significance. Elam and co-workers stated that aureomycin was found to increase egg production and hatchability (72). Carlson (34) found that feeding aureomycin at high levels, 200 mg. per kg. of diet, increased egg production by hens that previously had a low production rate.

The addition of 2 gm. of procaine penicillin per ton of diet was found to be followed by decreases in hatchability, egg production, egg weight and feed consumption in turkeys. The decreases were small but consistent; moreover, the differences in hatchability and egg production between the two groups were promptly reversed when the diets were "switched" (176a). Further studies indicated that turkeys hatched from hens receiving penicillin weighed slightly less at 8 weeks of age than those hatched from hens not fed the antibiotic (176b). A possible relation of these observations to secondary deficiencies in the basal diet may exist.

Miscellaneous effects

The addition of 10 mg. of aureomycin hydrochloride per kilo of diet did not significantly change the eating quality of roasted turkeys 14, 16 and 26 weeks old. The general quality of the flesh of all groups was high (184a). An increase in the deposition of subcutaneous fat in turkeys was observed to accompany the feeding of penicillin, 2 parts per million of diet (165a).

The effect of supplementation with aureomycin and vitamin B_{12} on experimental infections with *Ascaridia galli* in chicks was studied by Hansen *et al.* (89a) who found that fewer chicks became infected in the supplemented group than in the non-supplemented group when exposed to a measured dose of the embryonated ova of the parasite. The supplemented chicks also harbored fewer worms at autopsy.

THE ANTIBIOTIC GROWTH EFFECT IN PIGS

The first indications of the profound effects of antibiotics on the growth of pigs appeared in the summer of 1949. We had noted (183, 106) that it was possible by assay with chicks to standardize crude preparations from the aureomycin fermentation which supplied not only vitamin B_{12} but another growth factor. These preparations, known colloquially as "Animal Protein Factor", were furnished to various investigators who were studying the effect of vitamin B_{12} in swine nutrition. It was reported by Cunha and co-workers (65) that these preparations markedly improved the growth of pigs on all-vegetable diets even when vitamin B_{12} was ineffective. In one experiment, the gain on basal diet was 0.29 pound per day; with B_{12}, 0.25 pound per day; and with the aureomycin fermentation supplement, 0.73 pound per day. Similar observations were made at the same time by Catron and by Krider, who commented upon the properties of the preparations in controlling diarrhea. The implication of an antibiotic effect was thus made. The effect of antibiotics in controlling bacterial diarrhea was well known, but the concept that most pigs although apparently healthy are in fact suffering from various degrees of subacute intestinal infection was new. It was this concept, advocated strongly by Catron and by Carpenter, which came to the fore as it was established that the growth-promoting effects of the aureomycin "Animal Protein Factor" preparation on pigs were due primarily to its antibiotic content (109, 131, 35, 14, 15, 17).

Many experiments were carried out with antibiotics in swine nutrition in the ensuing three years. The field was reviewed recently by Braude and co-workers (25) and so many reports were published that these authors were able to summarize the results of 337 comparisons of the growth of pigs receiving antibiotics with that of controls. Table IX, which is condensed from their review, summarizes the data published by various agricultural experiment stations with respect to the effects of various antibiotics on the growth rate and the efficiency of feed utilization of pigs.

Wide variations may be expected to exist in the type and numbers of antibiotic-susceptible bacteria encountered in pigs, so that these animals may be expected to vary greatly in their response to antibiotics. When diarrhea is present, the antibiotic growth response in pigs often ranges up to a doubling or even tripling of the growth rate of the controls. Some variations, therefore, may be anticipated among experiments in which the effects of antibiotics upon special phases of nutrition, such as the requirement for proteins and vitamins, are measured.

Attention was soon drawn to the relation of the antibiotic response to the type and level of protein in the diet of pigs. The early reports by Cunha indicated that the response to crude "A.P.F." preparations from the aureomycin fermentation was far greater with peanut meal than with soybean meal as the major source of protein and that the use of fishmeal instead of soybean meal reduced the response still further. No increased growth was obtained when vitamin B_{12} was added to the peanut meal diet, but the possibility remained that the extent of the responses encountered was in part dependent upon the vitamin B_{12} content of the "A.P.F." preparations. In further studies, Cunha *et al.* (66) found that the growth rate of pigs in a corn-peanut meal diet without the crude aureomycin supplement was improved by increasing the protein level up to 19.6% which was the highest level tested. When, however, the aureomycin supplement was added, the growth rate was the same on a 17.9% level of protein as on the 19.6% level, leading to the conclusion that "animal protein factor" lowered the protein needs of the pig.

TABLE IX

*Growth and efficiency of feed utilization of pigs receiving various antibiotics (25)**

ANTIBIOTIC	GROWTH INDEX (UNSUPPLEMENTED = 100)	FEED REQUIRED PER UNIT OF GAIN (UNSUPPLEMENTED = 100)
Aureomycin	135.9 (187)	90.2 (146)
Penicillin	110.6 (53)	94.3 (44)
Streptomycin	115.2 (50)	94.4 (41)
Terramycin	123.7 (23)	93.9 (17)
Bacitracin	109.0 (12)	103.0 (10)
Chloramphenicol	105.5 (6)	98.2 (6)
Neomycin	93.3 (4)	87.6 (3)
Polymyxin	96 (1)	100 (1)
Subtilin	89 (1)	130 (1)

* Figures in parentheses indicate number of comparisons.

Catron and co-workers (44) studied the growth rate of pigs with and without aureomycin on diets containing various levels of protein which were lowered progressively as the pigs increased in weight. The protein was supplied in the form of corn and soybean meal which mixture is known to supply a well-balanced mixture of the indispensable amino acids for pigs. In the absence of antibiotic, the rates of gains varied among the several sets of protein levels. This variation was not observed among the groups fed the antibiotic (10 mg. of aureomycin per pound of diet). The results indicated that the protein level for rapid growth could be reduced by about 2 gm. per 100 gm. of diet if aureomycin was fed. Pigs receiving the antibiotic gained an average of 0.12 pound more per day and consumed 23 units less feed per 100 units of gain than the pigs not receiving antibiotic.

No effect of terramycin on the protein requirement of pigs was found by Hoefer and co-workers (94); however, in their experiments the rates of growth were the same on both levels of protein tested even in the absence of the antibiotic.

