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## Influence of Bile Acids, Vitamin K and Cincophen on Erosions of the Chick Gizzard Lining.

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We have reported<sup>1, 2</sup> the curative and preventive effect of bile acids, particularly cholic acid, in experimental deficiency lesions or erosions of the chick gizzard lining. These studies have been continued.

The basal, gizzard-factor-deficient diet employed, the care of chicks, and the methods of analysis for cholic acid and of scoring gizzard erosions were the same as previously described.<sup>2</sup> Chicks were deprived of food for 12 to 14 hours previous to killing since this practice causes a greater accumulation of bile in the gall bladder.

The results of further experiments (Table I) confirm our earlier report.<sup>2</sup> Cholic acid and dehydrocholic acid consistently show strong preventive action against gizzard erosions at ½% of the diet. Dehydrocholic acid, the only bile acid sufficiently non-toxic to be administered by injection, is not preventive when so given. Deoxycholic acid is undoubtedly the least effective of these bile acids.

Since our first reports, certain workers<sup>3, 4</sup> have suggested that the

TABLE I.  
Effect of Bile Acids on Chick Gizzard Erosion.

Supplement to basal diet	Level in diet, %	No. of groups†	Avg erosion score divided by basal group erosion score	Avg cholic acid content of dried gall bladder bile‡ mg per g
None	—	15	1.00	111
Cholic acid	0.50	9	0.15	542
" "	0.25	2	0.36	540
Dehydrocholic acid	0.50	5	0.16	104
" "	0.50*	2	0.92	86
Deoxycholic acid	0.50	5	0.73	85
Glycocholic acid	0.50	1	0.58	393

\*Administered by frequent intramuscular injection of the aqueous sodium salt in amounts equivalent to 0.50% of the diet consumed.

†Each group contained 7 or more chicks, in most cases 10.

‡Cholic acid was determined on pooled samples from each group.

1 Almquist, H. J., *Science*, 1938, **87**, 538.

2 Almquist, H. J., and Mecchi, E., *J. Biol. Chem.*, 1938, **126**, 407.

3 Lansing, A. I., and Miller, D., *Poultry Science*, 1940, **19**, 258.

4 Cheney, G., *Proc. Soc. Exp. Biol. and Med.*, 1940, **45**, 190.

mechanism of the effect of the bile acids is that of promoting the absorption of a fat-soluble, anti-erosion factor. A closer consideration of our results would have made it apparent that such explanation is unlikely, since deoxycholic acid is particularly known to facilitate absorption of the fat-soluble vitamins,<sup>5</sup> yet it is comparatively inactive against gizzard erosions.

The gall bladder bile analyses show that the anti-erosion activity is not merely a process of increasing the cholic acid in the bile. One-fourth percent of cholic acid causes the same cholic acid content per gram of dried bile but is less effective than 1/2% cholic acid in preventing erosions. It would appear in this case, as in the experiments with injected dehydrocholic acid, that the concentration of bile acid coming in direct contact with the lining is important. Dehydrocholic acid does not raise the bile cholic acid above that of the basal groups, yet has a strong preventive effect. This bile acid evidently has a specific action like that of cholic acid. That dehydrocholic acid is not, to any significant extent, converted to cholic acid is indicated by the cholic acid content of the gall bladder bile which is even lower than that of the basal groups.

Certain writers<sup>6, 7, 8</sup> have differentiated between kinds of gizzard lining abnormalities such as roughening and thickening, sub-surface hemorrhagic lesions, crater-like lesions and necrotic areas. All these we have observed in our chicks also. It is a question whether these conditions are really differences in kind or merely in degree. The strongest evidence for the latter view, that all the abnormalities are but manifestations of one primary deficiency, lies in the observation that one pure, natural substance, cholic acid, is capable of preventing all these conditions.

