

It is concluded that cell cultures are superior to embryonated hen's eggs in detecting viruria, that in the cell culture system cytopathic effect is a more sensitive indicator of virus than hemagglutination or hemadsorption, but that the latter is a simple and rapid confirmatory technic.

We are pleased to acknowledge the skillful technical assistance of Mr. Donald Hollabaugh.

1. Utz, J. P., Kasel, J. A., Cramblett, H. G., Szwed, C. F., Parrott, R. H., *New England J. Med.*,

1957, v257, 497.

2. Shelokov, A., Vogel, J. E., Chi, L., *Proc. Soc. Exp. Biol. and Med.*, 1958, v97, 802.

3. Utz, J. P., Szwed, C. F., Kasel, J. A., *Proc. Soc. Exp. Biol. and Med.*, 1958, v99, 259.

4. Am. Public Health Assn., *Diagnostic Procedures for Virus and Rickettsial Diseases*, 2nd ed., 578 pp., New York, 1956.

5. Bengston, I. A., *Pub. Health Rep.*, 1944, 402.

6. Rowe, W. P., Hartley, J. W., Cramblett, H. G., Mastrota, F. M., *Am. J. Hyg.*, 1958, v67, 57.

7. Hsiung, G. D., *Fed. Proc.*, 1962, v21, 465.

Received June 4, 1962. P.S.E.B.M., 1962, v110.

Amprolium 10. Influence of Egg Yolk Thiamine Concentration on Chick Embryo Mortality. (27667)

DONALD POLIN, ELIZABETH R. WYNOSKY AND CURT C. PORTER

Merck Institute for Therapeutic Research, Rahway, N. J.

A thiamine deficiency can be produced in adult chickens and eggs(1,2,3) by feeding sufficiently high dietary levels of the coccidiostat amprolium* [1-(4-amino-2-*n*-propyl-5-pyrimidinylmethyl) - 2 - picolinium chloride • hydrochloride]. The drug is deposited in egg yolks at concentrations related to its level in the diet(2) but is innocuous to developing embryos or newly hatched chicks over a wide range of diet levels studied(2,3). At very high levels of amprolium in feed, embryo mortality was observed and correlated with depressed free thiamine concentrations in the egg(3).

From the many experiments(1,2,3) used to evaluate effects of amprolium on reproduction of adult chickens, data were obtained on yolk thiamine concentrations, and hatch results associated with these yolk levels. These and other data in this report made it possible for the first time to estimate minimum free thiamine concentration in yolk for optimum development of White Leghorn chick embryos.

Methods and procedures. Adult White Leghorn female chickens were housed in single cages of 3-deck batteries in a constant temperature room at 72°F or on wire-floored

pens in a wood frame house. They were fed *ad libitum* for 21 days a commercial breeder ration containing amprolium and then non-medicated diet for 12 days. Daily collections of eggs from the hens were set weekly in an incubator at 99½°F and 85% humidity. Pooled samples of yolk from 3 eggs were obtained from each day's collection and analyzed for free thiamine and amprolium. In other studies, weekly collections were employed(1,2,3). Fertile eggs were obtained from battery-reared hens by use of artificial insemination, and from hens in wire-floored pens by use of 1 male to each 10 females. Hatchability was calculated from the number of normal chicks obtained from fertile eggs, and this proportionality was plotted after arc sin transformation, according to Snedecor(4).

Results. Free thiamine concentrations in yolks averaged 2.91 ppm during the first 3 days that .2% amprolium was fed to hens. Thereafter, its concentration declined to a low of .38 ppm by day 11 on medication (Fig. 1). On the other hand, the hatch showed no significant change ($P < .05$) until day 8, when it declined from a control of $85 \pm 2\%$ (mean $\pm$ standard error) to 65, and thereafter continued to decline reaching its lowest level by day 21 (Fig. 1). Preliminary (unpublished)

* Coccidiostat from Merck & Co., Inc., Rahway, N. J.

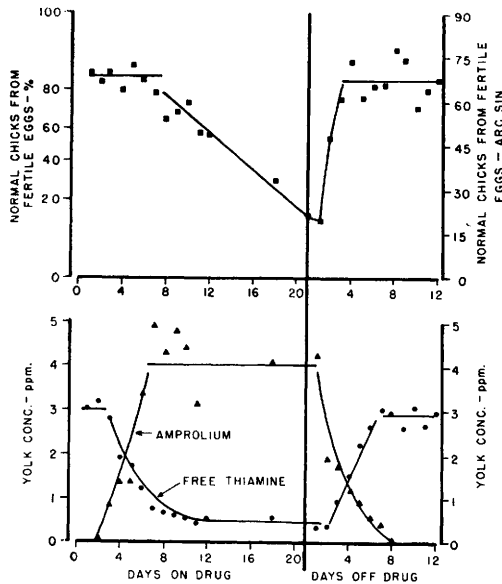


FIG. 1. Yolk concentrations of amprolium and free thiamine in relationship to percent of normal chicks from fertile eggs during 21 days hens were fed .2% amprolium, and during first 12 days after withdrawal of medication.

studies of 9 weeks duration established that maximal effect on the hatch occurred during the 3rd week on medication.

On the 2nd day after withdrawal of the amprolium diet, the hatch showed a marked improvement, but no increment was detected in yolk free thiamine. Thereafter, free thiamine levels progressively increased to attain

the original control value of about 3 ppm by day 6 off medication (Fig. 1). Hatchability returned to normal within 3 days after withdrawal of amprolium.