The growth-promoting effect of antibiotics in pigs is quite marked during the first few weeks *post partum*. Carpenter found that baby pigs which were allowed to eat a diet containing aureomycin supplementary to suckling during the first eight weeks weighed 1.43 times as much as controls receiving the supplementary diet without an antibiotic (37). If instead of suckling, the baby pigs are fed an "artificial milk" the response to antibiotics is also quite marked. Wahlstrom and co-workers (193) found that two-day-old pigs grew at the average rate of 0.74 pound per day for 8 weeks on a diet of lard, glucose, soybean protein, methionine, minerals and vitamins. With the addition of aureomycin, 100 mg. per kg. of dry matter in the diet, the growth was 0.97 pound per day. No growth-promoting effect was obtained from penicillin or phthalylsulfathiazole. Similar results were reported by Noland and co-workers (149).

An effect of aureomycin (20 mg. per kg. of diet) in increasing urinary output was noted by Braude and Johnson (24) in an experiment with young pigs. Increases in growth rate and efficiency of food utilization were also noted but there was no effect on nitrogen retention. The average 3-day water intake was 9017 ml. for the controls and 9181 ml. for the supplemented pigs. The figures for urinary excretion were respectively 3384 ml. and 3989 ml., for urine output as a percentage of water intake, 37.5 and 43.1, and for fecal output, 223.4 and 225.8, so that the increased urinary excretion by the antibiotic-fed pigs could not be explained on the basis of decreased feces. The authors suggest that the feeding of the antibiotic may have lowered the metabolic rate of the animals and they speculate that the suppression of a gastrointestinal infection by the antibiotic might lead to a lowering of the body temperature, thus explaining the difference in urinary volume.

An examination of the data of Richardson and co-workers (156) shows no consistent relationship between antibiotic feeding and urinary volume in pigs.

Pigs reared under practical conditions are subject to intestinal infections accompanied by diarrhea, sometimes with voiding of blood. The phenomenon termed "runtiness", the frequent presence of undersized or "unthrifty" baby pigs in otherwise normal litters, is well known in animal husbandry. It now appears that these disturbances may often be prevented or arrested by the addition of a suitable antibiotic to the diet. The effects of antibiotics on undersized or so-called "runt" pigs have been so noteworthy as to compel re-evaluation of "runtiness". It may well be that a large proportion of undersized animals are suffering from obscure subclinical infections of the digestive tract.

Carpenter (36) carried out an experiment with two groups of runt pigs, 16 in each group, averaging 16.5 pounds per pig, in weight. The group which received aureomycin had a growth rate of 1.0 lb. per day after 4 weeks while the controls were gaining 0.5 lb. per day. At the end of 6 weeks the supplemented pigs were using 2.65 units of feed per unit of gain as compared with 4.83 units of feed for the controls.

Catron and Cuff (43) described an experiment with groups each containing 8 runt pigs averaging 20 pounds in weight. The control group gained 0.70 pound per day while a second group receiving a supplement which furnished about 40 mg. of aureomycin per kg. of diet gained 1.16 pounds per day during the 9-week experimental period. A striking result was reported by Becker and co-workers (11) who studied two groups of pigs which were initially selected for "unthriftiness". The first group, which served as controls, grew at a rate of 0.55 pound per day over a 5-week period and consumed 4.75 units of feed per unit of gain while the second group, which received 11 milligrams of crystalline aureomycin per kg. of diet gained 1.65 pounds per day with 2.88 units of feed per unit of gain.

The addition of vitamin B_{12} to the diet of slow-growing unthrifty pigs did not significantly affect their gain but similar pigs receiving a supplement supplying 1.8 parts of aureomycin per million of diet grew more rapidly and were more uniform than controls. The response to aureomycin was primarily with the lighter and more unthrifty pigs (20).

In studies with pigs, it was found by Catron and co-workers (45) that the response curves to graded levels of aureomycin with and without vitamin B_{12} were parallel, indicating the absence of a sparing effect. As an example of their data, the rate of gain in pounds per day were with no vitamin B_{12} or aureomycin, 1.35; with aureomycin (20 mg. per lb. of diet) and no vitamin B_{12}, 1.57; with vitamin B_{12} (10 mg. per lb. of diet) and no aureomycin, 1.53; with vitamin B_{12} and aureomycin, 1.77; thus the response to aureomycin either in the presence or absence of added vitamin B_{12} was 16%.

Effect on composition of tissues

The increase in growth rate and the improvement in food utilization shown by pigs receiving antibiotics have led to investigations of the composition of the tissues of such pigs. The depth of dorsal fat of pigs which were fed aureomycin was measured by Vohs and co-workers (191) who found no differences between the antibiotic-fed and the control pigs. Vestal reported that pigs receiving aureomycin had back-fat which was 10% thicker than that of controls, but the pigs were allowed free choice of selection between shelled corn and a high-protein supplement with the result that the aureomycin-fed pigs selected a higher proportion of corn than did the controls (188). Bowland and co-workers found that diets containing an aureomycin-containing supplement resulted in a reduction of the carcass length and an increase of fat deposition in bacon-type pigs; the differences were not statistically significant at the 5% probability level (23). They attributed the increase in fat to the rapid gains during the later stages of growth.

No significant differences existed between antibiotic and no-antibiotic treatments in respect to back fat, depth, length or depth of body or per cent of lean when measured on 24 representative carcasses (44). Similar conclusions have been reached in the majority of investigations, including Wilson and co-workers (204), Robison and co-workers (158), Terrill and co-workers (184b), Heidebrecht and co-workers (91a) and Kline and co-workers (114), the exception being the Purdue group (153) who reported that the crude fat (ether extract) content of pigs receiving aureomycin was significantly higher than that of controls.

In a study of brucellosis, Cameron (33) fed pigs with aureomycin at the unusually high level of 250 mg. per kg. of body weight per day for 28 days. No toxic effects were noted and at the end of the experiment the pigs appeared to be in excellent condition. At autopsy the bone marrow was found to be yellow but showed no histological abnormality.

Effects on reproduction

The effect of antibiotics on gestation and lactation in pigs were studied by Carpenter (37) who found that a supplement containing aureomycin fed for 49 to 90 days prior to parturition did not affect the size of the litter or the survival rate of young pigs, nor was there any indication of transfer of aureomycin from the diet to the milk. Further studies (120) led to the conclusion that feeding a diet containing aureomycin from weaning through two gestation and lactation periods did not improve the reproductive performance of sows, although mothers fed the antibiotic weaned more and heavier pigs per litter than did the control dams. Improvements in reproductive performance have been recorded by Catron (42), in a summary of results with 157 sows in 7 experiments, as follows:

	NO ANTIBIOTIC	PLUS ANTIBIOTIC
	lbs.	*lbs.*
Av. birth wt.	2.82	2.85
No. pigs alive 56 days	6.0	6.5
Total weight of litter		
21 days	78	80
56 days	185	213

Further experiments were described by Ellis (74) who noted an increase in the per cent of pigs weaned, and by Vestal and co-workers (189) who observed an increase in birth weight. No effect on gestation or lactation was found by Dyer *et al.* (69) or by Hogan *et al.* (95).

