TABLE II.
Titration of Hamster-Adapted Newcastle Virus in Hamsters.

Virus	Dilution 10-1	10-2	10-3	10-4	10-5	Titer
Hamster brain 5th intradermal passage originally from 14th nasal passage	*4/4	4/4	4/4	0/4	0/4	$10^{-3.5}$
Hamster brain 3rd intradermal passage originally from 300th intracerebral passage	3/3	4/4	2/4	0/4	0/3	10^{-3}

* Numerator denotes No. of deaths.
Denominator denotes No. of animals inoculated.

the 5th intradermal passage of the 14th intranasal series. Specific immune chicken serum completely neutralized the intradermal brain virus from both series, whereas normal chicken serum had no effect.

The virus titered 10^{-3} in the first series (300th intracerebral) and $10^{-3.5}$ in the second series (14th intranasal), according to the Reed-Muench calculation.[3] Details are given in Table II. Paralysis of the pharynx, as evidenced by excessive salivation with wetting of the mouth and chin, is now a distinct symptom in hamsters.

It has been observed in other modified forms of this virus that day-old chicks are susceptible, whereas older individuals are resistant to infection.

Summary. After the 300th intracerebral and the 14th intranasal hamster passage, Newcastle disease virus, California strain No. 11,914 can successfully infect hamsters by intradermal injection. The virus in the brain material from each series was passed intradermally in hamsters. Virus was isolated from the brain and cord of hamsters of both series upon inoculation of embryonated chicken eggs. The results of the neutralization tests confirm the virus to be that of Newcastle disease. Hamsters infected intracerebrally, intranasally, and intradermally show similar symptoms, such as involuntary motor reactions, excessive salivation from pharyngeal paralysis, general paralysis, prostration, and death.

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[3] Reed, L. J., and Muench, H., *Am. J. Hyg.*, 1938, **27**, 493.

Effect of Oxythiamine on the Growth of Chicks.* (17364)

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Bergel and Todd[1] reported the synthesis of oxythiamine.‡ They found that 1 mg of this compound possessed no antineuritic ac-

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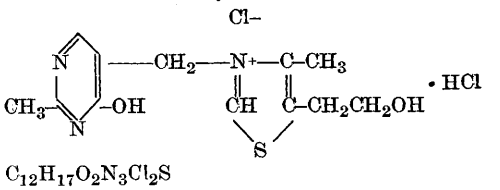
[1] Bergel, F., and Todd, A. R., *J. Chem. Soc.*, 1937, 1504.

‡ The formula of oxythiamine is



$C_{12}H_{17}O_2N_3Cl_2S$

tivity when assayed by the bradycardia method using the rat. Soodak and Cerecedo[2] prepared oxythiamine by deamination of thiamine with nitrous acid, and observed that the compound produced a marked toxic effect when fed to mice. The administration of 25 to 50 μg of oxythiamine per day for a 2-week period resulted in the death of young mice maintained on a thiamine-low synthetic diet supplemented with 1 μg of thiamine per day. In this report evidence is presented which shows that oxythiamine is also an active antagonist of thiamine in the nutrition of the chick.

Experimental. Four experiments were conducted with day-old White Leghorn cockerels, 10 or 15 per lot. They were housed in electrically heated battery brooders in a room in which the temperature was thermostatically controlled. All chicks were on wire mesh floors to prevent coprophagy. Feed and water were supplied *ad libitum*. The chicks were fed basal diet 653 described by Hill, Norris, and Heuser,[3] modified by adding 100 μg of synthetic folic acid per 100 g of diet and by omitting thiamine. In the first 3 experiments crude casein was used instead of purified casein. In these experiments the basal diet contained between 20 and 25 μg of thiamine per 100 g, as determined by the thiochrome method. The procedure used was that proposed by the Research Corporation Committee on the thiochrome method.[4] The basal diet fed in Experiment 4 in which purified casein was used contained 7 μg of thiamine per 100 g. The chicks were weighed individually at weekly intervals.

