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Relation of Milk, Cholesterol and Estrogens to Cholic Acid Formation in Chicks.

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Liquid milk products in the diet of the chick are known to exert a preventive effect on the development of gizzard erosions caused by either a dietary deficiency or the presence of cincophen in the diet.[1,2] This preventive action of milk products is evidently related in both cases to an enhanced secretion of cholic acid.[2] An earlier report linked the gizzard erosions with a deficient formation of bile in the chick and especially of cholic acid in the bile.[3]

Further studies have been made of the action of milk in promoting cholic acid formation in the chick. In general, the methods employed were the same as previously described.[2,3] Two types of diets were used, a complete practical diet containing 1% of cincophen and a deficient basal diet.[2,3]

Attempts were also made to concentrate the anti-erosion and cholic acid-producing activity from whey by a procedure essentially similar to those used for the extraction of estrogenic substances from urine.[4] The extract obtained yielded distinct color tests for estrogens.[4,5] Presence of such substances in milk and whey has been reported.[6] We have, consequently, investigated known pure estrogenic compounds, since the presence of estrogens in milk and their general structural relation to the bile acids are particularly suggestive of a possible role in bile acid formation in the chick.

The results given in Table I confirm an

TABLE I.
Effect of Milk Products, Cholesterol, and Estrogens on Cholic Acid Content of Chick Gall-bladder Bile.

Diet	Supplement	Level in diet	No. of groups	Avg cholic acid per g dried bile, mg
Practical	None		1	148
"	Skim milk	*ad lib.*	1	240
"	Cincophen	1%	1	118
"	Skim milk + cincophen	*ad lib.* 1%	1	203
Basal	None		6	104
"	Skim milk	*ad lib.*	4	168
"	Protein-free milk	"	3	154
"	Buttermilk	"	1	153
"	Lactic acid whey	"	1	155
"	Cholesterol	0.3%	2	100
"	Whey estrogen fraction	twice *ad. lib.* intake of whey	1	91
"	Theelin	100 mg/kilo	2	106
"	"	50 "	1	98
"	Estriol	50 "	1	105
"	Diethylstilbestrol	200 "	3	80
"	"	50 "	1	108

[1] Cheney, G., Proc. Soc. Exp. Biol. and Med., 1940, **45**, 190.

[2] Almquist, H. J., Mecchi, E., and Kratzer, F. H., Proc. Soc. Exp. Biol. and Med., 1941, **47**, 525.

[3] Almquist, H. J., and Mecchi, E., *J. Biol. Chem.*, 1938, **126**, 407.

[4] Bachman, C., and Pettit, D. S., *J. Biol. Chem.*, 1941, **138**, 689.

[5] Kleiner, I. S., *J. Biol. Chem.*, 1941, **138**, 783.

[6] Supplee, G. C., *Ind. Eng. Chem.*, 1940, **32**, 238.

earlier report[2] that milk products given in place of drinking water lead to an increased level of cholic acid in the gallbladder bile of the chick. In addition, it is shown that liquid buttermilk, whey and protein-free milk all exhibit an activity similar to that of skim-milk. The protein-free milk was made by isoelectric precipitation of casein, filtering, coagulation of remaining proteins by heating, filtering again and neutralizing. There remains no doubt that the greater part of the cholic acid-producing activity remains in milk after separation of the proteins of milk, and that the activity is resistant to heating to the boiling point at pH 4 to 5. Cholic acid-producing activity is not shown by the estrogen fraction from whey, or by the pure estrogens tested, or by another structurally related substance, cholesterol.

The results obtained in this study confirm previous reports of the existence in milk of a cholic acid-producing substance, possibly a precursor of bile acids, which is yet unidentified.

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Effects of Smallpox Vaccination (Vaccinia) on Serologic Tests for Syphilis.

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False positive serologic tests for syphilis have been attributed to many causes. More recently,[1,2,3,4] smallpox vaccination (vaccinia) has been added to the list. Lynch[3] and his associates, in a controlled study, showed that false positive reactions occurred in 16% of 263 vaccinated subjects. The Kolmer, Kline, Hinton and Mazzini tests were employed. One or more of these tests were positive, varying in intensity from ± to ++++. The reactions appeared in 2 weeks and remained positive for 2 months in most instances, but a few persisted for 4 months and one for 5 months.

An opportunity for a similar study was presented to us recently.[5] A group of individuals (mostly medical students and nurses) was chosen, in whom a serologic test had been performed within a reasonable time previous to the vaccination. There were 202 individuals, ranging in age from 3 weeks to 84 years. Over one-half were between 20 and 30 years of age. Forty-four individuals developed a primary cutaneous (non-immune) reaction at the site of inoculation, 134 developed a local accelerated (vaccinoid) form, and 24 were immune.

Out of 202 individuals on whom a single blood examination was performed, 19 were found to be positive, ranging in intensity from ± to ++++ by one or more of the Kolmer, Kahn and Mazzini tests. Not all persons were tested at the same time. They were examined from 10 days to 57 days following the vaccination, but 93.6% were done before the forty-third day. One month following vaccination 25 persons that gave a negative reaction were re-examined. Four out of this group were now positive. Undoubtedly, others would have been detected if the entire group had been examined more frequently.

In all, there were 24 individuals out of 202 who gave a false positive reaction for syphilis. This is an incidence of 11.8%. The majority of the reactions were ++ or less in intensity

1 Barnard, R. D., *Illinois M. J.*, 1940, **77**, 78.

2 Thomas, G. E., and Garrity, R. W., *U. S. Naval Med. Bull.*, 1941, **39**, 272.

3 Lynch, F. W., Boynton, R. E., and Kimbal, A. C., *J. A. M. A.*, 1941, **117**, 591.

4 Mohr, C. F., Moore, J. E., and Eagle, H., *Arch. Int. Med.*, 1941, **68**, 1161.

5 Favorite, G. O., *Phila. Med.*, in process of publication.