A study of reproduction in pigs receiving aureomycin was made through five litters and two generations (163). The first generation, a group of 12 sows, averaged respectively 8.1, 7.6 and 9.6 pigs per litter during 3 successive parturitions. A group of 20 daughters of the first group averaged 8.2 and 8.8 pigs per litter during two successive parturitions. All the pigs received a diet containing 10 mg. of aureomycin per pound from weaning until breeding, 5 mg. during gestation and 10 mg. post-gestation. The absence of any effect on reproduction was indicated by these results.

In summary, improvements in certain aspects of reproductive performance have been noted by certain investigators but others have found no effect. It seems probable that any beneficial effects are indirect by improving the health of the dam or following the consumption of small portions of her diet by the young. There is no indication of passage of dietary antibiotics through the placenta or into the milk.

ANTIBIOTICS FOR RUMINATING ANIMALS

After it had been established that aureomycin was of nutritional value for poultry and swine, investigations on ruminants were undertaken and reported in 1950. At first the picture was confusing. While Rusoff (163), Bartley *et al.* (7) and Loosli and Wallace (126) reported that aureomycin promoted growth of calves and eliminated scours, Bell *et al.* (12, 13) reported an adverse effect on steers and Colby *et al.* (57, 58, 59) found that aureomycin markedly depressed feed consumption and growth of lambs. This latter report is in contrast to an earlier report in 1950 which indicated that lambs exhibited a definite growth response to aureomycin (5). In 1951, Jordan and Bell (105) substantiated the finding that aureomycin promoted the growth of lambs and decreased the incidence of enterotoxemia.

TABLE X

Effect of aureomycin on growth of calves

INVESTIGATORS	CONTROLS	AVERAGE DAILY GAIN WITH AUREOMYCIN
	lbs./day	*lbs./day*
Bartley, Fountaine and Atkeson (7),	0.52	0.83 (1st 7 weeks)
Experiment I	1.02	1.35 (8 to 12 weeks)
Bartley, Experiment II	0.95	1.28 (1st 8 weeks)
	1.34	1.63 (9 to 12 weeks)
	1.75	2.20 (1 to 22 weeks)
Loosli *et al.* (126)	0.95	1.16 (0 to 8 weeks)
Rusoff (163), Experiment I	1.42	1.53 (14 to 22 weeks)
Rusoff, Davis and Alford (164),	0.99	1.21 (4 to 16 weeks—Jerseys)
Experiment II	1.40	1.69 (4 to 16 weeks—Holsteins)
Jacobson (101)	1.11	1.46 (0 to 16.5 weeks)
Bloom and Knodt (21)	0.54	1.00 (0 to 4 weeks—Holsteins)
	1.29	1.57 (0 to 12 weeks—Holsteins)

Effects of antibiotics on the growth of calves

Loosli and Wallace (126) and Loosli, Wasserman and Gall (128) studied 39 pairs of calves on a variety of diets including both milk and milk replacements, consistently noting a 20% increase in rate of gain with aureomycin.

Much of the early work on the effect of antibiotics on growth of calves is summarized in Table X. Bartley *et al.* (6) in one experiment utilized a building which had been previously demonstrated to be unsuitable for growth of healthy calves, since the contaminated quarters caused a high incidence of severe scours. Under these conditions, aureomycin gave a 70% increase in the rate of growth (Table X). The growth of the supplemented calves did not surpass the "Ragsdale standards" (155), but in a second experiment the calves were kept under more sanitary conditions and the growth of the unsupplemented group was superior to the "Ragsdale standards" while the group receiving crude aureomycin showed a growth rate 30% above the controls throughout the 22-week experiment. Rusoff *et al.* (164) added crude aureomycin to a vegetable diet which was fed with whole milk. Significant quantities of the diet and hence of aureomycin were not ingested until 30 days of age, at which time the milk was removed. Jersey and Holstein calves receiving the supplement over the controls showed a 20% increase in the rate of gain at 16 weeks of age. At 13 weeks, Jersey calves on the supplement showed a gain of approximately 25% over their controls, while the Holstein calves showed a gain of 15% over their controls. There was some question as to the possible effects of vitamin B_{12} and of other unknown growth factors present in the crude aureomycin supplement. However, since it had been reported that vitamin B_{12} was without effect on calves, (92) it appeared probable that the growth effects observed were due to aureomycin. The experiments of Jacobson *et al.* (101), in addition to those of Loosli and Wallace, showed that the growth effects observed were due to aureomycin. They supplemented the diet of a number of different breeds of calves paired according to breed, sex and weight with pure aureomycin and noted an increase in the rate of growth of 32% at 16.5 weeks.

In the experiments discussed above and tabulated in Table X, the control animals usually showed a certain degree of scouring. Several of the investigators suggested that the effect of aureomycin in promoting growth was achieved by the elimination of the infectious scours. However, Bloom and Knodt (21, 22) used healthy Holstein bull calves showing very little or no scouring. As shown in the last experiment listed in Table X, there was a very definite response to aureomycin with these healthy animals, particularly in the first four weeks of the experiment. Knodt and Ross (117) obtained good growth responses through 8 weeks of age in calves which received several different levels of aureomycin. There was no definite relationship between the level of aureomycin and the rate of gain in body weight or the efficiency of food utilization. No scouring was observed. McGilliard (137) investigated the effect of rumen inoculation on the response of young calves to aureomycin. Two groups of calves received aureomycin for 5 weeks, both gaining 23.1 lbs. as compared to the controls which gained 12.4 lbs. Aureomycin was then discontinued and one of the aureomycin groups was inoculated orally at 5 weeks and at 6 weeks with cud material. During the second 5-week period, the calves receiving a cud inoculation gained 45 lbs., while the controls and the other group which had received aureomycin gained approximately 40 lbs. After 14 weeks of observation there appeared to be little effect of the feeding of cud material since both groups which had received aureomycin gained approximately the same weight. Rusoff (164) reported that calves on three protein sources, gossypol-free cottonseed meal, soybean oil meal and cottonseed meal, responded quantitatively to aureomycin in a very similar fashion.

The experiments of Bartley *et al.* (9) indicated that large doses of aureomycin from 200 to 800 mg. per day per 100 lbs. of body weight did not produce anorexia, weight loss or any other deleterious effects in calves 12 to 16 weeks old. One calf received 2500 mg. of aureomycin daily for 4 weeks, starting at 16 weeks of age.