Exp. 1 and 2. Two preliminary experiments were conducted to study the effects of graded levels of oxythiamine§ on the growth of chicks to 4 weeks of age. Oxythiamine at the levels of 0.5, 1, 2, and 8 mg was added to 125 μg of thiamine per 100 g of diet. The results indicated that oxythiamine retarded growth at all levels used; the 8 mg level was so highly toxic that only one chick survived to 3 weeks of age. The chicks showed the typical thiamine deficiency symptoms of head retraction, convulsions and inability to stand. Two chicks with these severe symptoms were given 100 μg of thiamine by injection, and within 2 hours they were on their feet and eating. The average 4-week weights of the chicks ranged from 289 g for those receiving 125 μg of thiamine to 182 g for those receiving 125 μg of thiamine plus 2 mg of oxythiamine per 100 g of diet.

Exp. 3. This experiment was designed to obtain a quantitative estimate of the effectiveness of oxythiamine as an antagonist of thiamine. The data from Exp. 1 and 2 indicated that the levels of oxythiamine to feed with approximately 125 μg of thiamine per 100 g of diet for the best results were between 1 and 2 mg. Exp. 3 was conducted using an experimental diet containing 120 μg instead of 125 μg of thiamine per 100 g plus 3 levels of oxythiamine. The basal diet contained 5 μg less thiamine per 100 g than the diet fed in Exp. 1 and 2. Eight levels of thiamine were included in order to establish a growth curve. The results of Exp. 3 are presented in Table I.

When oxythiamine was fed in addition to 120 μg of thiamine a marked reduction in growth occurred. By plotting a curve for the effect of varying amounts of thiamine on growth, it was possible to determine the amount of thiamine that was counteracted by the different amounts of oxythiamine. An index was calculated by determining the ratio of the amount of inhibitor to the amount of vitamin antagonized by the inhibitor. The range of the 3 indices obtained in this experiment was 27-39.

Exp. 4. This experiment was designed to study the reversibility of this antagonism by additions of thiamine. The basal diet contained purified casein. Increasing levels of thiamine were added to a level of 2 mg of oxythiamine per 100 g of diet. This study was continued for a period of 4 weeks. The

[2] Soodak, M., and Cerecedo, L. R., *J. Am. Chem. Soc.*, 1944, **66**, 1988.

[3] Hill, F. W., Norris, L. C., and Heuser, G. F., *J. Nutrition*, 1944, **28**, 175.

[4] Hennessy, D. J., *Cereal Chemists' Bull.*, 1942, **2**, 1.

§ The authors are indebted to Dr. Gustav J. Martin, The National Drug Company, Philadelphia, Pa., for the oxythiamine used in these experiments.

TABLE I.
Inhibitory Action of Oxythiamine on Chick Growth.

Treatment, μg thiamine	Avg wt 3 wk, g	Avg wt 4 wk, g	Index*
1. 45†	— (0)‡	— (0)	
2. 70	142 (4)	161 (4)	
3. 80	152 (9)	218 (7)	
4. 90	162 (12)	251 (10)	
5. 100	176 (14)	264 (13)	
6. 110	187 (15)	250 (15)	
7. 120	180 (15)	264 (15)	
8. 220	175 (15)	266 (14)	
9. 120 + 1 mg oxythiamine	179 (15)	233 (14)	27
10. 120 + 1.5 mg oxythiamine	136 (15)	191 (15)	34
11. 120 + 2 mg oxythiamine	118 (14)	157 (12)	39

* Index is the ratio of amount of inhibitor to amount of vitamin inhibited.
† Crude casein basal diet contained 20 μg thiamine per 100 g. The levels of thiamine indicate the total thiamine present per 100 g of diet.
‡ Numbers in parentheses represent the surviving chicks of original 15.

TABLE II.
Reversal of Inhibitory Effect of Oxythiamine by Thiamine.

