Antibiotic Potentiation

A Review

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Shortly after the discovery that antibiotics in feeds would increase growth of chickens, it was found that higher levels could be used economically for therapeutic purposes. The therapeutic use of antibiotics orally has been a great boon for the poultry and livestock industry and today many feed manufacturers provide high-level antibiotic feeds. In addition, a higher concentration of antibiotics may be administered orally by means of the drinking water.

High levels of oral antibiotics are applied for two purposes: (1) to attack non-specific detrimental organisms in the digestive tract, i.e. those that cause enteric disorders and (2) to prevent or treat systemic diseases, i.e., those of the respiratory system, blood stream, synovial tissues, etc. To attack organisms in the digestive tract antibiotics do not necessarily have to be absorbed into the blood stream, but to inhibit systemic diseases it is essential that the antibiotic be present at effective levels in the blood. Systemic diseases in poultry that have been subjected to antibiotic treatment include Chronic Respiratory Disease, sinusitis, synovitis, ornithosis, etc. Of the oral antibiotics used for therapeutic purposes those with broad spectra of antibacterial activity are most effective against systemic diseases due to their more rapid rate of absorption into the blood stream.

Recently there has been wide interest in antibiotic potentiation. This term refers to any technique which will increase the level of antibiotic in the blood stream following oral administration.

The discovery of antibiotic potentiation in animals came about indirectly. Several years ago it was observed in studies with disease organisms causing mastitis that a higher level of Terramycin was required to inhibit organisms grown in milk as compared with those grown in a pure nutrient broth (1). Further investigations showed that the factors in milk which inhibited the antibiotic activity were the divalent ions calcium and magnesium. It was shown that if the calcium and magnesium of milk were removed by absorption on resins, the minimal inhibitory level of antibiotic against test organisms in the treated milk was much less — in other words, its effectiveness was increased. Furthermore, it was shown that by adding calcium-binding agents to whole milk, the amount of antibiotic required to treat a specific disease organism in the milk was also decreased. Such calcium-binding agents as ethylenediamine tetra-acetic acid (E.D.T.A.), oxalic acid, citric acid, etc. were studied. All increased the activity of Terramycin against disease organisms in milk.

These findings suggested that, since calcium forms a relatively large portion of poultry diets, substantial quantities of Terramycin might be "tied up" by the calcium in the digestive tract of the bird and thereby not available for absorption into the blood. As was expected, the Terramycin blood serum levels markedly increased in birds fed diets low in calcium as compared to those fed high calcium diets (2, 3, 4). A further step in this line of reasoning was to include in the ration an organic acid which could form an insoluble compound with the calcium and thereby allow the Terramycin to be absorbed (5). Organic acids such as oxalic, citric, etc. did increase the blood antibiotic levels when included in medicated feeds.

Among a long series of organic acids studied as calcium-binding agents, tereph-

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thalic acid was discovered to have exceptional antibiotic potentiating properties. In order to study the antibiotic absorption mechanism more closely, a technique was established whereby birds were anaesthetized and the duodenal loop exposed through a surgical opening posterior to the rib cage of the chicken. Each end of the duodenal loop was tied off in order to prevent movement of contents. Then solutions of materials under study were injected by an inoculating needle into this isolated duodenal loop. Subsequently the loop was replaced in the bird and the incision clamped. The bird was then revived and subsequently at fixed intervals samples of blood were taken by puncture of the wing vein. By this method it was possible to demonstrate the absorption rate of the antibiotic which had been injected into the loop.

Using the above technique, the effect of injecting calcium along with the Terramycin (4 mg.) is observed in Table 1.

It will be observed that the Terramycin blood level is significantly reduced when calcium is injected simultaneously with the antibiotic into the duodenal loop. Terramycin urine levels are similarly reduced by the calcium.

Evidence that organic acids tie up the calcium and allow the Terramycin to be absorbed from the loop at a faster rate is indicated in Table II.