In spite of published evidence<sup>9</sup> that gizzard erosions are independent of vitamin K deficiency, a relation has again been suggested,<sup>3</sup> without supporting data. We had noted that prolonged massive doses of the highly potent antihemorrhagic compound, 2-methyl-1,4-naphthoquinone, tended to reduce the discoloration due to decomposed blood around the erosions, but not the actual erosions. The more potent, water-soluble, 2-methyl-1,4-naphthohydroquinone

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<sup>5</sup> Cohn, E. T., and Schmidt, C. L. A., *Proc. Soc. Exp. Biol. and Med.*, 1939, **41**, 443; Schmidt, C. L. A., *Pac. Coast Med.*, 1938, **5**, 7.

<sup>6</sup> Lansing, A. I., Miller, D., and Titus, H. W., *Poultry Science*, 1939, **18**, 475.

<sup>7</sup> Blount, W. P., *Veterinary J.*, 1939, **95**, 301.

<sup>8</sup> Bird, H. R., Oleson, J. J., Elvehjem, C. A., Hart, E. B., and Halpin, J. G., *Poultry Science*, 1937, **16**, 238.

<sup>9</sup> Almquist, H. J., and Stokstad, E. L. R., *Nature*, 1936, **137**, 581; *J. Nutrition*, 1937, **13**, 339.

TABLE II.  
Effect of Various Forms of Vitamin K on Gizzard Erosions.

Supplement to basal diet	Level in diet, mg per kg	Avg erosion score divided by basal group erosion score	Avg prothrombin clotting time, sec.
Vitamin K <sub>1</sub> *	100	0.82	28.9
'' ''*	½ }	0.18	34.0
Cholic acid	5000 }		
2-methyl-1,4-naphthohydro- quinone diphosphoric acid†	50	1.27	
Phthiocol‡	2,500	0.90	
Practical chick mash	total diet	0.00	28.1

\*No alfalfa extract present in basal diet in these cases.

†Hydrated tetra sodium salt. Fed at approximately 500 times the required level for normal prothrombin clotting time as determined by previous assay.

‡Fed at approximately 100 times the required level for normal prothrombin clotting time as determined by previous assay.

diphosphoric acid ester and the synthetic vitamin K<sub>1</sub>, 2-methyl-3-phytyl-1,4-naphthoquinone, were fed in large doses, but had no effect in reducing gizzard erosion. In the presence of a sub-optimal amount of vitamin K<sub>1</sub> (½ mg per kg of diet) cholic acid was able to maintain the erosion score at a low value (Table II). We have repeatedly measured blood clotting times and blood prothrombin levels of groups of chicks that proved to have severe gizzard erosions, finding these blood characteristics quite normal. It would be hard to obtain more conclusive evidence of a fundamental difference in the two deficiencies.

A large number of substances have been tested and found ineffective. These include cholesterol, phytosterol, yeast sterols,  $\alpha$ -tocopherol, phytol, glucuronic acid, gum arabic, agar, choline, creatine, phytic acid, aloin, mucin powder, linoleic acid, dried stomach lining, butter, artichoke, avocado, papaya, garlic, whole-milk powder, and copper sulphate. Chondroitin and certain samples of wheat germ oil showed a slight protective effect at levels of 5% of the diet. Hog bile salts which contain relatively little cholic acid proved less effective than beef bile salts which are a good source of cholic acid.

It is well known that the compound cincophen, administered orally or intravenously, causes gastric ulcers in the dog.<sup>10</sup> Recently it has been reported that cincophen also produces lesions or erosions of the chick gizzard lining.<sup>4</sup> Preliminary tests showed that the addition of one-half percent of cincophen to our basal deficiency diet caused a slight increase in severity of the lesions. The lesions remained, superficially at least, the same as those usually observed, with the exception of an occasional instance of cylindrical penetration

<sup>10</sup> Bradley, W. B., and Ivy, A. C., *PROC. SOC. EXP. BIOL. AND MED.*, 1940, **45**, 143.

TABLE III.  
Effects of Additions of Cincophen and of Bile Acids to a Practical Chick Ration.