These experiments indicated that it was not necessary for calves to be "conditioned" to the feeding of aureomycin and that high levels may be beneficial to calves which have not previously received any antibiotic.

Almost all of the work reported with calves was with aureomycin, but a few experiments have been made with terramycin and penicillin. Bloom and Knodt (22) found that potassium penicillin fed at approximately 6 mg./day per calf decreased the growth rate of Holstein bull calves. In a second experiment, procaine penicillin failed to affect significantly the growth rate of calves (116). In both instances the penicillin salt was fed in an artificial milk mixture.

The first reported study on terramycin was by Cason and Voelker (40). Calves were fed terramycin at the dosages of 15 and 30 mg./100 lbs. body weight. At the end of 8 weeks there was no growth response to these two amounts of terramycin. In further experiments (190) these workers found a slight growth response to terramycin fed at 30 mg./day per 100 lbs. of body weight. In a separate experiment the amount of terramycin was increased to 100 mg./100 lbs. body weight per day and a 28% increase in the rate of growth was observed without an effect on the efficiency of feed utilization. In a third experiment at a rate of 90 gm. per ton of grain supplement to calves with an average age of 4.8 months and which were on pasture, the animals receiving aureomycin gained 17% more rapidly than the unsupplemented animals. This experiment is of much interest since the response was obtained with older animals. Terramycin was also studied by Kesler and Knodt (113). Calves receiving 20 mg. per 100 lbs. of body weight per day showed a 29% increase of growth rate during the first 8 weeks of the experiment. However, 8 weeks later, when the experiment was terminated, there was no difference between the terramycin-supplemented and the unsupplemented groups.

To summarize, it is well established that aureomycin causes an increase in rate of growth of approximately 15 to 35% and in a few cases up to 70% under a variety of experimental conditions and rations. Results with other antibiotics have been too few and variable for satisfactory evaluation (115).

Effect of antibiotics on calf scours

The disease called calf scours or "white scours" of calves is a diarrhea in calves which is coincidental with poor growth, a rough hair coat, frequent presence of respiratory disease and a generally unhealthy animal. One of the consistent effects of aureomycin in calves has been a marked reduction in the incidence of scours, a disease which sometimes terminates fatally. Bartley *et al.* (7) distinguished between mild scours and severe scours (Table XI) and it is interesting to note that the antibiotic had the greatest effect upon the incidence of severe scours or the type that is sometimes fatal.

The studies of Loosli and Wallace (126) are noteworthy since they utilized two artificial milk diets upon which a high incidence of scours was obtained. As shown in Table XII, the presence of aureomycin was associated with a marked decrease in the scours in the artificial milk groups (diets 11 and 13), particularly with diet 13. The calves on whole milk were almost completely free from scours when aureomycin was given.

The data on the effect of other antibiotics on calf scours are very limited. The experiments of Bloom and Knodt (22) and Knodt and Ross (117) with potassium and procaine penicillin showed no effect on scouring. In a study by MacKay *et al.* (133) the effect on scours was not significant, perhaps because of the low incidence of scours in both the controls and the animals receiving terramycin.

TABLE XI

Effect of aureomycin on calf scours (7)

	CONTROLS	WITH AUREOMYCIN
Incidence* of mild scours	1.58	0.83
Incidence of severe scours	2.83	0
Incidence of all scours	4.41	0.83

* Average number of days of scouring per calf.

TABLE XII

Effect of aureomycin on calf scours (126)

DIET	AVERAGE INCIDENCE OF SCOURS*	
	Controls	With Aureomycin
Milk replacement—Diet 11	21.3	12.7
Milk replacement—Diet 13	12.2	5.5
Milk	7.5	0.2
Average (on all diets)	6.9	2.5

* Number of days of scouring during the 8-week experimental period.

Other beneficial effects from antibiotic feeding

Even though observations on the appearance of calves receiving antibiotics were admittedly subjective, investigators have consistently mentioned improved appearance in calves receiving aureomycin. Murley (146) studying calves on different diets containing aureomycin states, "In each group the aureomycin-fed calves were superior in physical appearance to the controls." Rusoff *et al.* (164) mention the "smooth hair, sleek solid muscular appearance" of the calves receiving aureomycin in his experiments. Bloom and Knodt (21, 22) mention the "smooth glistening hair coat and improved musculature" of the calves in their experiments which received aureomycin.

Effect of antibiotics on lambs

In an experiment by Colby *et al.* (58, 59) 100 mg. of aureomycin was fed daily by capsule and, in a second experiment, penicillin and streptomycin were used.

The animals receiving antibiotics lost appetite and weight, showing a diarrhea for about a week followed by a week of gradual recovery.

At the end of the experiment, the lambs receiving antibiotics weighed slightly less than the controls. In another experiment with the same amount of aureomycin there was a slight but definite increase in growth rate. Jordan and Bell (105), using considerably smaller amounts of aureomycin, reported a beneficial effect on lambs. The animals were given 5 mg. of aureomycin daily for 6 weeks; during this time they gained an average of 0.64 lbs. per day, as compared to the control lambs which gained 0.54 lbs. daily. A third group of 4 lambs was given 15 mg. daily and made an average daily gain of 0.59 lbs. The lambs receiving aureomycin were healthy and normal and other experiments gave similar results. The authors also noted a decrease in the instance of enterotoxemia in herds of lambs receiving aureomycin. This result is not unexpected since the organism presumably causing enterotoxemia, *Clostridium perfringens*, is extremely sensitive to aureomycin.

The effect of antibiotics on adult ruminants

Bell *et al.* (13) investigated the effect of comparatively high levels of aureomycin on steers weighing about 600 pounds. Preliminary experiments indicated that steers receiving 600 mg. of aureomycin daily developed anorexia and diarrhea, so the aureomycin intake was reduced in the second experiment to 200 mg. On this intake, two animals developed mild symptoms of anorexia and diarrhea. The experiments were not well controlled since two of the three steers receiving aureomycin also received urea while the controls did not. Neumann *et al.* (148) fed 15 mg. of aureomycin daily to beef cattle for 150 days; diarrhea was completely absent and there were no signs of abnormal paunch distension, although there was a slight reduction in appetite for the first few days on the experiment, followed by recovery. All groups of animals continued to gain approximately 2 pounds per day; there was no significant difference between the controls and the group receiving aureomycin. Haq *et al.* (90) supplemented the ration of dairy cows in full lactation with aureomycin and found no effect on the appetite. There was no influence upon milk production of the cows receiving aureomycin nor did aureomycin appear in the milk. Bartley *et al.* (8) fed lactating dairy cows 32 mg. of aureomycin per 100 lbs. of body weight. This dosage of aureomycin did not affect milk or butter-fat production, water consumption, body weight, rumination, pulse rate, body temperature and general health. Loosli and Warner (127) reported that 100 mg. of aureomycin daily fed to milk cows did not affect the milk production nor feed consumption.

