confuse the results obtained. The number of pupae present at the time of final reading is also stated.

Upon consideration of these data, the first fact that stands out is that neither tumor strain has even 50% viability at 30° C, since both fail to reach the end point. Whether this represents sterility induced by high temperature or a failure to develop which may be regarded as a lethal or semilethal condition is not at present determined. It is clear, however, that in these tumor strains the reduction in tumor incidence at 30° C is accompanied by reduced viability.

TABLE 1

GROWTH RATE OF LARVAE AT THREE CONSTANT TEMPERATURE LEVELS

Run No.	No. of eggs	No. of pupae at final reading	No. of hrs to final reading
		30° ± .5° C	
		st sr stock	
1	203	92 (total pupation for run)	144–4
2	105	39 " " " "	158–3
3	271	92 " " " "	143–4
		bw tu stock	
1	200	79 (total pupation for run)	160–14
2	240	104 " " " "	141–2
3	225	93 " " " "	147–3
		wild stock	
1	530	296	113–14
		25° ± .5° C	
		st sr stock	
1	60	31	138–3
2	90	46	150–4
3	220	111	160–14
4	200	109	147–4
		bw tu stock	
1	140	72	142–3
2	120	61	165–4
3	311	200	142–4
4	110	62	142–4
		wild stock	
1	625	313	122–4
		20° ± .5° C	
		st sr stock	
1	744	414	213–4
		bw tu stock	
1	355	190	192–4
2	304	155	200–4
		wild stock	
1	712	356	260–2

As a second point, it would seem that the developmental rate of larvae of all three strains is, as might be expected, markedly slower at 20° C than at 25° C or 30° C, the control showing the slowest development of the three at 20° C and the fastest at the other two levels. On the basis of the results obtained, a comparison of control and experimental strains at 30° C is not justified, since the figure noted under control represents pupation of 50% of the eggs started, while those noted for the tumor strains represent total pupation. This total is, of course, less than 50%. Therefore, restricting present consideration to the 20° C and 25° C levels, it is interesting that both tumor strains develop more slowly at the higher temperature than does the wild, but more rapidly at lower temperature. The bw tu strain is evidently the least retarded at 20° C.

Finally, as far as can be noted in this work, viability is no different in the three strains at the lower temperature levels. In terms of tumor incidence this is confusing, since bw tu shows high incidence at 20° C, while st sr shows much-reduced incidence at this temperature. The fact that viability is not reduced in st sr probably indicates that this reduction in tumor incidence is dependent on other factors than that noted in the two strains at 30° C. Some clue bearing on this may lie in the fact that these tumor strains do have different developmental rates at 20° C, and this is now being investigated along with other phases of the tumor problem.

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High Insulin Tolerance in an Inbred Strain of Mice

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A case of extreme tolerance to insulin has been found in an inbred strain of mice. It is inherited. Cases of high insulin resistance are of interest in connection with carbohydrate metabolism and the possible mechanism of insulin action, and inherited variations are important in view of the recognized procedure of assaying the potency of insulin by determining the convulsive dose in white mice (*5, 6, 9*). Although controls involving temperature and food consumption prior to injection are generally practiced, controls involving possible hereditary variations in these white mice are not considered. An hereditary difference of the magnitude found in our strain is also of interest in connection with the sort of physiological differences that may be inherited and the possible extent of such variations in man.

Differences in insulin tolerance have been described for some classes of vertebrates. Many birds (quail, pigeon, fowl, etc.) have a higher tolerance to insulin than do mammals (*2, 3, 4*). Likewise, certain differences in insulin tolerance have been noted within a single species. In humans, cases of extremely high insulin resistance have been observed among some diabetic patients (*8*). McIntyre and Burke (*7*) found in albino rats one strain which had a tolerance 10 times as high as the standard strains. In mice, Allen (*1*), using an unspecified number and type of mice, reported the dose necessary for beginning shock to be 1,000 units/kg. These results are con-

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siderably higher than those reported by Chen, *et al.* (*3*), who found the convulsive dose to range from 7 to 40 units/kg.

In the present study death is taken as the criterion of tolerance. Convulsive doses parallel the death results but are more irregular. Three inbred strains of the University of Illinois colony were used: KL—albinos, derived and inbred for 15 brother-sister generations at Illinois; Zr—pink-eyed, brown (café-au-lait) and white spotted, derived and inbred for 10 brother-sister generations at Illinois; and C57 Black—the stock of Jackson Memorial Laboratory, Bar Harbor, Maine, representing total inbreeding of over 40 generations.

