

TABLE 2
RESULTS OF BABY-CHICK TESTS USING A SYNTHETIC,
FOLIC ACID FREE DIET

Chemical injected	Micrograms per day	Total chicks	Per cent chicks with tumor
None (four tests)	...	40	0
Folic acid	500	10	80
	100	10	90
	10	10	60
Folic acid with d(—) glutamic acid	100	10	0
	10	10	0
N-methyl-folic acid	100	10	80
Pteroyldiglutamic acid	500	10	90
Pteroyltriglutamic acid	500	10	100
Pteroylaspartic acid	100	10	0
Pterotic acid	100	10	0
N-methylpterotic acid	100	10	0
P.A.B.-triglutamic acid	100	10	0

TABLE 1
PROTOCOL OF A BABY-CHICK TEST USING A SYNTHETIC,
FOLIC ACID FREE DIET

Bird No.	Selected results from the record of daily observations				
	8th Day	10th Day	12th Day	14th Day	16th Day
<i>Effect of synthetic, folic acid free diet alone</i>					
2348
2349
2350
2351
2352
2353
2354
2355
2356
2357
Per cent	0	0	0	0	0
<i>Effect of injecting 100 micrograms/day of folic acid</i>					
2360	?	P	2P	3P	4P
2361	?	P
2362	2P	3P
2363	P	P	2P	3P
2364	P	2P	3P	Dead
2365	?	2P	2P	3P	4P
2366	P	2P	3P	4P
2367	P	2P	3P	4P
2368	?	2P	2P	3P	4P
2369	P	2P	3P	4P
Per cent	30	80	80	90	90

P: palpable swelling.
2P: 1/2 to 2/3 full size.
3P: 2/3 to 3/4 full size.
4P: full size.

a preliminary inspection of a series of tumors in different stages of development.

The earliest evidence of tumor usually consisted of a slight elevation of the lower margin of the breast. This symptom was noted, in the record of results, as "questionable." Thereafter, almost daily changes occurred and were recorded in the following manner:

Symbol	Meaning
P	Palpable swelling
2P	Tumor $\frac{1}{4}$ to $\frac{1}{2}$ full size
3P	Tumor $\frac{1}{2}$ to $\frac{3}{4}$ full size
4P	Tumor full size

Observations were made daily, beginning with the eighth day and continuing until the differences had been clearly demonstrated, at which time the birds were sacrificed and examined for internal evidence of tumor. The pathological studies were made by Dr. E. Woll.

As demonstrated in TABLE 1, the synthetic, folic acid free diet completely prevented the occurrence of breast tumors in the baby chicks, but nearly all of the chicks developed tumor when injected daily with folic acid. This result was regularly confirmed in the different tests in which we studied the virus-stimulating effect of other chemicals.

TABLE 2 shows the result of giving baby chicks, maintained on synthetic, folic acid free diet, daily injections of chemicals which are closely related to folic acid. It will be noted that folic acid with d(—) glutamic acid had no virus-stimulating effect. L(+) glutamic acid is the naturally occurring form found in the body and in the vitamin. N-methyl-folic acid, pteroyldiglutamic acid, and pteroyltriglutamic acid all contain the natural form of glutamic acid and showed as great a virus-stimulating effect as folic acid. Pteroylaspartic acid, pteric acid, N-methyl-pterotic acid, and P.A.B.-triglutamic acid had no virus-stimulating effect. Chemicals which will be dealt with subsequently as folic acid antagonists also had no virus-stimulating effect.

This part of our report would not be complete without presentation of the formula of the synthetic, folic acid free diet used. As shown in TABLE 3, this diet contained adequate amounts of the thirteen vitamins which, together with folic acid, are required for complete nutrition of the chicken. Oleson and associates (1946) have described the effect of this diet on the feathering of chickens.

TABLE 3
FORMULA OF THE SYNTHETIC, FOLIC ACID FREE
DIET USED IN CHICK TESTS

(This diet was made up by Dr. J. J. Oleson
of our laboratories)

Cerelose	53.0%
Alc. ext. casein	22.0%
Salt mixture	4.3%
Calcium gluconate	3.0%
Gelatin	8.0%
Ruffex	4.0%
Soybean oil	5.0%
Cholic acid	0.25%
Cystine	0.45%
Choline chloride	200 mg. %
Calcium pantothenate	3 mg. %
Nicotinic amide	3 mg. %
Pyridoxine	0.5 mg. %
Thiamin chloride	0.3 mg. %
Biotin	0.03 mg. %
Riboflavin	0.5 mg. %
Inositol	100 mg. %
P.A.B.	5 mg. %
Vitamin E	5 mg. %
Vitamin K	0.2 mg. %
Vitamin A	3,500 units %
Vitamin D	200 units %

The Virus-Inhibiting Effect of Chemicals. TABLE 4 illustrates the results of tests in which baby chicks were maintained on a diet of ordinary chick-feed for the purpose of determining the virus-inhibiting effect of chemicals. The standard brand of prepared feed contained alfalfa meal, corn gluten meal, wheat bran, distillers dried solubles, dried buttermilk, fish meal, meat scrap, ground yellow corn, bone meal, pulverized oats, wheat middlings, soy bean oil meal, ground limestone, iodized salt, fortified vitamin A and D feeding oil, vitamin D-activated animal sterol, manganese sulphate, and riboflavin supplement.