Supplement to practical ration	Level in diet, %	Avg erosion score*	Gall bladder bile†	
			Vol. per chick, cc	Cholic acid in dried bile, mg per g
None	—	0.10	0.19	91
Cincophen	1.0	0.93	0.20	113
Cincophen	1.0	0.13	0.67	478
Cholic acid	1.0			
Cincophen	1.0	0.36	1.16	413
Ox bile salts	2.0			

\*Average erosion score for 15 chicks.

†Pooled samples from each group.

into the underlying tissues and, in a few cases, perforation. Cholic and dehydrocholic acids were found to exert a protective effect even in the presence of cincophen.

Chicks reared on a practical mash which ordinarily permitted no erosions were found to develop erosions closely resembling the deficiency type, even to the usual discoloration, when fed cincophen. At least 1% in the diet was required to cause severe erosions. Two percent of cincophen was decidedly inhibitory to growth and caused heavy mortality. Experiments were continued with the practical mash as it was felt that the lesions produced could be attributed entirely to cincophen. Some results are given in Table III.

Development of the cincophen-induced erosions was largely prevented by the simultaneous feeding of cholic acid and of beef bile salts. In overcoming the effects of cincophen on the gizzard lining, cholic acid evidently functions in a similar way as in the prevention of deficiency erosions. The fundamental rôle of cholic acid in the maintenance of the lining is, therefore, further emphasized.

It is of interest to mention reported indications that cincophen tends to reduce cholic acid synthesis in the dog.<sup>10</sup> The analyses of the chick gall bladder bile do not, however, give any indication of a reduction of amount of cholic acid present in the chick when cincophen is fed as compared to no cincophen. On the other hand, the increased bile volume and cholic acid content of bile in the remaining groups may have been assisting factors in the reduction of the erosion score.

The above evidence is in accord with our previously expressed view,<sup>2</sup> based on the presence of bile pigment and of cholic acid in combination with the lining, that bile and especially cholic acid,

coming in periodic direct contact with the lining serves naturally to aid in maintaining the lining in sound condition.

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### Effect of Adrenalectomy on Intestinal Absorption Involving Osmotic Work in Rats.

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The abnormalities of the mineral metabolism of adrenalectomized animals are common knowledge, and have been the subject of several comprehensive reviews (*c. g.*, Kendall<sup>1</sup>). In the kidney it has been possible to study the course of interference with the normal sodium and potassium balance in some detail. Further analyses in the kidney are however confronted with great difficulties. Fortunately, disturbances of reabsorption by the kidney tubuli can often be demonstrated by analogous experiments with the small intestine. Phloridzin interference with sugar reabsorption in proximal parts of the tubuli can be seen for example in the inhibitory effect of phloridzin on active sugar absorption in the intestine (Wertheimer<sup>2</sup>). Visscher and coworkers<sup>3</sup> in a series of investigations have shown that an active transport of ions in a direction opposite to that of the concentration gradient can regularly be demonstrated in the small intestine under specified conditions. This finding seems to be of general physiological significance; a similar transport of ions against the direction of the gradient has been shown to exist in plant roots by Lundegardh<sup>4</sup> and in several freshwater animals by Krogh.<sup>5</sup> In this connection mention may also be made of the papers of Steward<sup>6</sup> and Keys.<sup>7</sup> It seemed of some importance in view of these results to ascertain whether an active transport of ions against the direction

<sup>1</sup> Kendall, E. Z., Congress Bericht 1, 16 Intern. Physiol. Congr., August, 1938, Zurich, p. 47.

<sup>2</sup> Wertheimer, E. *Pflüger's Arch.*, 1933, **233**, 514.

<sup>3</sup> Ingraham, R. C., and Visscher, M. B., *Am. J. Physiol.*, 1938, **121**, 771.

<sup>4</sup> Lundegardh, H., *Biochem. Z.*, 1937, **290**, 104.

<sup>5</sup> Krogh, A., *Scand. Arch. Physiol.*, 1937, **76**, 60.

<sup>6</sup> Steward, F. C., *Trans. Faraday Soc.*, 1937, **33**, 1006.

<sup>7</sup> Keys, A., *Trans. Faraday Soc.*, 1937, **33**, 930, 972.