ammonium chloride increased the urinary excretion of ascorbic acid. The mean daily urinary ascorbic acid excretions for the 3 periods are: C. C. 1st period, 26±1.3 mg, 2nd period, 19±2.0 mg, and 3rd period, 44±4.1 mg; and J. G. 1st period, 41±2.7 mg, 2nd period, 34±7.6 mg, and 3rd period, 46±4.3 mg. The differences between the periods were significant for C. C. but not J. G. tested according to Livermore's formula: differences must be 2 times the standard deviation of the differences.[8] The mean daily plasma ascorbic acid levels were consistently and significantly[8] decreased from the levels of the regular control diet with the addition of sodium bicarbonate or ammonium chloride. The mean daily plasma ascorbic acid values for the 3 periods are: C. C. 1st period, 0.98±.03 mg %, 2nd period, 0.87±.02 mg %, 3rd period, 0.89±.02 mg %; and J. G. 1st period, 0.98±.02 mg %, 2nd period, 0.81±.04 mg %, and 3rd period, 0.79±.01 mg %.

Hathaway and Meyer[9] have defined "utilization" as the difference between intake and excretion. By this definition the subjects' apparent "utilization" on the 3 periods was as follows: C. C., 61%, 72%, and 34%, and J. G., 39%, 49%, and 31%, respectively. Apparent "utilization" is then increased with the administration of sodium bicarbonate and decreased with ammonium chloride ingestion. However, the lowered mean plasma ascorbic acid values and the varied plasma and urinary excretion responses to the administration of a test dose at the completion of each period (Fig. 1) are not consistent with the apparent percentage "utilization."

Summary. This study indicates that the changes in the excretion of ascorbic acid are not accurate indications of utilization. The ingestion of both salts significantly lowered the mean plasma ascorbic acid content of the subjects indicating an interference with normal utilization. The reduced excretion of ascorbic acid with the ingestion of sodium bicarbonate both daily and in response to a test dose would seem therefore to represent more accurately increased destruction of ascorbic acid in the excretory process; and conversely, the increased excretion with ammonium chloride would seem to represent increased preservation in the excretory process.

[7] Farmer, C. J., and Abt, A. F., PROC. SOC. EXP. BIOL. AND MED., 1936, **34**, 1625.

[8] Hauck, H. M., personal communication, 1943.

[9] Hathaway, M. L., and Meyer, F., *J. Nutrition*, 1941, **21**, 503.

16337

Application of a Metabolic Inhibitor to the Developing Chick Embryo.

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This research is the first of a series of attempts to apply a new method to the study of chemical embryology. The usefulness of metabolic analogs (anti-vitamins, anti-metabolites) in the study of the metabolism of microorganisms has been fully demonstrated by Shive and his co-workers in this laboratory by the development of the method of "inhibition analysis".[1,2] An outgrowth of these findings, which has been in prospect in this laboratory for some time, is an attempt to block metabolic reactions in embryonated and cancer-bearing eggs and to apply the same methods of study to these tissues. A study of the effect of precursors of the competing metab-

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[1] Shive, W., and Macow, J., *J. Biol. Chem.*, 1946, **162**, 451.

[2] Beerstecher, E., and Shive, W., *J. Biol. Chem.*, 1946, **164**, 53.

olite on such an inhibitor in embryonic tissue holds promise of information as to the embryo's biochemical abilities, as well as affording a means of following the development of the biochemical potentialities of the embryo in much the same manner that the morphological development has been traced.

The inhibitor, 3-acetylpyridine, used in this investigation has already found use in the production of pellagra-like symptoms in the mouse, an animal normally requiring no exogenous source of nicotinic acid.[3] It has been reported that its toxicity on this animal can be nullified by feeding nicotinic acid or tryptophan which have been postulated as precursors of nicotinamide.[4,5]

Developing chick embryos are inhibited by 3-acetylpyridine and this inhibition can be reversed competitively by nicotinamide, but nicotinic acid and tryptophan are much less active reversing agents. Details of some of these experiments with embryonic chicks and their implications are presented below.

