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Genetic Susceptibility to Goldthioglucose-Induced Obesity in Mice.* (25953)

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A single injection of goldthioglucose at appropriate concentrations produces a persistent obesity in mice(1,2,3,4,5). The weight gain is correlated with an increase in food intake (6). Lesions found in the hypothalamus of goldthioglucose-injected mice are considered to be the basis for the hyperphagia and ensuing weight gain(7,8,9), and localization of tissue destruction in ventromedial nuclei of the hypothalamus has been related to the postulated existence of a "glucoreceptor" center in this particular area of the diencephalon(7). However, lesions have been found in areas of the brain that lie outside the confines of the hypothalamus following an obesity-inducing dose of goldthioglucose(8,10). Liebelt and Perry(8) have shown a genetic difference in the susceptibility of CBA and C_{57} Black mice to goldthioglucose-induced obesity. The purpose of this investigation was to extend these findings to other strains of inbred mice, thus studying the influence of genetic factors in goldthioglucose-induced obesity.

Methods. All strains of mice used in these experiments had been inbred by brother-sister mating for over 20 generations,† and included C_{58}, CBA, DBA/2, BALB/C, RIII as well as the following F_1 hybrids: (RIII x CBA), (CBA x RIII), (CBA x C_{58}), (C_{58} x CBA), (CBA x BALB/C). Only male mice were used and were housed in groups of 4-6 animals per wooden cage (6 x 12 x 6 in.). Purina lab chow was fed *ad libitum*. Crystalline goldthioglucose (Solganol B)‡ was dissolved in saline, and injected intraperitoneally at 90-110 days of age. All injections were made between 9:00 and 11:00 a. m.; the animals were not starved prior to treatment. A minimum of 10 and a maximum of 40 animals were injected at each of the various dose levels tested for each strain and F_1 hybrid combination; mean weight gain of the surviving animals was determined at 60 days post-injection. Susceptibility to the *toxic effects* of goldthioglucose was expressed only as the LD/100 dose.

Results. Table I shows that both gain in body weight and lethal toxic effects in any given strain vary with dose of goldthioglucose injected. C_{58} mice appear to be most "susceptible" to goldthioglucose in terms of weight gain and toxicity as compared to the more "resistant" BALB/C, RIII and DBA/2 strains. Mice of the CBA strain fell within these two extremes. Weight gain response and mortality were characteristically variable, especially as LD/100 dose was approached, in all except the CBA strain. CBA mice appeared to be unique in that a uniform weight gain response could be predicted for each dose level over a range of 0.2 to 0.6 mg/g body weight with less than 10% mortality at the latter dose.

In general, all animals showed a greater weight gain as dose of goldthioglucose was increased. However, as the LD/100 dose was approached in the DBA/2, RIII, and BALB/C strains, animals actually lost weight when

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† Mice obtained from Kirschbaum Memorial Research Lab., Dept. of Anatomy, Baylor Univ. College of Medicine, Houston, Texas.

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TABLE I. Body Weight Gain (g) and Mortality following Various Concentrations of Goldthioglucose (mg/g Body Weight) at 60 Days Post-Injection.

Strain	Control	.1	.2	.3	.4	.6	.8	1.0	1.2	1.4	1.5
C_{58}	3.0 ± .52* (20)	7.33 ± 1.72 (18)	11.33 ± 1.66 (15)	10.16 ± 2.43 (21)	All dead (25)						
CBA	2.5 ± .50 (30)	2.0 ± .82 (30)	9.62 ± 1.21 (40)	13.25 ± 2.23 (40)	22.50 ± 1.52 (40)	23.12 ± 1.32 (20)	All dead (15)				
RIII	2.75 ± .83 (15)				3.0 ± .12 (16)	6.33 ± 1.24 (17)	15.0 ± 4.0 (20)	2.0 ± 1.2 (14)	All dead (10)		
DBA/2	2.83 ± .62 (15)					5.75 ± 1.32 (15)	14.0 ± 2.37 (18)	15.5 ± 5.31 (20)	4.2 ± 4.81 (12)	All dead (10)	
BALB/C	3.62 ± .42 (20)		3.75 ± .96 (18)		4.25 ± 1.68 (12)	7.62 ± 2.82 (14)	10.25 ± 3.12 (14)	12.50 ± 2.68 (12)	13.0 ± 2.89 (13)	3.2 ± 4.41 (10)	All dead (12)

* Stand. dev.

No. of mice in parentheses.

compared with controls. Some of these animals have remained alive as long as 8 months post-injection at these reduced weights. Animals dying within 2-3 days post-injection besides demonstrating extensive hypothalamic damage and gastric ulcers also showed tubular necrosis in the kidney and parenchymal destruction in the liver.