Two related papers by Chance *et al.* (48, 49) described the effects of aureomycin on digestion and synthesis of various nutrients in the rumens of fistulated steers. The first paper was concerned with the rate of passage of certain nutrients from the rumen and the effect of digestion in the rumen. Aureomycin was fed at the rate of 0.5 gm. per day for 15 days and then 1.0 gm. per day for the next 15 days. Neither of the two steers under study showed signs of anorexia or diarrhea at any time during aureomycin supplementation. The rate of removal of dry matter, crude fiber, crude protein and nitrogen-free extract was the highest when 0.5 gm. of aureomycin was fed. There was a slight depression in the rate of removal of these constituents when 1.0 gram of aureomycin was fed. Crude fat, as measured by total ether extractives, accumulated during the very early part of aureomycin administration in both animals. The increase in rate of disappearance of nutrients when the animals were receiving 0.5 gm. of aureomycin coincided with an increase in the number of bacteria in the rumen, indicating that the antibiotic may have stimulated bacterial action. A second report indicates that the ten essential amino acids in the rumen were decreased when 0.5 gram of aureomycin was given per day. Since the amino acids appeared to be derived from dietary protein, the decrease was attributed to increased removal from the rumen. The amount of riboflavin in the rumen was decreased during aureomycin administration and there was a slight decrease in the amount of synthesis of niacin.

EFFECTS OF ANTIBIOTICS ON THE GROWTH OF CHILDREN

The growth of human beings takes place so much more slowly than that of the common animals that marked growth responses to antibiotics were not to be anticipated in infants; for example, a pig grows to a weight of 50 kg. in about one fiftieth of the time taken for a human being to reach this weight. However, by analogy with animals which are undersized due to unidentified bacterial infections, it was thought that antibiotic feeding might improve the growth and survival rate in underweight and sickly infants. Perrini (152) fed aureomycin to ten premature infants at a rate of 25 mg. per kg. per day in four doses at 6-hour intervals. After an initial 2-day drop in weight, their growth was more rapid than that of 23 controls. On the tenth day the supplemented infants were 8% above their birth weight as compared with the controls whose average weight was the same on the tenth day as at birth.

The effect of aureomycin, 50 mg. per kg. of body weight daily, on premature infants was studied by Robinson (157) who gave the antibiotic to the weaker one of twins or the weakest of a set of triplets, the stronger ones serving as controls. Five of the fifteen controls died from intercurrent infections while all the babies who received a full course of aureomycin survived. The average daily gain was 29.5 gm. in the treated group compared with 18 gm. in the controls. Snelling and Johnson (177) found that aureomycin lowered morbidity, speeded growth and shortened hospital stay in premature babies. There was 1 death in a group of 47 who received 50 mg. of aureomycin daily as compared with 8 deaths in a control group of 48.

The effects of aureomycin on the growth of poorly-developed children were studied in Guatemala by Scrimshaw and Guzman (167) who found that children receiving daily either 20γ of vitamin B_{12} or 50 mg. of aureomycin increased in both height and weight at a significantly greater rate over an 18-month period than that observed for a control group. No abnormal hematological values were found in any of the subjects.

A study of the effects of aureomycin, 75 mg. twice daily, on 20 mentally defective children was carried out by Carter (39) for periods ranging from one to three years. The children all had cerebral palsy and mental deficiency of such severity that they were almost entirely helpless. The average yearly gain for the supplemented group was 6.5 pounds as compared with 1.9 pounds for the controls. There were no significant blood changes in either group. There was a marked absence of gastrointestinal upsets, especially diarrhea, in the supplemented group, which was contrasted in this respect with the controls. There appeared to be no significant development of aureomycin-resistant organisms for in all cases tried the infection was controlled by increasing the dose of aureomycin to the full therapeutic level.

EFFECTS ON ANEMIA

A megaloblastic anemia has been shown by many investigators to follow certain cases of intestinal stricture, or it may be produced by the surgical segregation of a loop of small intestine. The subject was reviewed by Cameron and co-workers (32) who concluded that the anemia was probably due to "stagnation of intestinal contents and the absorption of toxic substances." Watson and Witts (198) found that aureomycin, 0.0625 gm. per 200 gm. of diet, prolonged the lives of rats which had developed anemia following the formation of a diverticulum in the small intestine and cured the anemia in all treated rats in a group of 16 except one which died after two days from acute obstruction. This anemia responded to folic acid but the response to B_{12} was "equivocal." The only clue to a possible cause of the anemia was a heavy infection of the small intestine with colonic flora, perhaps suggesting that these bacteria produced some factor which interfered with the normal utilization of folic acid.

The possible involvement of a bacterial "toxin" in producing pernicious anemia has been discussed by various authors cited by Lichtman and co-workers (121) who investigated the effects of dietary antibiotics on this disease. Four patients with pernicious anemia in relapse and one patient with nutritional macrocytic anemia responded to the oral administration of aureomycin, 2 to 3 gm. daily. A fifth patient with pernicious anemia failed to respond to 0.6 gm. of aureomycin given intravenously daily for 20 days, thus indicating that the aureomycin was not contaminated with significant amounts of hemopoietic factors such as vitamin B_{12} and folic acid. Microbiological assay of the aureomycin with *Euglena gracilis* indicated a vitamin B_{12} content not greater than 0.17γ per gm; furthermore, case No. 3 did not respond to vitamin B_{12} alone, 3γ orally per day, during a 10-day period of pre-treatment. It seems evident, therefore, that the effects observed by Lichtman *et al.* (121) were not due to the presence of vitamin B_{12} or folic acid in the aureomycin but were more probably caused by changes in the bacterial flora. The possible effects of such a change include increases in the production or liberation of folic acid or vitamin B_{12} in the intestinal tract or an improvement in the uptake of these vitamins. The response of the nutritional megaloblastic anemia case indicates that folic acid was not involved.

Foy and co-workers (79, 80) described good responses in two anemic patients who were treated with injections of penicillin; one patient had pernicious anemia and the other had megaloblastic anemia of pregnancy.