It is noteworthy in the citric acid experiment that the urine level also increased as the blood level increased due to faster absorption of Terramycin. However in Table III presenting a similar experiment using terephthalic acid, it will be observed that where the blood level was increased by the presence of terephthalic acid in the duodenal loop, the urine level did not parallel this. The same is true whether calcium was present or absent from the duodenal loop.

This leads one to suspect that there are two different avenues of antibiotic potentiation: the first in which the antibiotic is tied up by calcium in the intestinal tract and therefore cannot be absorbed as readily into the blood stream; the second type of potentiation apparently brings about not only increased blood levels but decreased excretion rate of the antibiotic from the blood stream. This results in a higher overall antibiotic content in the blood for a longer period of time.

It was of interest to determine the effect of altering the calcium content of a chick ration as well as the effect of additional terephthalic acid in the diet on the antibiotic blood level. A summary

TABLE I

The Influence of Calcium on Absorption of Terramycin From The Ligated Duodenal Loop of Chickens

Specimen	Mcg. Terramycin/ml. or Gm. Treatment TM	TM + 16 mg. Ca.
Serum (90 min.)	0.414	0.162*
Serum (180 min.)	0.351	0.212*
Liver	0.807	0.563*
Urine (180 min.)	60.0	20.6*
Duodenal Loop (1)	3301.9	3686.6

*Significant decrease from TM
(1) Unabsorbed TM

TABLE II

Effect of Citric Acid on Absorption of (4 MG.) Terramycin from the Duodenal Loop in the Presence and Absence of Exogenous Calcium (4 MG.)

Citric Acid (mg.)	Specimen	Terramycin Blood Levels (Mcg/ml or Gm) Treatment: Terramycin	Terramycin & Citric Acid	Terramycin & Calcium	Terramycin & Citric Acid & Calcium
0.4	Serum	0.67	0.71	0.40(1)	0.53(1)
	Liver	1.06	1.39	0.88	1.04
	Urine	68.10	75.80	36.30(1)	62.40
0.8	Serum	0.54	0.56	0.37(1)	0.55(2)
	Liver	1.47	1.35	1.14	1.45
	Urine	81.00	68.00	26.80(1)	71.00(2)

(1) Significant decrease from TM (2) Significant increase over TM & Ca.

TABLE III

Effect of Terephthalic Acid (TPA) on Absorption of Terramycin (4 Mg.) from) The Duodenal Loop in The Presence and Absence of Exogenous Calcium (4 Mg.

Teraphthalic Acid Conc. (Mg.)	Specimen	Terramycin Blood Levels (Mcg/ml or Gm) Treatment			
		Terramycin	Terramycin & TPA	Terramycin & Calcium	Terramycin & Calcium & TPA
0.4	Serum	0.54	0.81(2)	0.28(1)	0.36
	Liver	1.12	1.64(2)	0.85(1)	1.02
	Urine	87120	50.00(1)	26.70(1)	21.50(1)
0.8	Serum	0.70	1.03(2)	0.27(1)	0.53(2)
	Liver	1.10	1.42(2)	0.80(1)	0.93
	Urine	81.80	58.90	42.40(1)	31.50(1)

(1)Significant decrease from Terramycin
(2)Significant increase over Terramycin
(2)Significant increase over Terramycin & Calcium

of several practical feeding experiments with chickens is shown in Table IV. It may be observed that Terramycin is increased in the blood stream either by decreasing the calcium or by the use of terephthalic acid, maximum effect being accomplished when both potentiating techniques are used simultaneously.

When antibiotics are used in oral therapy, the higher the blood level that can be attained the better, particularly against systematic diseases. In studies at the Pfizer Agricultural Research Centre, potentiation mechanisms were evaluated practically in synovitis trials with chickens (4). Synovitis is an excellent disease for this purpose, since it is easy to infect a bird by inoculating synovitis organisms into the hocks or joints. Moreover the degree of severity can be numerically evaluated. This is done by setting up a lesion score based on the seriousness of the involvement in the hock, foot pad, etc. A score of 0 to 9, as suggested by West Virginia researchers (8), was used, 0 being no lesions and 9 being extremely severe lesions.