Treatment, thiamine	Avg wt 3 wk, g	Avg wt 4 wk, g	Index*
1. 62 μg†	75 (1)‡	— (0)	
2. 72 μg	99 (4)	86 (1)	
3. 82 μg	128 (8)	126 (5)	
4. 92 μg	140 (7)	168 (6)	
5. 102 μg	147 (9)	194 (9)	
6. 112 μg	177 (10)	250 (10)	
7. 122 μg	185 (10)	259 (10)	
8. 1 mg	172 (10)	246 (10)	
9. 122 μg + 2 mg oxythiamine	105 (9)	124 (9)	49
10. 147 μg + 2 mg oxythiamine	116 (10)	153 (10)	34
11. 172 μg + 2 mg oxythiamine	121 (10)	172 (9)	25
12. 1 mg + 2 mg oxythiamine	160 (10)	244 (9)	

* Index is the ratio of amount of inhibitor to amount of vitamin inhibited.
† Purified casein basal diet contained 7 μg thiamine per 100 g. The levels of thiamine indicate the total thiamine present per 100 g of diet.
‡ Numbers in parentheses represent the surviving chicks of original 10.

results of this experiment are presented in Table II.

The addition of 2 mg of oxythiamine to 122 μg of thiamine caused a 135 g retardation in growth. However, as more thiamine was added to this level of oxythiamine growth was improved. Fifty μg of added thiamine resulted in a 48 g increase, while the addition of 1 mg of thiamine produced normal growth. It is possible that much less than 1 mg of thiamine would have given maximum growth. The inhibitory index for the three levels of thiamine ranged from (25-49).

At the end of the 4th week the chicks of lots 9, 10, and 11 of Exp. 4 were divided into 3 groups. Three chicks from each lot were continued as controls, 3 received injections of 200 μg of thiamine per day into the breast muscle, and 3 received 200 μg of thiamine per day by stomach tube. These chicks were selected to give groups of equivalent weights. All chicks were continued on the diets they had received from the start of Exp. 4. Lot 7 receiving 122 μg of thiamine was continued as the positive control group. The study was carried out for 12 days. The results are summarized in Table III.

The toxicity produced by oxythiamine can be at least partially corrected by additional thiamine. The data show that by injecting or giving thiamine orally a growth response was produced. Injection was somewhat more

TABLE III.
Effect of Parenteral and Orally Administered Thiamine on Oxythiamine-Toxic Chicks.

	Treatment	Avg initial wt,* g	Avg final wt,† g	Avg change in wt, g
1.	Control	160‡	262	102
2.	Injected§	153	304	151
3.	Oral‖	153	290	137
4.	Positive control¶	286	490	204

* Weight at 4 wk of age.
† At end of 12-day experimental period.
‡Avg of 8 chicks, rest are avg of 9 chicks.
§ 200 μg thiamine inj. per day into breast muscle.
‖ 200 μg thiamine per day by stomach tube.
¶ Group 7 of Exp. 4 received 122 μg thiamine per 100 g of diet from hatching.

effective than oral administration. In a 12-day period the chicks receiving thiamine gained 35 to 49 g more than the controls, although as should be expected, their growth rate was less than that of the positive controls.

Discussion. Oxythiamine produced a thiamine deficiency state in chicks, which could be prevented, or cured to some extent, by administering increased amounts of thiamine. This antagonist was very effective as evidenced by the inhibitory index of 25-49. Although this index is an approximate value it is quite low and comparable to that obtained by Woolley and White[5] for the pyrithiamine: thiamine index in mice of 40. It has been shown by Woolley and White[6] that a low pyrithiamine:thiamine index is obtained with bacteria that require thiamine as the intact molecule. If a microorganism can utilize one or both moieties of the thiamine molecule, the index is 10 to 100 times greater. The chick requires the entire thiamine molecule, thus a low oxythiamine:thiamine ratio is to be expected.

Whether the oxythiamine-thiamine inhibition is a strictly competitive one is doubtful. In Table I it is noted that the chicks in lot 2 averaged 161 g at 4 weeks of age with 4 chicks surviving of the original 15, whereas those in lot 11 grew at the same rate, 157 g, but 12 chicks survived. The same effect is observed in Table II, where chicks receiving low levels of thiamine showed a greater mortality than chicks receiving both oxythiamine and a higher level of thiamine, although the growth rates were similar. (Compare lots 3 and 9, 4 and 11). If simple competition were involved similar mortality would be expected in these lots of chicks.