In contrast to these reports, Ungley obtained no response in patients with pernicious anemia who were treated with streptomycin and aureomycin (187). A patient with pernicious anemia was given an intensive 6-day course of antibacterial drugs by mouth including phthalylsulfathiazole, aureomycin and dihydrostreptomycin, during which *E. coli* disappeared from the stools by the third day and cocci by the fifth day. An oral dose of 80γ of vitamin B_{12} was given on the fifth day. There was no response over a 10-day period, following which a second dose of 80γ of vitamin B_{12} was given together with gastric juice. A response in reticulocytes and hemoglobin then occurred. This experiment indicated that the defect in the uptake of vitamin B_{12} from the intestine in pernicious anemia is *not* due to an effect of *E. coli* in "scavenging" the vitamin, the presence of intrinsic factor in normal subjects being postulated as having a protective effect against such "scavenging." Ungley's case appears to differ from the series reported by Lichtman *et al.* (121) which responded to aureomycin; however, it should be noted that Lichtman *et al.* found it necessary to administer the antibiotic for 24 to 28 days in pernicious anemia before obtaining a reticulocyte peak. One is tempted to speculate that the effect observed by Lichtman *et al.* (121) was due to a slowly-developing change in the bacterial flora, leading to vitamin B_{12} or folic acid or both being more available to the patient. It is also conceivable that such a change might diminish the bacterial production of an inhibitory factor, such as the one postulated by Callender and Lajtha (31); indeed, the results reported by Watson and Witts are suggestive of such an explanation. The prolonged nature of such a change may be indicated by the results of Sizemore *et al.* (174) who found that an improvement in the hatchability of the eggs of hens on a diet deficient in vitamin B_{12} occurred when aureomycin was fed and that the improvement persisted for several weeks after the withdrawal of the antibiotic.

EFFECT ON AMINOPTERIN TOXICITY

The interrelationship between aureomycin and Aminopterin, an antagonist of folic acid, is of interest because some of the deaths of experimental animals receiving Aminopterin may be due to infections resulting from the penetration of microorganisms through the intestinal wall, to which Aminopterin may cause damage (154). It was found by Waisman and co-workers that aureomycin counteracted the toxic effects in rats of 0.5 to 1.0 mg. of Aminopterin per kg. of diet (197), while Schwartz (166) found that dietary aureomycin increased the concentration of citrovorum factor in the liver and ceca of rats. These observations suggested that aureomycin may promote growth by causing an increased synthesis of citrovorum factor which is known to alleviate the toxic effects of aminopterin in mice and rats. However, further experiments by Waisman *et al.* (197a) showed that penicillin, streptomycin, chloramphenicol and terramycin, in contrast to aureomycin, were ineffective in reversing Aminopterin toxicity. Furthermore, aureomycin increased the rate of growth of rats even when an excess of citrovorum factor was in the diet. Obviously, if citrovorum factor was involved in the mechanism of growth promotion by antibiotics, it would appear likely that Aminopterin should be reversed by more than one of the antibiotics which promote growth. Citrovorum factor was fed and injected into chicks (203) which were fed a purified diet containing an excess of the known vitamins; in spite of this treatment, aureomycin produced a growth response. Recent experiments with rats (110) showed only a slight effect of aureomycin in protecting against the toxic effects of Aminopterin contained in the diet. Aureomycin was less effective than injected citrovorum factor. Our findings corroborate those reported by Sauberlich (165) who has repeatedly failed to demonstrate any protective effect of aureomycin or penicillin, 100 mg. per kg. of diet, for two strains of rats fed Aminopterin, 1 mg. per kg. of diet. In these same experiments, daily injections of 100 micrograms of citrovorum factor completely prevented the Aminopterin toxicity.

EFFECT ON LIVER INVOLVEMENTS

Some beneficial effects with aureomycin have been described in various liver diseases including the late manifestations of acute viral hepatitis (170), cholangiolitic hepatitis (141), hepatic coma (77), posthepatic cirrhosis (162) and chronic hepatitis and cirrhosis (169). The beneficial effect of the antibiotic on patients with hepatitis was concluded to be due to its antibacterial effect (83). The nutritional implications of these findings were indicated by Gyorgy and co-workers (89) who described the prevention of experimental hepatic necrosis in rats by aureomycin. The disease was produced by a diet consisting principally of corn starch and yeast and could be prevented by cystine, methionine or vitamin E. In further studies (88) it was found that a delay in, rather than the prevention of, the production of hepatic necrosis by the starch-yeast diet was obtained with aureomycin and to a lesser extent with terramycin and streptomycin; sulfaguanidine had a temporary effect, while chloramphenicol, polymyxin and penicillin were ineffective. It was postulated that necrosis eventually appeared in the rats receiving aureomycin because organisms later reappeared in the intestinal flora which were resistant to aureomycin. This temporary effect of aureomycin on the prevention of hepatic necrosis may therefore be contrasted with its effects on growth which are commonly maintained throughout the growing period of animals.

The mechanism for this effect was postulated (89) to be an inhibition of toxin-producing bacteria in the lower intestine, because the necrosis was found in the unsupplemented animals to occur mainly in the left lobe of the liver which derives its portal blood from the large intestine and stomach while the right lobe which was relatively free of necrosis drains portal blood from the small intestine. However, Luckey (130) has recently found that "germ-free" rats developed liver necrosis so that, even if the intestinal bacteria are concerned with the production of this disease, other mechanisms must also be involved.

Prevention of hepatic cirrhosis and renal changes by aureomycin in rats fed a low-casein-high-fat diet were recorded in the investigation by Gyorgy *et al.* (88), perhaps indicating a sparing effect of aureomycin on methionine. A possible mechanism for lipotropic effects of aureomycin was suggested by the observations of de la Huerga and Popper (99, 100) who found that the antibiotic reduced the urinary excretion of trimethylamine which followed oral dosage with large amounts of choline in dogs. The inference was that choline was to a considerable extent broken down to trimethylamine by bacteria in the intestine and that this action of the bacteria was abolished by aureomycin. It was found by Baxter and Campbell (10) that aureomycin, 5 gm. per kg. of diet, had a protective effect against the renal lesions produced by choline deficiency in paired-fed rats on high-fat-low-choline diets. Vitamin B_{12} was partially effective and vitamin E was inactive. The mechanism of this effect was obscure; there was only a slight increase in fecal choline.