Using an experimental strain of syno-

TABLE IV

Effect of Terephthalic Acid on Antibiotic Blood Serum Concentrations of Chicks Fed Rations Containing Various Calcium & Phosphorus Levels

Antibiotic Level in Feed	Calcium	Phosphorus	Terephthalic Acid*	Terramycin Serum Lev. (mcg/ml)
g/Ton	%	%		
100	1.20	0.70	−	.150
100	1.20	0.70	+	.289
100	0.18	0.70	−	.401
100	0.18	0.70	+	.548
200	1.20	0.70	−	.210
200	1.20	0.70	+	.408
200	0.18	0.70	−	.687
200	0.18	0.70	+	.828
200	0.70	2.35	+	.710
500	2.25	0.55	−	.178
500	2.25	0.55	+	.304
1000	1.20	0.70	−	.617
1000	1.20	0.70	+	1.200
1000	0.18	0.70	−	1.237
1000	0.18	0.70	+	2.790
1000	1.20	4.08	−	1.435
1000	2.25	0.55	−	.368

*8 lbs./Ton Feed

vitis organism 200 grams of Terramycin per ton of feed fed continuously was needed to control the disease completely. However the use of a low calcium — high phosphorous ration with terephthalic acid along with Terramycin at 50 grams per ton gave complete control of the disease with a treatment period as short as one week.

In another synovitis test run under similar conditions, the infected untreated control birds developed lesions very rapidly. In the case of the birds fed the normal calcium diet (1.18 per cent calcium), Terramycin at 100 grams per ton fed for 10 days reduced the severity of lesions somewhat but not as well as the 200 gram per ton level. At both 100 grams and 200 grams of Terramycin, with 7.5 pounds of terephthalic acid per ton of feed, good control of synovitis lesions occurred during the medication period. Best results were obtained with the higher level of antibiotic. It was observed that when medication was stopped at 10 days lesions reappeared rather rapidly with all treatments except in the group given the higher level of antibiotic plus terephthalic acid. In the case of the chickens fed low calcium (0.18 per cent), all treatments maintained a very low lesion score during the period of medication. Complete inhibition of lesions was obtained in the group administered the higher level of antibiotic plus terephthalic acid. When medication was stopped, lesions did not appear as rapidly in those treatments containing terephthalic acid, and good control of the disease continued for as long as two weeks following the termination of treatment.

Besides synovitis, the diseases coccidiosis, fowl typhoid and CRD have been studied with antibiotics at high level combined with antibiotic potentiation techniques (6). In all cases, antibiotic potentiation — either lowered calcium or addition of terephthalic acid to the ration — has improved the therapeutic effect of the antibiotic. Field studies have shown that PPLO can be completely eliminated by this technique (7). Further work is being continued in an effort to determine the optimum method of eliminating PPLO along with the least cost for the procedure.

Concerning alteration of dietary calcium, it is important to note that the calcium cannot be completely eliminated from the diet, especially for long periods of time. Work has been conducted to determine the length of time that calcium may be removed from the diets of both growing and laying chickens so as to evaluate the practicality of this antibiotic potentiation technique (2).

For growing chicks, calcium was altered to different levels in two tests with results shown in Table V.

TABLE V

Influence of Dietary Calcium on Chick Growth to Two Weeks

Calcium	Weight	Index
% of Diet	gm.	%
	Test 1	
0.473	181	86
0.702	191	90
0.930	211	100
	Test 2	
0.138	122	63
1.138	194	100
2.138	186	96

It will be observed that when calcium is decreased to approximately 0.5 per cent in a growing diet, the growth rate was lowered but not to a radical degree. However, when all of the calcium sources of the diet were removed so that the ration contained only 0.13 per cent calcium, about 1/3 of the growth was lost in a two-week period. It would be important in broilers not to eliminate calcium for periods of two weeks since this would decrease growth rate. However, for replacement chicks, the rate of growth in early stages would not be as economically important, and it would probably be better to lower the calcium and at the same time use a high level antibiotic feed in order to obtain the maximum antibiotic effect against a particular disease.

