to detect certain quantitative and qualitative alterations, which frequently escape other non-specific serological tests, which are dependent upon different abnormalities in proteins. Thus the presence of disease is easily discovered and diagnostic and prognostic conclusions may be drawn(3). Measurement of the thermal coagulation of serum with determination of the thermal coagulation point is simpler than other technics. It has a smaller intrinsic error because it does not require an additional measurement of protein concentration, nor the calculating of a ratio. The results obtained by either method are equally significant for the detection of abnormal thermal coagulation; the scope of both is determined by factors inherent in the basic principle.

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Effect of Antibiotics on Intestinal Microflora and on Growth of Turkeys and Pigs.* (18375)

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A marked increase in growth of turkey poults fed a diet supplemented with a whole dried aureomycin fermentation mash was reported by McGinnis *et al.*(1). This response was obtained under conditions where vitamin B_{12} was ineffective in promoting growth. Stokstad. *et al.*(2) reported that a similar product gave increased chick growth over that obtained with crystalline vitamin B_{12}. Subsequent reports have shown that crystalline aureomycin(3,4) and streptomycin(5) were effective in promoting growth of chicks, turkeys and pigs. Unpublished data from this laboratory confirm these reports and have also shown that penicillin, terramycin, bacitracin and 3-nitro-4-hydroxybenzene arsonic acid were effective in promoting growth of turkey poults.

Several possible explanations have been offered for the growth-promoting properties of antibiotics: (a) the inhibition of toxin-producing bacteria; (b) the reduction in total numbers of intestinal bacteria and lowering of competition between host and microflora for nutrients; and (c) the selective inhibition of a group of the microflora permits increased growth of other microorganisms that synthesize unidentified essential nutrients. The experiments described in this paper were conducted to determine the effects of antibiotics on the intestinal microflora of turkey poults and pigs and to find whether the changes could be correlated with increased animal growth.

Procedure. The turkey poults used in these studies were kept in electrically heated wire-floored battery brooders and were fed the experimental diets *ad libitum* immediately after hatching. Drinking water was available in metal pans at all times. The basal diet was composed of ingredients ordinarily used in making practical feeds; its composition is given in Table I. Turkey poults fed

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TABLE I. Composition of Basal Diet.

Ingredients	Parts
Ground corn	45.
Soybean oil meal (solvent)	30.
Fish meal	10.
Dehydrated alfalfa meal	2.5
Limestone	2.
Dicalcium phosphate	1.
Salt	.5
Riboflavin concentrate (500 μg/g)	.5
Vit. A & D supplement (5000 A, 1000 D/g)	.2
Choline	.25
$MnSO_4$*	—

* 8 g/100 lb.

diets supplemented with 15 p.p.m. of penicillin G (K salt) or terramycin and a combination of these antibiotics were sacrificed and the cecal contents removed for enumerating some of the various bacterial types. Even though this level of antibiotics gave a marked increase in poult growth, no consistent and reproducible differences in the microflora being enumerated could be obtained. When the level of antibiotic supplementation was increased to 100 p.p.m., it appeared that some differences were obtained.

In Exp. 1, turkeys were sacrificed after the second and third weeks and the cecal contents removed for counting of bacteria present. The cecal contents of 5 poults were thoroughly mixed and a 1 ml aliquot was used for serial decimal dilutions which were plated in triplicate on the following media:

1. Eugonagar (BBL)—for "total anaerobes" and "total aerobes". 2. Violet red, bile agar (Difco)—for Coliforms. 3. SS agar (Difco)—for lactose negative, gram negative rods.

A set of plates on medium 1 was incubated under aerobic and a duplicate set under anaerobic conditions using the procedure of Weiss and Spaulding(6) (with the inclusion of 10% CO_2) to give an index to the total number of "aerobes" and "anaerobes". The plates for coliforms and lactose-negative rods were incubated for 18-24 hours under aerobic conditions at 37°C. Five tubes of the "SF" medium of Hajna and Perry(7) were inoculated with each dilution and incubated 36 hours at 45°C for the enumeration of enterococci. The most probable numbers were determined from the tables of Halvorson and Ziegler(8). The poult growth data and bacterial counts on cecal contents at 2 and 3 weeks of age are given in Table II.

The number of bacterial types found in the cecal contents was influenced by the antibiotic supplements, but the effects of penicillin and terramycin were not consistent even though both antibiotics gave increased poult growth. In general, the coliform count was lowered. One of the most interesting observations was the increase in lactose-negative bacteria in the ceca of birds fed terramycin. In a subsequent trial, a greatly increased number of such bacteria was also found in ceca of birds fed penicillin. A more thorough examination of the lactose-negative colonies from SS agar demonstrated that members of the genus *Proteus* were present. These colonies were seeded on Kliglers iron agar and found to be H_2S producers which exhibited a strong urease activity as indicated by the medium of Rustigian and Stuart(9).

The presence of *Proteus* in ceca of birds fed the basal diet could not be demonstrated by using the urea ricinoleate medium of Zarett and Doetsch(11). In contrast, when this medium was used to plate cecal contents of poults fed antibiotics *Proteus* could be recognized and enumerated after 24 hours incubation at 37°C. This confirms the results of Harrison and Hanson(12) who did not find *Proteus* in the cecal feces of turkeys which were probably fed a diet without antibiotic supplementation. The change in flora, indicated by the presence of *Proteus,* that followed the administration of terramycin appears to be similar to that caused by aureomycin, since Metzger and Shapse(10) found an immediate and marked increase of *Proteus* in

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TABLE II. Exp. 1. Effect of Antibiotics on Turkey Growth and Microflora of Cecal Contents.