The requirement of thiamine for White Leghorn cockerels on a purified casein diet is considerably greater than on a crude casein diet. On the purified casein diet the requirement for optimum growth to 4 weeks of age was between 102 and 112 μg of thiamine per 100 g. Whereas, on the crude casein diet the requirement was between 80 and 90 μg per 100 g. The thiamine values obtained by the thiochrome method on replicate samples of each diet were very consistent, although the concentration was low, 0.07 μg per g and 0.2 to 0.25 μg per g, respectively. The explanation for these different requirements is not known, although it may be that the purification process removed from casein some substance that affects the requirement of thiamine. The addition of liver and fish meal supplements to the purified diet produces a significant growth response.[7] It is possible that one of the unknown growth factors present in these materials and crude casein and not in purified casein to any great extent, may affect the requirement of thiamine.

Summary. Oxythiamine, the 4'-OH analogue of thiamine, has been shown to be an effective thiamine antagonist in the nutrition of the chick. By increasing the level of oxythiamine fed, the chick weight was decreased until typical thiamine-deficiency symptoms occurred and death ensued. The addition of

[5] Woolley, D. W., and White, A. G. C., *J. Biol. Chem.*, 1943, **149**, 285.

[6] Woolley, D. W., and White, A. G. C., *J. Exp. Med.*, 1943, **78**, 489.

[7] Unpublished data, Agricultural Experiment Station, Cornell University, Ithaca, N. Y.

large amounts of thiamine to the diet prevented the toxicity due to oxythiamine, and the parenteral and oral administration of thiamine to oxythiamine-toxic chicks tended to overcome the inhibition.

The chick, as well as the mouse, has been shown to be very sensitive to the addition of oxythiamine to the diet. It seems probable, therefore, that oxythiamine is an antagonist of thiamine in the nutrition of all species that require this vitamin as an essential nutrient.

Evidence has been presented which indicates that the thiamine requirement of growing White Leghorn cockerels is greater in the absence of sufficient of the unidentified chick growth factors.

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The Virostatic and Virucidal Action of α-Haloacylamides on Vaccinia Virus *in Vitro*. (17365)

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During the course of an investigation dealing with the possible antiviral effects of pyrimidine derivatives[1] it was found that amides of 5-aminouracil[1] inhibit the multiplication of vaccinia virus in the tissue culture system previously described.[2] Based on antagonisms demonstrable using *Lactobacillus casei*[3] it was predicted that the climax of this series would be reached with 5-chloroacetamidouracil or amides of 5-aminouracil with other acids having pKa values near that of chloroacetic acid. Actually the activity of chloroacetamidouracil was greater by a factor of 10 or more than the predicted value. Further investigation revealed a high degree of antiviral activity to be a function of all the chloro- and bromoacylamides and dichloroacetamides which were tested including chloroacetamide itself. This paper presents the data on the *in vitro* tests.

Experimental. Screening tests with vaccinia virus in chick embryonic tissue culture were carried out as described previously.[2]

Discussion. It will be seen (Table I) that bromo- and chloroacylamides usually inhibit growth of the vaccinia virus. The inactivity of the fluoroacetamidouracil (Compound 14, Table I) suggests that an alkylation reaction may be involved in the activity.

When the high degree of activity of 5-chloroacetamidouracil was discovered attention was turned first to the embryonic tissue test system. It was a recognized weakness of the screening test that substances capable of poisoning the embryonic tissue undoubtedly would be expected to prevent the multiplication of the virus.[2] Thus known poisons, such as azide and cyanide, could be shown to inhibit viral multiplication.[2] On the other hand, 5-chloroacetamidouracil was no more toxic to mice than related substances of much lower antiviral potency. It is ineffective against the majority of bacteria, and in reasonable concentration fails to affect the respiration and glycolysis of tissue slices or the glycolysis of washed cell suspensions of *Lactobacillus casei*.†

* These studies were begun at Western Reserve University Medical School (1945) and continued at the Medical College of Virginia (1946-7).

1 Thompson, R. L., Wilkin, M. L., Hitchings, G. H., Elion, G. B., Falco, E. A., and Russell, P. B., *Science*, 1949, **110**, 454.

2 Thompson, R. L., *J. Immunol.*, 1947, **55**, 345.

3 Hitchings, G. H., Elion, G. B., Falco, E. A., Russell, P. B., and Vander Werff, H., *Annals N. Y. Acad. Sci., in press.*

† We are indebted to Doris Lorz for these determinations.