Testing Methods. All solutions injected into eggs were adjusted to a pH of 7 and were equivalent in osmotic pressure to .58% saline. Sterilization was accomplished by autoclaving and the same precautions of aseptic technique were used in yolk-sac injection as are described for tumor in egg cultivation.[6] Deaths were determined by candling. Time and temperature of incubation are given in the tables.

Results. The results shown in Table I indicate that 600 γ of 3-acetylpyridine per egg is lethal in 24 hours when injected into the yolk-sac. Sub-lethal doses of 3-acetylpyridine or lethal doses rendered just sub-lethal by the addition of nicotinamide or precursors of this vitamin, cause a mal-development of the chick. Some of the symptoms associated with the toxicity were undersized deformed legs, and a general edematous-like condition over the surface of the body. These symptoms could be prevented by the simultaneous injection of large amounts of nicotinamide with the 3-acetylpyridine.

Reversal of the Lethal Effect. The amount of nicotinamide necessary to just nullify the lethal effect of 3-acetylpyridine increases as the concentration of this inhibitor increases. Further, from Table I it can be seen that the relationship of these 2 substances is a competitive one and that the inhibitor-substrate (3-acetylpyridine-nicotinamide) ratio is approximately 14. In order to determine this ratio, it is necessary to measure the total inhibitor and substrate which is just non-lethal to the egg. However the egg itself contains some nicotinamide and for this a correction must be made by deducting from the total 3-acetylpyridine that amount which corresponds to the maximum non-lethal dose.

The justification for determining the inhibition constant in this manner can be demonstrated concisely if we allow:

I = Total inhibitor
S = Total substrate
S_a = Added substrate
S_o = Substrate present in egg initially
I_o = Maximum non-lethal level of inhibitor
E = Enzyme
K = Inhibitor-substrate ratio
P = Product of substrate

The competition of inhibitor and substrate for some specific enzyme has been represented by the following expressions:

$$S + E \rightleftharpoons ES \rightarrow E + P$$
$$I + E \rightleftharpoons EI$$

For such a competitive system by definition, the ratio of I to S is a constant

$$(1) \qquad \frac{I}{S} = K$$

Since S represents the total amount of nicotinamide present, and this is composed of the vitamin already in the yolk plus that which has been injected then:

$$(2) \qquad S = S_a + S_o$$

From expression (1) and (2) one obtains:

$$\frac{I}{S_a + S_o} = K$$

or by rearrangement:

$$(3) \qquad I = KS_a + KS_o$$

Now from equation (3) it is obvious, that when the added substrate is zero, the amount of inhibitor which the eggs will tolerate is

3 Woolley, D. W., *J. Biol. Chem.*, 1945, **157**, 455.

4 Woolley, D. W., *J. Biol. Chem.*, 1946, **162**, 179.

5 Rosen, F., Huff, J. W., and Perlzweig, W. A., *J. Biol. Chem.*, 1946, **163**, 343.

6 Taylor, A., Thacker, J., and Pennington, D., *Science*, 1942, **96**, 342.

TABLE I.
Effect of 3-Acetylpyridine and Nicotinamide on 4-day-old Chicks.

Nicotinamide γ per egg	3-Acetylpyridine γ per egg	No. of eggs* injected	Exper. fraction dead in 24 hr	Control† fraction dead in 24 hr	$\frac{I - I_o}{S_a}$‡
—	400	8	0.00	0.00	—
—	450	8	0.13	0.00	—
—	500	7	0.14	0.00	—
—	550	7	0.14	0.14	—
—	600	8	1.00	0.00	—
80	2000	7	0.72	0.00	—
90	2000	7	0.43	0.00	—
100	2000	7	0.00	0.00	14.5
160	3000	7	0.72	0.00	—
180	3000	7	0.00	0.00	13.6
200	3000	7	0.00	0.00	—
200	4000	8	1.00	0.00	—
220	4000	8	0.63	0.00	—
230	4000	7	0.57	0.14	—
240	4000	8	0.00	0.00	14.4
280	5000	8	0.50	0.00	—
313	5000	8	0.00	0.00	14.2
325	5000	8	0.00	0.00	—
350	6000	8	1.00	0.00	—
370	6000	8	0.75	0.00	—
380	6000	8	0.00	0.00	14.3

* 4-day-old eggs were used, incubated at 37°C.
† Control eggs were injected with .58% sterile saline.
‡ These values were calculated; I and S correspond to the inhibitor and substrate added and I_o is the maximum non-lethal dose experimentally determined as 550 γ per egg.