Supplement to basal diet			No. of bacterial types in cecal contents (millions per ml of sample)				
Penicillin, p.p.m.	Terramycin, p.p.m.	Avg poult wt, g	Total anaerobes	Total aerobes	Coliforms	Lactose-negative rods	Enterococci
			2 wk				
—	—	142(31)*	7170	663	126	.0002	171
100	—	191(34)	8200	69	9.3	.0002	5.42
—	100	191(48)	4730	66	4.9	.016	349
100	100	198(46)	4630	—	6	.68	349
			3 wk				
—	—	287(26)	323	69	16.6	.000	1.09
100	—	389(29)	6600	167	.4	.0004	.0493
—	100	359(38)	1213	450	4.9	.079	17.1
100	100	410(35)	3130	910	63.0	7.2	91.8

* No. of poults in parentheses.

TABLE III. Exp. 2. Effect of Penicillin and Terramycin on Growth and *C. perfringens* Counts of Cecal Contents.

Supplement to basal diet		Avg wt, g		Counts of black colonies/ml	
Penicillin, p.p.m.	Terramycin, p.p.m.	7 days	14 days	7 days	14 days
—	—	84(21)*	170(9)	3800	25230
100	—	95(22)	246(11)	<100	<100
—	100	96(28)	229(11)	<100	<100
100	100	103(26)	244(11)	<100	<100

* No. of poults in parentheses.

human intestinal contents following administration of aureomycin.

Proteus did not always increase following the feeding of antibiotics to turkeys. It therefore does not appear that the growth-promoting effect of antibiotics was due to an influence on *Proteus* alone, if at all.

Since clear-cut results were not obtained in Experiment 1, a second experiment was conducted. Because the possibility existed that antibiotics promote growth by preventing the production of bacterial toxins in the intestine, it was decided to make counts of certain *Clostridium* in the cecal contents of turkeys fed the basal diet with and without antibiotics. For this purpose a sulfite-glucose-iron agar medium was used which had previously been employed by Wilson and Blair(13) and Skinner and Baskin(14) for counting *Clostridium perfringens (C. welchii)* in water and in soils, respectively. The plates of this medium were incubated anaerobically at 37°C for 12-18 hours. After this time, large black colonies were counted. Typical colonies were inoculated into milk and were found to produce "stormy fermentation" characteristic of *C. perfringens*. This evidence along with the experience of others indicates that these organisms probably belong to the genus *Clostridium* and may possibly be *C. perfringens*. The results on poult growth and *C. perfringens* counts are given in Table III.

Following the observations that penicillin and terramycin inhibited the growth of *C. perfringens* in the ceca of turkeys, samples of feces were collected from pigs fed a purified diet with and without terramycin supplementation. A count was made of *C. perfringens* and the results are presented in Table IV. The average weights of pigs fed the diet are also given.

The reduction in the number of *C. perfringens* brought about by terramycin was

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TABLE IV. Effect of Terramycin on Growth and *C. perfringens* Count of Pig Feces.

Supplement to basal diet			Counts of black colonies/g	
Vit. B_{12}, g/kg	Terramycin, mg/kg	Avg wt at 8 wk, lbs.	1st count	2nd count
—	—	57.0	—	33700000
50	—	66.3	34300000	79400000
50	15	75.8	10	10

* 6 pigs/group.

very marked. A second count showed even more striking results. These findings that penicillin and terramycin inhibit *C. perfringens* agree with the results reported by Bliss and Warth(15) who showed penicillin and aureomycin to be inhibitory to members of this genus *in vitro* and *in vivo*.

Conclusions. In view of the inhibitory effect of penicillin and terramycin on *C. perfringens* in the intestinal tract of turkeys and of terramycin in pigs, it appears possible that these antibiotics promote growth by preventing enterotoxemia. This possibility is supported by data gathered by Merchant(16) which show that enterotoxemia in sheep is caused by *C. perfringens.*

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Effect of Niacin and Tryptophan in Counteracting Toxicity of Crystalline Borrelidin for Rat.*† (18376)

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The production of pellagra-like signs in animals fed toxic materials has been described by several investigators. Stockman and Johnston(1) reported the isolation of an organic acid from corn which produced coma and death in monkeys, rabbits and guinea pigs. No attempt was made to counteract the effects with niacin. Woolley(2) concentrated a nitrogenous base from corn meal which, on addition to a synthetic ration, inhibited the growth of mice. Indolacetic acid was claimed to inhibit growth of rats(3), but the experiments were not confirmed(4). It was shown that adenine at high levels produced the symptoms of black tongue in dogs (5), but no attempt was made to counteract this effect with niacin. Krehl *et al.*(6) demonstrated the role of corn in the production of niacin deficiency in rats.

Berger, Jampolsky and Goldberg(7) have reported the isolation of a toxic crystalline antibiotic, borrelidin, from the culture broth

* A preliminary report was presented before the Fed. Am. Soc. for Exp. Biol., Apr. 1950, Atlantic City.

† Roche Publication No. 221.

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