The results with low calcium in rations of layers is shown in Table VI where it is observed that even on an extremely low calcium diet, egg production was not drastically reduced over a 5-day period. It would appear, therefore, that should an outbreak of a systemic disease occur in laying birds, it would be better to eliminate calcium for a period of 2—3 days and tolerate a slightly reduced egg production than to have the disease progress and thereby result in a greater production loss.

With regard to terephthalic acid use in foods, it appears to be safe at the level required for optimum activity. Terephthalic acid has been fed to chickens at levels up to 80 pounds per ton without in-

TABLE VII

Effect of Terephthalic Acid Salts on Potentiation of Terramycin

Terramycin Feed Level	Water Level	Acid or Salt Used(1)	Terramycin Serum Levels
g/Ton	mg/Liter		mcg./ml
—	—	—	0.150
200	—	—	0.150
500	—	—	0.355
500	—	Terephthalic acid	0.686
500	—	Disodium terephthalate	0.715
500	—	Dimethyl terephthalate	0.538
—	100	—	0.150
—	250	—	0.275
—	250	Doisdium terephthalate	0.538
—	250	Monoethanolamine terephthalate	0.582
—	250	Triethnolamine terephthalate	0.535

(1) 0.375% in feed and 0.1875% in water.

TABLE VI

Influence of Low Calcium Diet on Egg Production

Pen No.	6-Week Pre-Exptl. Period	Exptl. Period 5-Days		6-Week Post Exptl. Period
	E.P.(1)	Ca	E.P.	E.P.
	%	%	%	%
1	65.4	1.34(2)	64.4	56.6
2	68.8	1.34(2)	69.5	61.9
3	55.8	0.12	50.0	55.7
4	68.2	0.12	62.1	57.4

(1) E.P. — Egg Production

(2) Plus limestone grit **ad libitum**

creased mortality (6). However, at 50 pounds per ton, noticeable decreases in growth rate appear. Optimum levels of terephthalic acid in feeds appear to be about six to eight pounds per ton regardless of the level of antibiotic used.

More recently (9) it has been shown that certain salts of terephthalic acid have antibiotic potentiation activity and may be administered by way of the feed or drinking water. Table VII indicates results of these tests.

In all cases the salts gave similar results to those obtained with terephthalic acid alone whether administered in the feed or drinking water. It does not appear that solubility of the compopund is related to its potentiating ability since terephthalic acid is less soluble than the salts tested; nor was the relative potentiating activity of any compound proportionate to its insolubility.

The mode of action of terephthalic acid in enhancing antibiotic blood levels is thought to be related to an effect of decreasing urinary excretion of the antibiotic (4). Additional information to advance this postulation was shown in work where the antibiotic and potentiation agent were both administered by subcutaneous injection (9). The amount of disodium terephthalate required to bring about maximum terramycin blood levels varied from 25—200 mg per 1½ lb. broiler. Regardless of the terephthalate level, there was a definite increase in terramycin blood concentrations in all instances, indicating the mode of action to be within the blood system of the bird.

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(4) **PRICE, K. E.,** and **ZOLLI, JR. Z.** The Influence of Dietary Calcium — Phosphorous and Terephthalic Acid on Antibiotic Control of Experimental Infectious Synovitis. Axian Diseases **3,** 135, 1959.

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(6) **PRICE, K. E.** Antibiotic Potentiation. Proceedings 7th Agricultural Research Conference, Chas. Pfizer & Co., Inc. Terre Haute, Ind. 1959.

(7) **LUGINBUHL, R. E.,** University of Connecticut. Personal Communication.

(8) **OLSON, N. O., et al.** Synovitis Control. A Comparison of Levels of Antibiotics. Am. Journal Vet. Res. **18,** 200, 1957.

