lationship was found between those PPLO giving rise to diphtheroids and the derived diphtheroids but no serological cross reaction between PPLO strains not giving rise to diphtheroids and the derived diphtheroids. Although it is apparent that the Campo and V73 strains can be converted to diphtheroids, final confirmation of the phenomenon cannot be made until serological tests show the diphtheroids from PPLO cultures are similar or identical with one another but different from contaminant diphtheroids and until the diphtheroids are converted to a PPLO similar or identical to the parent PPLO.

Eventual establishment of a relationship between all PPLO strains and some microbial species is not improbable. The conversion of bacteria to a stable L form can be considered to involve a mutation or mutations, either spontaneous or induced. Conversion of a PPLO, which can be considered a stable L form, although the related bacterium is unknown, to a bacterium can likewise be considered to involve a mutation or mutations. In the case of the 3 strains which exhibited conversion to diphtheroids in this study, spontaneous mutation could be operative. Other strains, which have not shown a tendency to convert may have such a low mutation rate that conversion is not evident. If the proper conditions were employed conversion might occur in all PPLO strains. Presently these conditions are unknown. Additional evidence that PPLO may be related to some microbial species is the widespread ability of bacteria to form L colonies and protoplasts.

Summary. A high frequency of appearance of diphtheroids in liquid culture of 3 established strains of pleuropneumonialike organisms has been noted. Statistical, morphological, biochemical and serological tests have shown that a relationship exists between these diphtheroids and the pleuropneumonialike organisms in which cultures the bacteria appear. All PPLO strains tested are serologically related. PPLO strains not giving rise to diphtheroids do not cross react with diphtheroids. Diphtheroids appearing in PPLO cultures differ from oral and air diphtheroids.

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Received September 19, 1957. P.S.E.B.M., 1957, v96.

Chlortetracycline in the Nutrition of Guinea Pigs.*† (23537)

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Numerous reports indicate that chlortetracycline (aureomycin) is harmful to the guinea pig. Roine *et al.*(1) have reviewed the literature and have explained the toxicity of orally administered aureomycin on the basis of a change in the intestinal flora. They found in treated animals an abundance of organisms that belong to the genus *Listeria,* and believed this organism to be the cause of death. Eyssen *et al.*(2) found penicillin, baci-

* Contribution from Mo. Agric. Exp. Station, Journal Series No. 1776. Approved by the Director.

† The aureomycin HCl was generously supplied through the courtesy of Dr. T. H. Jukes, Lederle Laboratories Division, Amer. Cyanamid Co., Pearl River, N. Y.

TABLE I. Chlortetracycline and Growth Rate of Weanling Male Guinea Pigs.

Chlortetracycline HCl, mg/kg diet	No. of animals	Avg daily gain, g (8 wk)
A. Purified diet		
0	12	6.3
25	8	5.5
50	4	6.5
75	8	5.3
100	4	6.4
200	4	5.7
B. Stock diet		
0	4	5.9
25	12	6.0

tracin, streptomycin and chlortetracycline to be toxic when administered singly or in various combinations, but their observations did not correspond to those of Roine *et al.*(1). They were unable to isolate *Listeria monocytogenes,* and penicillin and chloramphenicol did not protect against chlortetracycline. Chlortetracycline is not toxic under all laboratory conditions. Hewes(3) observed that it stimulated the growth of weanling guinea pigs and had no adverse effect on reproduction.

The purpose of this communication is to present additional evidence that chlortetracycline is not harmful to guinea pigs under all conditions and, in fact, is helpful in the control of certain infections. Chlortetracycline has been fed to guinea pigs in this laboratory for a period of about three years without evidence of toxicity. The experimental data presented here show its effect on growth and reproduction.

Methods. Two types of diets were used. One was a practical type diet composed of ground wheat 26; wheat germ 6; alfalfa meal 34; dried yeast 15; iodized salt 1; steamed bone meal 2; soybean oil meal 15; vitamin A-D concentrate 1; $MnSO_4 \cdot H_2O$ 0.025 and ascorbic acid 0.05%. The purified diet was similar to ration 3884 previously described (4), but contained 2.7% potassium acetate and 0.5% magnesium oxide in place of an equal amount of sucrose. For growth studies, groups of male guinea pigs weighing from 170 to 200 g were fed the respective diets for eight weeks. Feed and water were supplied *ad libitum* and the animals were weighed weekly. The reproduction data are based on all animals in the stock breeding colony during two periods—one year before the use of chlortetracycline and the year following. It was included in the stock diet at a level of 25 mg/kg. The animals were kept in battery cages with one male and three females per cage. The litters were born in the colony cage so that mating usually occurred immediately after parturition.