The effects of aureomycin on dogs with ligated pancreatic ducts were studied by Kaplan *et al.* (112). These animals retain the endocrine secretion of the pancreas while losing the exocrine "anti-fatty-liver" factor which is present in pancreatic juice (47) and which may be replaced by free choline or methionine. Aureomycin was given in doses of 0.75 or 1.0 gm. daily and its administration was accompanied by a restoration of the abnormal blood pattern to normal as shown by the serum total and ester cholesterol levels; this change was paralleled by an increase in phospholipids, while the alkaline phosphatase activity decreased. These changes are characteristically produced in these animals by lipotropic substances such as choline. The changes with aureomycin reached a maximum in 3 or 4 weeks and then retrogressed to a plateau, thus recalling Gyorgy's observation (89) that the effect of aureomycin on liver necrosis was temporary. In one animal, vitamin B_{12} and folic acid were administered after the plateau had been reached and a "lipotropic response" was obtained. No such response was obtained when these vitamins were given to non-aureomycin-fed dogs. Kaplan *et al.* (112) noted that aureomycin produced no consistent depression of choline destruction as measured by trimethylamine, in contrast to the results of de la Huerga and Popper (99).

Antithyrotoxic effects. It was reported by Ershoff (75, 76) that crude supplements derived from the aureomycin or penicillin fermentation, but not crystalline aureomycin hydrochloride, prolonged the survival of immature rats fed toxic doses of thyroid. However, Meites and Ogle found that penicillin or neomycin was effective in overcoming the growth inhibition induced in rats by adding iodinated casein (142) to diets containing an adequate supply of vitamin B_{12}. The antibiotics did not change the effects of the hormone on oxygen consumption, adrenal weight or spleen weight.

Laboratory animals. The addition of aureomycin to the diet was found to improve the postoperative survival of hypophysectomized rats (46). Three rats out of 35 receiving aureomycin died in a period of 14 days as compared with 8 out of 20 controls.

It was reported that in experiments with rats the uptake of a casein hydrolysate from an isolated segment of ileum was immediately increased by adding a mixture of aureomycin and penicillin (78).

The use of antibiotics in the diets of laboratory animals may improve growth and decrease mortality. Care should be taken that such diets are not fed when the animals are to be used for purposes such as testing for syphilis, where the antibiotic may interfere with the test by destroying the pathogen. Apart from such phenomena associated with increased resistance or improved utilization of food, no disturbing effects have been noted in laboratory animals.

TABLE XIII

Aureomycin in tissues of chickens

EXPT.	GROUPS (5 CHICKENS/ GROUP)	AUREOMYCIN ADDED PER KG. DIET	AUREOMYCIN FOUND IN		MUSCLE
			Serum	Liver	
			γ/ml.	*γ/gm.*	*γ/gm.*
1	1A to 1E	None	Neg.	Neg.	—
	2A	0.2 gm.	<0.01	Neg.	—
	2B	0.2 gm.	<0.01	Neg.	—
	2C	0.2 gm.	Neg.	Neg.	—
	2D	0.2 gm.	Neg.	Neg.	—
	2E	0.2 gm.	<0.01	Neg.	—
1	3A	0.6 gm.	0.021	Neg.	—
	3B	0.6 gm.	0.015	Neg.	—
	3C	0.6 gm.	0.019	<0.05	—
	3D	0.6 gm.	0.024	0.10	—
	3E	0.6 gm.	0.011	0.065	—
1	4A	2.0 gm.	0.039	0.15	<0.05
	4B	2.0 gm.	0.046	0.24	0.165
	4C	2.0 gm.	0.054	0.30	0.16
	4D	2.0 gm.	0.044	0.165	<0.05
	4E	2.0 gm.	0.046	0.135	<0.05
2	5A to 5E	None	Neg.	Neg.	—
2	6A	2.0 gm.	0.092	0.49	0.0625
	6B	2.0 gm.	0.105	0.43	0.100
	6C	2.0 gm.	0.092	0.38	0.075
	6D	2.0 gm.	0.084	0.20	0.077
	6E	2.0 gm.	0.064	0.11	0.050
2	7A	6.0 gm.	0.260	0.39	0.165
	7B	6.0 gm.	0.090	0.18	0.08
	7C	6.0 gm.	0.100	0.23	0.20
	7D	6.0 gm.	0.350	0.37	0.33
	7E	6.0 gm.	0.088	0.24	0.20

STORAGE IN TISSUES

The extensive feeding of antibiotics to farm animals used for human food brings up the question of the presence of antibiotics in the tissues and organs of the animals so fed. The detection and estimation of an antibiotic in tissue depend on the sensitivity and accuracy of the assay method employed. Broquist and Kohler (29) using a modification of the pad-plate method (85) found that, when chicks were fed a diet containing 0.2 gm. of aureomycin per kg., barely detectable amounts of aureomycin were in the liver and serum of some of the chicks (Table XIII-Exp. 1). The level fed is ten to 20 times greater than the amounts of aureomycin commonly used for "nutritional" purposes. As shown in Table XIII, 600 and 2000 mg. per kg. of chick diet gave readily measurable but still extremely low amounts of aureomycin in serum, liver and muscle, the lowest amounts being in muscle. The second experiment, summarized in Table XIII, indicated that at very high levels of aureomycin, 0.2% or 0.6% of the diet, the accumulation of aureomycin varied between 0.1 to 0.3 microgram per gram of wet muscle tissue. Eisner *et al.* (70) have reported that citrate included in the diet increased the amount of aureomycin in the serum of rats. Broquist and Kohler observed that citrate had no effect on the serum levels obtained in the chick (Table XIV). In an experiment in cooperation with Rusoff it was found that calves receiving 20 mg. of aureomycin per kg. of diet did not show detectable amounts of aureomycin in the blood or urine while a significant amount was contained in the feces (Table XV). Four hundred mg. of aureomycin injected weekly per calf resulted in a urinary excretion of 0.2 microgram per ml., while none was found in the blood. Broquist and Kohler (29) examined three adult pigs which had not previously received an antibiotic and which were fed 200 to 2000 mg. of aureomycin per kg. of diet for 3 weeks. In comparison to chicks at equal levels the tissues of hogs fed aureomycin contained 5 to 10 times

TABLE XIV

Aureomycin in tissues of chickens fed sodium citrate in the diet

GROUPS (5 CHICKENS/ GROUP)	ADDITIONS TO BASAL DIET*		SERUM γ AUREO./ML.	LIVER γ AUREO./GM.	MUSCLE γ AUREO./GM.
	Aureomycin mg./kg. diet	Na citrate mg./kg. diet			
1	—	—	Neg.	Neg.	Neg.
2	—	600	<0.01	0.36	Neg.
3	200	—	<0.01	0.20	Neg.
4	200	200	<0.01	0.13	Neg.
5	200	600	<0.01	0.04	Neg.

* Additions to diet of 4-week old chickens; chickens then sacrificed at the end of 10 days.

