In units per kilogram this is 250 to 9,240 to 18,300 in an interval of less than 20 days.

In genetic tests between KL and Zr (Table 2) there is a suggestion that extreme tolerance is a single factor recessive. Using 80 units as a test dose, the F_1s all died, the F_2s approximate a 3:1 ratio ($2.3 \times SE$), the combined first backcross 18:30 fits a 1:1 ratio ($1.7 \times SE$), and the second backcross 4:8 agrees with the first. It should be pointed out, however, that 80 units used as the test dose might arbitrarily divide the distributions due to multiple factors in approximately this manner. Zr and KL would then consist of distributions widely separated and on opposite sides of this arbitrary threshold. In agreement with this possibility are 7 animals of the first backcross which, at 100 days, lived with 5 units, 2 with

TABLE 2

CROSSES BETWEEN KL AND ZR*

	Alive	Dead
KL strain	All	
Zr strain		All
F_1		7
F_2	15	21
BC to KL♀ (F_1♂ × KL♀)	15	9
BC to KL♂ (F_1♀ × KL♂)	15	9
2BC to KL♂ (BC♀ × KL♂)	8	4

* Test dose used is 80 units of insulin. Numbers refer to number of animals. ♀, used in second backcross (2BC), died when tested with 80 units.

200 units, and 4 which died with 500 units (one, however, died only at 3½ days, typical of KL). Also, with 50 units 3 F_1 mice lived and 1 died, although none lived with 80 units. Segregation with 80 units is demonstrated, but this does not necessarily constitute a proof of one factor with dominance. A more plausible explanation involves many factors with little or no dominance, the 80-unit test dose giving an artificial cleavage which simulates single-factor segregation.

The magnitude of the difference is too great to be explained on the basis that KL is mildly diabetic or hypoglycemic. However, a blood sugar analysis was made (Folin and Malmros micro method). The results are very similar for all strains tested. In milligrams per cent the sugar levels for uninjected mice are 170 for Zr, 172 for C57 Black, and 171 for KL.

In conclusion, the KL strain of mice has an extremely high insulin tolerance. For a given age or weight, the tolerance is of the order of a few hundred times that of strain Zr or C57 Black. Blood sugar levels for uninjected mice are essentially the same for these three strains. Evidence from crosses between KL and Zr, using an 80-unit test dose, simulate for F_1, F_2, first and second backcrosses, a single factor recessive for high tolerance; but more probably this is a dichotomy by the test dose of continuous distributions due to many factors. The necessity of control of the genetic constitution of animals used in the bioassay of insulin is self-evident.

Insulin tolerance or resistance of such a magnitude can be used to study the nature of insulin action in carbohydrate metabolism or, more specifically, the nature of antagonistic or neutralizing reactions.

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Introduction of Radioactive Sulfur (S^{35}) Into the Penicillin Molecule by Biosynthesis[1]

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It has been found possible to introduce radioactive sulfur into the penicillin molecule by biosynthesis. The radioactive penicillin was produced by surface growth of the mold, *Penicillium notatum* NRRL 1249 B 21, on a synthetic medium (*2*) containing radioactive sulfur as sodium sulfate. The total sulfur available in the 272 liters of medium was 16.475 gm of S^{32} and 0.528 γ, or 24.2 mc of S^{35} present as inorganic sulfates. The ratio of S^{35} to S^{32} was therefore 3.12×10^{-8} in the original medium. The mold was harvested 10 days after inoculation of the medium and the crude or amorphous penicillin extracted. The total yield was 53.31 gm of amorphous penicillin having an average antibiotic activity of 2.47×10^5 Oxford units/gm and a specific radioactivity of 15.56 μc/gm. Hence, 3.4% of the original radioactivity was found in the amorphous penicillin.

A small portion of the amorphous penicillin was purified by G. T. Barry and Y. Sato, National Institute of Health Fellows, at the Rockefeller Institute for Medical

[1] Summary of a paper presented before the Antibiotic Study Section, National Institute of Health, Bethesda, Maryland, October 1, 1947.

The radioactive sulfur used in this investigation was supplied by Monsanto Chemical Company and obtained on allocation from the U. S. Atomic Energy Commission.

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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[1] The material used in this study was kindly supplied by the Lederle Laboratories through the courtesy of Y. Subba-Row and B. L. Hutchings.

[2] Startena (Ralston Purina Co.).