Amprolium concentrations averaging 4 ppm in yolk were reached by day 6 on medication and drug was no longer detected in yolk by the 9th day after withdrawal (Fig. 1).

It was previously demonstrated(3) that embryo mortality occurred whenever thiamine concentrations in the yolk were far below normal (Fig. 1). Furthermore, supplemental thiamine in amprolium diets, or injection of the vitamin into eggs from hens fed the medicant, reversed embryo mortality(3). This occurred even though the concentration of amprolium ranged as high as 48 ppm in some eggs(3), a concentration 10-fold greater than values presented in Fig. 1. This led to the conclusion that amprolium, *per se*, was not toxic to the embryo, but was mediating its effect by preventing thiamine deposition in yolk. Hatch results from the many experiments using graded levels of amprolium from .006 to .8% were related to the yolk concentrations of free thiamine found in yolk samples representing each hatch. The data, along with those from control groups, are presented in Table I. Normal hatches, ranging from 72 to 85% were obtained when free thiamine concentrations in yolks were at least .63 ppm,

TABLE I. Relation Between Free Thiamine Concentration in White Leghorn Eggs and Resultant Hatch Expressed as Normal Chicks from Fertile Eggs.

Interval, ppm	Yolk-free thiamine		Amprolium in yolk observed, ppm	No. of settings*	No. of fertile eggs	Hatch, %
	Observed mean, ppm					
.0 - .125	.04	22.6	1	0	—	—
.126 - .250	.23	24.8	2	0	—	—
.251 - .375	.32	9.7	9	172	35.8†	—
.376 - .500	.47	5.8	12	333	42.3†	—
.501 - .625	.59	6.5	9	221	64.0†	—
.626 - .750	.68	6.0	3	77	77.1	—
.751 - .875	.80	12.3	4	151	78.3	—
.876 - 1.00	.98	1.7	3	61	84.3	—
1.01 - 1.50	1.24	3.1	11	390	78.6	—
1.51 - 2.00	1.68	4.1	10	283	85.8	—
2.01 - 2.50	2.25	0.8	6	103	72.2	—
2.51 - 3.00	2.78	control	16	410	84.6	—
3.01 - 3.50	3.24	control	11	337	78.6	—
3.51 - 4.00	3.81	control	10	286	82.2	—
4.01 - 4.50	4.33	control	4	36	84.3	—

* Also indicates number of pooled yolk samples analyzed for thiamine and amprolium.

† Weak and dead chicks at hatch.

observed mean = .68 ppm. Lower concentrations than this were associated with a decline in hatchability evidenced by either an increase in embryo mortality during the last 3 days of incubation or the presence of weak or dead chicks in the hatching tray.

Very few eggs were obtained with thiamine concentrations in yolk below .25 ppm. Hens which were expected to lay such eggs manifested symptoms of a thiamine deficiency and were usually out of production.

Discussion. The minimum free thiamine concentration in yolk for optimum hatch was found to be .63 ppm. This value is far below the 3.0 or 2.8 ppm of the vitamin found in control yolks when hens receive commercial or laboratory stock rations containing, respectively, 5.8(3) or 5.4(5) ppm. Thus, a surplus of thiamine was available for embryo development. This was indicated by the delay in onset of a decrease in hatch while free thiamine concentrations in yolk declined (Fig. 1).

Only free thiamine was analyzed in yolk but there were indications that another form of thiamine may be physiologically important. The presence of a non-free or "bound" thiamine could account for the failure of the hatch and free thiamine curves to fall and rise together. Thus, a decline in non-free thiamine would explain the continued decline in the hatch even though free thiamine levels had reached their minimum at an earlier point. Also, replenishment of non-free thiamine in yolk prior to deposition of the vitamin in the free state could account for the significant rise in the hatch on day 2 after removal

of the medicated diet, whereas not until the next day did free thiamine levels start their increase toward normal.

These data apply only to adult females of light breed chickens. No inference can be made with respect to heavy breeds because these birds deposit significantly less thiamine in the yolk while fed the same diet as light breeds(5,6). An additional study would be needed to establish their minimum thiamine level required for optimum hatch.

Summary. Free thiamine concentrations in yolk decreased from 3 to .4 ppm when adult White Leghorn females were fed .2% amprolium. The lowest free vitamin concentration in yolk was reached by day 11 on medication, but maximum effect on the hatch did not occur until the 3rd week on medicated diets. On withdrawal of medication, free thiamine in yolk returned to original concentration in 6 days while normal hatches were obtained in 3 days. Minimum free thiamine in yolk for optimum hatch was found to be about .63 ppm.

1. Polin, D., *Poultry Sci.*, 1960, v39, 1284.
2. Polin, D., Porter, C. C., Wynosky, E. R., Cobb, W. R., *ibid.*, 1962, v41, 372.
3. Polin, D., Wynosky, E. R., Porter, C. C., *J. Nutrition*, 1962, v76, 59.
4. Snedecor, G. W., *Statistical Methods*, ed. 5, Iowa State Coll. Press, Ames, Iowa, 1956.
5. Scrimshaw, N. S., Hutt, F. B., Scrimshaw, M. W., Sullivan, C. R., *J. Nutrition*, 1945, v30, 375.
6. Howes, C. E., Hutt, F. B., *Poultry Sci.*, 1956, v35, 1223.

Received June 4, 1962. P.S.E.B.M., 1962, v110.