TABLE XV

Aureomycin content in calf blood, bile, urine and feces

GROUPS	AUREOMYCIN CONTENT						
	Blood—γ/ml.	Bile—γ/ml.		Urine—γ/ml.		Feces—γ/gm.	
		Assay 1	Assay 2	Assay 1	Assay 2	Assay 1	Assay 2
Control..................	None	<0.01	0.02	<0.01	<0.01	Neg.	0.15
Injected (400 mg. aureo./ wk).....................	None	0.08	0.07	0.23	0.26	<0.1	<0.1
Oral (20 mg./kg. of diet)..	None	0.05	0.05	0.05	0.05	0.66	0.61

as much aureomycin (Table XVI). The experiments indicated that in chicks, calves and pigs the amount of aureomycin in the diet required to give detectable amounts in the tissues was at least twenty times as great as that commonly added to animal diets for nutritional purposes. Cooking destroyed aureomycin in all the tissues tested.

With an assay method utilizing the "cup" technique instead of pads, and spores of *B. mycoides* in place of vegetative cells of *B. cereus*, Broquist and Kohler (29) in collaboration with DiLorenzo and Welch (67a) have been able to detect extremely low amounts of aureomycin. The blood serum of chicks fed 50 mg. of aureomycin per kg. of diet for 12 weeks varied from .012 to .037 microgram per ml. With this assay method, aureomycin was not detected in the livers and muscle tissues of chickens receiving 50 or 200 mg. of aureomycin per kg. of diet, leading to the conclusion that less than 1 part per 100 million was present in these tissues.

TABLE XVI

Aureomycin in tissues of pigs fed aureomycin

GROUPS (1 PER GROUP)	ADDITIONS TO BASAL DIET	SERUM γ AUREO./ML.	LIVER γ AUREO./GM.	MUSCLE γ AUREO./GM.
1	—	Neg.	Neg.	Neg.
2	200 mgs./kg. diet	0.10	0.13	Neg.
3	2000 mgs./kg. diet	0.62	1.05	0.30

RADIATION INJURY

Cronkite (64) suggested that penicillin might be helpful in the treatment of radiation injury as a result of experiments with goats; however, no controls were available.

Antibiotics in "clinical" doses given in the diet or drinking water were found to exert a temporarily protective effect on rats and dogs exposed to lethal doses (LD_{90}) of 250 KVP x-radiation (200). Aureomycin alleviated the diarrheal state in rats following the x-radiation and was superior to chloramphenicol, streptomycin and penicillin. Survival was prolonged an additional 5 to 7 days, following which the animals died of a hemorrhagic syndrome which was not considered typical of a radiation death for this species. A lowering of the count of gram-negative organisms in the intestinal tract was noted. The effects with dogs were even more marked; it was found that these animals, when treated with clinical doses of aureomycin or aureomycin plus streptomycin, showed no evidence of radiation sickness and had bleeding tendencies associated only with local trauma. Three of six dogs receiving the antibiotic treatment survived large doses of x-radiation over a period of months. The authors suggested that aureomycin might have an effect in maintaining the integrity of the bowel against the entrance of "bacteria, bacterial products or substances of some order which act in turn as the causative agents in the production of those symptoms of radiation disease manifest in other organ systems."

Furth and Coulter (81) reported that the mortality four months after radiation was 4 out of 5 control dogs which received an LD_{90} (450 r) of whole body radiation while 3 out of 5 dogs receiving aureomycin died. Streptomycin and penicillin in combination were effective in reducing the mortality and morbidity and in prolonging the survival time of rats given lethal doses of P^{32} (19).

Streptomycin and, to a lesser degree, aureomycin, were found to reduce post-irradiation mortality in mice (143a) but it was noted that the beneficial effect was lessened if infections with *Salm. typhimurium*, *Ps. aeruginosa* or *Proteus* were developed during the experimental period, thus appearing to show a difference between any effect of the antibiotic against these microorganisms and the effect against radiation sickness. No reduction of mortality in rats following irradiation was produced in rats by aureomycin in studies by Furth et al. (81a) and Smith et al. (176c) although the latter authors observed an increase in survival time. Further results with dogs by Furth and co-workers (81b) with a large group of dogs failed to confirm the earlier conclusions of Howland et al. (cited above) and in the more recent study the results indicated that aureomycin had no effect on dogs which had received an LD_{88} or LD_{62} of total body irradiation.

SUMMARY

The addition of certain antibiotics to the diet at low levels increases the rate of growth of young animals and the efficiency with which they utilize food as measured by the ratio between food intake and increase in body weight. This effect of antibiotics is not obtained in environments in which bacteria are absent, such as in the chick embryo or in "germ-free" chicks, and the effect may be reduced when the animals are kept under highly sanitary conditions. Evidently certain widely-distributed types of antibiotic-sensitive intestinal microorganisms interfere with the utilization of food. Aureomycin, terramycin, penicillin, streptomycin and bacitracin produce the "antibiotic growth effect" in chicks when added to the diet at levels as low as 1 to 20 parts per million. The effect with other species of animals depends on the antibiotic used; the "wide spectrum" antibiotics produce the most consistent effects over a range of species. A decrease in gut weight without a change in length has been reported to be associated with the antibiotic growth effect in chicks; Coates (50) has recorded a decrease of 18% in gut weight accompanying an increase of 9% in body weight. This gives rise to the speculation that a mild "early-sprue-like" condition, engendered by certain intestinal bacteria, may exist in so-called normal animals and that this condition is corrected by the dietary use of antibiotics which inhibit or eliminate the causative bacteria. Such a change might account for some of the "dietary" effects reported for antibiotics, including improvements in the utilization of food, sparing effects on the requirement for proteins and for certain minerals and vitamins, and anti-anemic effects under conditions which respond to folic acid. However, it is possible that other dietary additions may give rise to decreases in gut weight without increasing body weight (150a) The antibiotic growth effect may be obtained when the diet is supplemented with more than sufficient amounts of the known vitamins. The tissues of antibiotic-fed animals are of normal composition.

The practice of feeding antibiotics to farm animals is widespread. No untoward effects on public health have resulted; for example, detectable amounts of aureomycin are not found in meat even when the animals are fed antibiotics at levels far higher than usual; furthermore, many antibiotics are destroyed by cooking. In any event, the daily administration of small amounts of aureomycin for prolonged periods to children has not been found to have untoward effects. Resistant strains of pathogenic bacteria have not been reported to have made their appearance in animals as a result of feeding antibiotics.

Reproduction in animals does not appear to be affected by feeding antibiotics, except that 1) hatchability of hen's eggs may be improved if the hens are on certain deficient diets, 2) the survival and early growth of young pigs and rabbits may be improved when an antibiotic is fed to the mother, perhaps because the number of pathogenic bacteria in the environment of the young animals is thus reduced.

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