The insulin used was Iletin (Insulin, Lilly), in concentrations of 20, 80, 100, and 500[2] units/cc. Injections were intravenous, intraperitoneal, or subcutaneous. Control injections of comparable volumes of mammalian Ringer's solution were used. Animals were tested at different ages and weights.

Results of the experiments indicate that survival of the tolerant strain is the same for intraperitoneal and subcutaneous injections, even though the latter must involve slower absorption. No sex differences are found. Young mice up to 15 days give nearly the same response for all strains (some individuals will tolerate 5 units), but from 20 to about 35 days there is a rapid divergence of tolerance which appears to consist of a somewhat increased sensitivity for the Zr strain and a greatly decreased sensitivity for the KL strain. Table 1 groups the data involving the three strains. A further peculiarity of the KL strain is that with high doses, death is sometimes delayed up to 3 and 4 days as compared with the usual insulin death within 24 hrs (maximum, 30 hrs). Other strains have been compared at some of the comparable ages and weights. An albino strain (Zrc), a wild type (Surv A), the black silver strain (Si), all agree with the C57 Black results. Only strain L (from "Swiss" albinos and one parent of the KL strain) approached the high tolerance level.

At 30–40 days it is evident that some KLs will tolerate up to 750 times the amount tolerated by Zrs or, since KL is heavier, up to 590 times/kg (31–18,300 units). Taking approximately 17 gm as a typical weight used in insulin assays and studies, we find maximum amounts tolerated at that weight to be 1, 3, and 300 units for Zr, C57 Black, and KL, respectively. In terms of units per kilogram (calculated from Table 1) this becomes 59 for Zr, 201 for C57 Black, and 18,300 for KL, an amount tolerated by KL which is 310 times that by Zr. Or, if the lowest dose which kills is considered for this approximate weight, we find 1 unit for Zr and C57 Black and 240 units for KL. In units per kilogram this becomes 59, 67, and 14,640, respectively.

In the extremely rapid increase in tolerance of the KL strain from 20 days to 30–40 days, the units tolerated increase from 2 to 120 to 300 for 20, 25, and 30–40 days.

TABLE 1
INSULIN TOLERANCE OF 3 STRAINS*

Age (days)	Av. wt. (gm)	Strains	Alive	Dead
20	8	Zr	.05, .1, .8	.5(3), 1(3)
		C57 Blk		
	8	KL	.4, .8, 1, 2(2)	.8, 5
25	9	Zr		1(4)
	8	C57 Blk	.2	.8, 2
	13	KL	.5, .8, 4, 15, 50, 120	190
30–40	13	Zr	.3, .4	.8(2), 1.2, 1.6, 2(2), 5, 80
	15	C57 Blk	.1, .5, .8, 1(2), 1.5, 3	1, 1.5, 2, 3, 5(3), 10, 15
	16.5	KL	50, 60, 100(4), 160, 180, 200, 300	240, 250, 260, 500
45–85	17	Zr	.3, 1	1, 5
	23.5	C57 Blk	.6, 3, 4, 8(2)	2, 3(2), 4, 5, 8(3), 10, 12(3), 16(2), 20, 25, 32
	27	KL	40, 70, 100(2), 160(3), 200(4) 240, 300(2), 400, 500(4)	400, 750†, 1250†(2)
95–175	24	Zr	5(3)	5, 80(5)
	25.5	C57 Blk	5(2), 10, 20(2)	15, 40(3), 50, 80
	34	KL	80(6), 300, 500(3), 600	400, 500†, 1000, 1000†, 1500
365	26	Zr	5(2)	
	26	C57 Blk	20	4, 40
	36	KL		500, 1000

* Numbers are units of insulin given. Numbers in parentheses indicate number of animals given that dose, otherwise one animal for each dose.

† Died late (3 and 4 days).

[2] The 500-unit insulin used was Iletin (Insulin, Lilly) U–500, of lot number W–1819–2, and was obtained through the courtesy of F. B. Peck, associate director, Medical Division, Research Laboratory, Eli Lilly and Company, Indianapolis, Indiana.

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.