K S_o and represents the largest non-lethal dose. Designating S_oK as I_o and substituting in expression (3) one has by rearrangement:

$$\frac{I - I_o}{S_a} = K \tag{4}$$

In this last equation the inhibitor-substrate ratio, K, is expressed in all experimentally measurable quantities.

From the experimental value of I_o and the average experimental value of K, the quantity of S_o can be estimated, since by designation in expression[4]

$$I_o = S_oK$$

The value of S_o so estimated for eggs averaging 56 g was found to be approximately 39 γ. The value of Cheldelin and Williams[7] determined by microbiological assays is .72 γ per gram or 40.3 γ per 56 g eggs.

[7] Cheldelin, V. H., and Williams, R. J., *The University of Texas Publication*, 1942, No. 4237, 105.

Effect of Nicotinic Acid and Tryptophan. The effects of nicotinic acid and tryptophan on the toxicity of 3-acetylpyridine on 4-day-old embryos is shown in Table II. It is striking that these two substances while active against low levels of inhibitor are much less potent than the nicotinamide. This does however demonstrate that the 4-day-old chick has the mechanism for the biochemical conversion of nicotinic acid and tryptophan to nicotinamide and that the ability is very feeble. This might indicate that at this stage of development the concentration of the enzymes necessary for the conversion of nicotinic acid to its amide is very low.

Summary. 1. Embryonic chicks were inhibited by the direct injection of 3-acetylpyridine into the yolk-sac. 2. It was shown that this inhibition could be reversed competitively by nicotinamide over the range of 2000 to 7000 γ per egg. 3. Evidence is presented showing that 4-day-old chick embryos have

TABLE II.
Effect of Nicotinic Acid and Tryptophan on the Toxicity of 3-Acetylpyridine.

3-Acetylpyridine γ per egg	Nicotinic acid γ per egg	Tryptophan γ per egg	No. of eggs* injected	Fraction dead in 24 hr
1500	0	0	6	1.00
1500	2000	0	6	0.50
1500	2500	0	6	0.67
2000	0	0	7	1.00
2000	2000	0	7	1.00
2000	2500	0	7	0.88
600	0	0	7	0.86
600	0	7500	7	0.00
650	0	0	7	1.00
650	0	7500	7	0.00
700	0	0	7	1.00
700	0	7500	7	0.14

* All eggs incubated at 37°C and were 4 days old at time of injection.

a very limited capacity to convert nicotinic acid to the corresponding amide. 4. Sublethal levels of 3-acetylpyridine are shown to cause a mal-development of the chick embryo.

16338

Effect of pH on MM Virus.

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MM virus was originally isolated from a hamster which had been injected intracerebrally with cord and medulla of a fatal case of poliomyelitis. Typical and severe poliomyelitic lesions are induced by it in the central nervous system of hamsters and albino mice.[1]

Serologically MM virus appears to be similar to the SK virus of Jungeblut and Sanders[2] but differs from the Lansing-like poliomyelitic strains and the Theiler virus.[3]

The virus is highly virulent for mice by the intraperitoneal as well as the intracerebral route. Thus it affords an opportunity for testing the effects of antibiotic agents on small amounts of a virus which induces poliomyelitic lesions in the test animal. In relation to a problem of this kind[4] an investigation was made of the effect of pH on MM virus.

Procedure. Mouse brains from the 45th to the 48th generation stored in 50% glycerol were ground, made up in a 10% suspension usually in salt solution containing 10% of infusion broth or occasionally in distilled water, and centrifuged to remove large particles. The supernatant fluid was used at once or after

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1 Jungeblut, C. W., and Dalldorf, G., *Am. J. Pub. Health*, 1943, **33**, 169.

2 Jungeblut, C. W., and Sanders, M., *J. Exp. Med.*, 1940, **72**, 407.

3 Dalldorf, G., to be published.

4 Schatz, A., and Plager, H., *Bull. Torrey Bot. Club*, 1948, **75**, 256.