Results. Chlortetracycline was first used in the colony to treat an acute infectious enteritis of unknown etiology. It was effective in controlling this epidemic and it seemed desirable to include the antibiotic in the diet routinely. Since chlortetracycline had been reported to be toxic to guinea pigs, it was fed to weanling animals at different levels to check toxicity under our conditions. The results of this trial are shown in Table I. The number of animals was small and consequently there was a high variability in rate of growth, but under the conditions, chlortetracycline had no significant effect on rate of gain. There was no mortality during the eight-week period. In view of these results, 25 mg/kg of diet seemed well within the tolerance of the guinea pig and this level has been included in experimental diets fed to over 1000 weanling guinea pigs with no obvious adverse effect. In fact, it appears helpful in the control of certain types of infectious enteritis, but it has not prevented *Salmonella* infections.

The data in Table II show the reproductive

TABLE II. Effect of Chlortetracycline on Reproductive Performance of Guinea Pigs.

	Period I*	Period II†
Chlortetracycline HCl	0	25 mg/kg
No. females	161	138
No. mortalities	94	51
Cases of lymphadenitis	21	1
No. of abortions	24	7
No. of litters	352	248
No. of young born	998	822
% weaned	91	97
Avg birth wt (g):		
2 in litter	117 (75)‡	117 (48)
3 " "	97 (120)	107 (95)
4 " "	98 (61)	96 (49)

* Period I extended from 9/1/53 to 9/1/54.
† Started aureomycin supplement 9/1/54. Period II from 10/1/54 to 9/18/55.
‡ No. in parentheses indicates No. of litters.

performance of the stock colony during the year before use of chlortetracycline and the year following. The number of females indicated includes all animals that were in the colony during the year regardless of the period of time. The number in period I was greater because of higher mortality and hence a larger number of replacements. During period I, about 13% of the animals were affected with cervical lymphadenitis‡ or lumpy jaw, but there was only 1 case after the use of chlortetracycline. Since that time there have been no cases in the colony. The percentage of abortions was markedly lower in period II. The litter size, birth weight and weaning percentage were slightly, but perhaps not significantly, higher during period II. At the present time a large proportion of our experimental animals are among the fourth generation since chlortetracycline has been routinely used in the colony diet.

In view of the observations of Roine *et al.* (1), one might suspect that the successful use of dietary chlortetracycline may be due to the absence of certain types of bacteria in the intestinal flora of our animals. Listeria was thought to be the causative agent in the Finland colony. In view of widespread occurrence of *Listeria monocytogenes,* it seems unlikely that our colony is free of this organism. In fact, in at least one case it has been cultured from an animal during a routine pathological check.

Summary. Chlortetracycline hydrochloride has been included in various diets of a guinea pig colony for 3 years without evidence of toxicity. It had no effect on growth rate and caused no mortality when added to a purified diet at levels from 25 to 200 mg/kg. At the routine level of 25 mg/kg, the antibiotic decreased abortions and adult mortality and eliminated cervical lymphadenitis from the breeding colony. Although chlortetracycline may be toxic to guinea pigs under some conditions, in our laboratory it has been beneficial in the maintenance of a breeding colony and has been used routinely in purified experimental diets for growth studies.

‡ The causative organism, which belonged to the genus Streptococcus, was isolated and identified by Dr. L. D. Kintner, Veterinary Pathology Department.

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Received August 16, 1957. P.S.E.B.M., 1957, v96.

Accumulation of Calcium^{45} by Salivary Glands. (23538)

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Many investigators have compared Ca^{45} exchange in various tissues(1,2,3), but none appear to have included the salivary glands in their studies. Chemical determinations of calcium concentration in the salivary glands of cats has revealed that the concentration was approximately twice that of plasma(4). In dogs, no difference was apparent(4). Histochemical determination of calcium in mouse tissues did not reveal a greater concentration in salivary glands than in other tissues(5). The present report compares the changes in specific activity with time after administration of calcium^{45} in the submaxillary gland, kidney, and blood of female rats. Total calcium in submaxillary glands, kidneys, and blood of rats is also determined.

Methods. Calcium^{45} chloride* in a dose of 10 μc was given to ether-anesthetized 6-month-old virgin female Long-Evans rats by injection into a foot vein. At intervals from

* Calcium^{45} was purchased from Union Carbide Nuclear Co., Oak Ridge, Tenn. under license number 4-918-1. The specific activity was 92 Mc/g.