Research, using the countercurrent distribution method (*1*). In one run, for example, a 24-tube transfer study was made with 508 mg of amorphous penicillin. The weight and antibiotic activity curves showed that the major portion of the penicillin was type G with a little F and dihydro F but no K or X. The yield of crystalline triethylamine penicillin G (m.p., 140–150° C) was 50 mg, having 60.54% carbon and 7.68% hydrogen. This crystalline penicillin G and the corresponding amorphous penicillin were assayed antibiotically, chemically, and radioactively, giving the following percentage recoveries in going from the amorphous to the crystalline material:

Weight	*Oxford units*	S^{32}	S^{35}
8.1	27.0	31.1	31.9

Hence, (1) the radioactive sulfur is incorporated into the penicillin molecule, and (2) all the sulfur in the amorphous penicillin must be present in the penicillin molecule, since the sulfur assay agrees with the antibiotic assay. The smaller weight recovery for the penicillin G reflects the presence of phenyl acetic acid and pigments in the amorphous material.

A comparison of the radioactivity of the purified salt with the calculated sulfur gave a ratio of S^{35} to S^{32} of 3.04×10^{-8} for the example cited above. This compares favorably with 3.12×10^{-8} in the original medium. Hence, the radioactive sulfur behaves just as ordinary sulfur in the biosynthesis. This is also shown by the table above.

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Interference With Estrogen-induced Tissue Growth in the Chick Genital Tract by a Folic Acid Antagonist

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Earlier observations (*4, 5*) indicated that the characteristic tissue-growth responses to estrogen in the genital tract of both the female monkey and the chick require adequate dietary intake of folic acid. Moreover, a close quantitative relationship between the level of folic acid ingestion and the response to estrogen was shown.

Extensive reports have described the nature of competitive metabolites which interfere with the biological activity of various members of the B-complex and certain amino acids (*8*). However, interference with the biological activity of a hormone by the competitive displacement of an essential dietary factor has not hitherto been described. We wish to report that the ingestion of the folic acid antagonist[1] described by Franklin, Stokstad, and Jukes (*2*) markedly reduces the tissue-growth response to maximally effective doses of diethylstilbestrol in the chick maintained on an otherwise normal stock diet.[2] Representative findings are presented in Table 1.

The data indicate that this antivitamin possesses the capacity to reduce the formation of new tissue in an organ which is under maximal hormonal stimulation for rapid growth. It is particularly noteworthy that such an inhibitory effect can be obtained in animals fed a natural grain diet and that this inhibition is promptly and completely reversed by the administration of an excess of synthetic folic acid (pteroylglutamic acid).

TABLE 1

Series	Additions to stock diet	Stilbestrol injected*	No. of chicks	Oviduct weight (mg)	Body weight (gm)
A	1% Antagonist	+	9	67 ± 16	56 ± 5.9
	1% Antagonist plus folic acid†	+	13	315 ± 52	62 ± 7.1
	None	+	10	263 ± 52	74 ± 9.6
B	1% Antagonist	+	7	65 ± 32	47 ± 5.2
	None	+	7	243 ± 47	73 ± 11.2
	"	−	10	16 ± 3	76 ± 12

* All stilbestrol-treated chicks given 0.5 mg of stilbestrol daily in 0.1 cc of corn oil subcutaneously for 4 days preceding autopsy.

† Each chick given 4 mg of folic acid (synthetic pteroylglutamic acid) in 0.5 cc of 0.01 N sodium hydroxide subcutaneously daily during 4 days of stilbestrol treatment only.

All chicks are New Hampshire Reds from the same flock and autopsied on 12th day after hatching.

Substantial retardation of cancer of the prostate and breast has been shown to result from the partial elimination from the body of the hormones involved in the normal metabolism of these organs (*1, 6*). Accordingly, the further exploration of any mechanisms which may even more effectively interfere with the physiological activity of such hormones in the body seems desirable. The direct reduction of the nutritive value of certain vitamins and amino acids with a view to the impairment of tumor growth has been suggested previously (*3, 7*). Our observations offer the additional possibility of interference with hormone-induced tissue growth by nutritional means.

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[2] Startena (Ralston Purina Co.).