(9) **PRICE, K. E., ZOLLI, JR. Z.,** and **DONOVAN, G. A.** The Influence of Orally and Parentally administered Salts of Terephthalic Acid on Oxytetracycline Serum Levels in Chickens. In Press.

Paul Marois, M. V., Named Regional Correspondent

The Editorial Board of this Journal is pleased to announce the appointment of Paul Marois, M.V., as Regional Correspondent for the Province of Quebec. This is the first such appointment to be made since the Journal's editorial board was reconstituted late in 1959.

Dr. Marois was born in Montreal in February, 1919. He obtained his doctorate in veterinary medicine from the University of Montreal in 1940, his Science degree in 1944, and his Master's degree in Science from the same university in 1946.

Employed at the Institute of Microbiology and Hygiene at the University of Montreal since 1941, he is now chief of the veterinary section and in charge of research. From 1945 to 1951, Dr. Marois was in charge of the course in microbiology at the School of Veterinary Medicine of the Province of Quebec and from 1953 to 1959 in the same capacity at Marianapolis College.

Dr. Marois has been president of the Veterinary Medicine Association of the Province of Quebec since 1957, and was secretary of the group from 1946 to 1955.

He is a member of a number of microbiological and veterinary medicine associations on this continent.

C'est avec grand plaisir que le Comité de la Rédaction de cette publication annonce l'appointement du Dr. Paul Marois, M.V., comme correspondant régional pour la province de Québec. Celle-ci est la première augmentation de notre groupe depuis qu'il fut ré-organisé vers la fin de l'année 1959.

Le docteur Paul Marois, M.V., est né à Montréal le 22 février, 1919. Il obtint son doctorat en médecine vétérinaire de l'Université de Montréal en 1940, sa license ès Sciences en 1944 et la maîtrise ès Sciences à la même université, en 1946.

A l'emploi de l'Institut de Microbiologie et d'Hygiène de l'Université de Montréal depuis 1941, il y est maintenant chef de la section vétérinaire et chargé des recherches.

De 1945 à 1951, le docteur Marois a été chargé du cours de Microbiologie à l'Ecole de Médecine Vétérinaire de la Province de Québec et de 1953 à 1959, au Marianapolis College.

Il est président de la Société de Médecine Vétérinaire de la Province de Québec depuis 1957 et en fut le secrétaire de 1946 à 1955.

Il est membre de plusieurs sociétés de microbiologie et de médecine vétérinaire du continent Nord-Américain.

The Use of Bephenium Chloride in the Treatment of Ancylostoma caninum infections in dogs when other Anthelmintics are Contraindicated.

In a number of reports which have appeared recently bephenium compounds have been shown to be effective against Ancylostoma caninum infection in dogs and Nematodirus and other trichostrongyle nematodes in lambs. Bephenium hydroxy napthoate has also been reported to be superior to tetrachlorethylene in the treatment of the common hook worm Necator americanus in man. In this study the results of treatment of twenty dogs with heavy infestation of Ancylostoma caninum with Bephenium is reported. In each case the number of eggs per gram of faeces was determined immediately before treatment and again three to twelve days after treatment, using Stoll's dilution technique. In some dogs, where this was not possible, the results were assessed by the presence or absence of eggs seen after flotation. The drug was given orally in gelatine capsules each holding 100 mg. All the dogs in this series had some disease other than Ancylostomiasis and were selected for this treatment because they could not normally have been given any other anthelmintic. All the animals improved, and seventeen of the twenty dogs treated showed no eggs after a period of three to seven days or more. Only one dog showed a few eggs eight days after treatment. Two dogs showed a slight nausea on administration of the drug. The results indicated that Bephenium Chloride is an anthelmintic which is safe for dogs suffering from severe Ancylostromiasis and other complications. This safety is attributed to the fact that Bephenium salts are very scarcely absorbed from the intestine. The results are similar to those reported in man where it was found that Bephenium could be given to patients with advanced anaemia, diarrhoea, hepatitis and cirrhosis.

P. Seneviratna, The Vet. Record 72:200-203.