All F_1 hybrid mice responded to a dose range of goldthioglucose similar to that characterizing CBA mice (Table II). This was found whether the CBA genetic influence was of maternal or paternal origin. Maximum mean weight gain at 60 days post-injection was 28.4 g for (RIII x CBA) F_1 hybrids, which was greater than maximum body weight attained by either of the parent stocks.

Discussion. The facility with which obesity may be induced in genetically different strains of mice is of both biological and practical importance. C_{58}, DBA/2, RIII and BALB/C mice showed a relatively unpredictable and variable weight gain and mortality as compared to the CBA strain over the dose range of administered goldthioglucose. Brecher and Waxler(1) indicated that of albino mice injected with an LD/50 dose (0.8 mg/g body weight) of goldthioglucose only ⅓ of the surviving animals became obese. Mortreuil-Langlois(11) reported that 1.5 mg/g body weight was necessary to induce a significant, yet variable weight gain in the XVII strain of mice, and LD/100 dose ranged between 1.6 and 1.7 mg/g body weight. Inbred strains of mice are considered to be genetically identical within a given subline; variation in response to goldthioglucose in these particular strains suggests the operation of non-genetic factors in addition to genetic determinants. The nature of such factors necessitates further study. The uniform response of CBA mice to the effects of goldthioglucose appears to be unique. It would be of interest to determine whether sublines of this strain in other laboratories respond similarly.

The susceptibility to the toxic effects of goldthioglucose expressed as LD/100 dose also varied with the different strains. Animals dying within 2-3 days post-injection demonstrated gastric ulcers and extensive hypothala-

TABLE II. Body Weight Gain (g) and Mortality in F_1 Hybrids following Various Concentrations of Goldthioglucose (mg/g Body Weight) at 60 Days Post-Injection.

Strain	Control	.1	.2	.4	.6	.8
RIII ♀ × CBA ♂	4.2 ± .6* (10)	4.1 ± .9 (15)	7.23 ± 1.05 (13)	17.3 ± 2.19 (12)	28.4 ± 2.02 (10)	All dead (10)
CBA ♀ × RIII ♂	3.9 ± .5 (10)			19.4 ± 1.86 (16)	25.6 ± 2.26 (13)	All dead (14)
CBA ♀ × C_{58} ♂	4.0 ± .72 (10)	5.1 ± .9 (18)		18.3 ± 2.25 (12)	All dead (11)	
C_{58} ♀ × CBA ♂	4.1 ± .64 (10)	4.8 ± .44 (17)		15.4 ± 1.87 (14)	All dead (14)	
CBA ♀ × BALB/C ♂	3.3 ± .9 (10)			12.3 ± 2.1 (12)	23.4 ± 2.2 (14)	All dead (12)

* Stand. dev. No. of mice in parentheses.

mic damage(8), as well as severe tubular necrosis of the kidney and parenchymal destruction in the liver. The latter type of lesions could account for the marked weight reduction seen in certain animals of DBA, RIII and BALB/C strains at dose levels approaching LD/100.

All F_1 hybrid combinations showed a weight gain response similar to that of the CBA strain for a given dose irrespective of whether the other parental strain was "resistant" or "susceptible." The LD/100 dose for F_1 hybrids was also similar to that of the CBA parent. It would appear that genetic factors involved in goldthioglucose obesity, and derived from the CBA parent, are expressed as the dominant influence in F_1 hybrids.

The 5 inbred strains of mice showed some variation as to maximum body weight attained for the range of doses of goldthioglucose utilized. (RIII x CBA) F_1 hybrids showed a weight gain to a given dose of goldthioglucose greater than that seen in either of the parent strains. These data suggest the operation of genetic and/or non-genetic factors which may influence extent of hypothalamic involvement by a given amount of goldthioglucose or may regulate peripheral factors involved in lipid deposition.

Thus the site(s) of gene action involved in "susceptibility" or "resistance" to goldthioglucose-induced obesity must be investigated with respect to 1) metabolism of goldthioglucose in organs other than the brain, 2) development of lesions in the hypothalamus and other areas of the brain, and 3) peripheral response of various organs including the fat depots to the induced hyperphagia.

Summary. Five inbred strains of mice (C_{58}, CBA, RIII, DBA and BALB/C) and 5 F_1 hybrid combinations were studied with respect to effects of single injections of various concentrations of goldthioglucose on body weight gain and lethal toxicity. Gain in body weight and mortality following a single injection of goldthioglucose was dependent on strain of mice and amount injected. In general, there was a greater increase in weight gain when larger doses of goldthioglucose were administered. The C_{58}, RIII, DBA and BALB/C strains of mice showed a relatively variable weight gain and mortality as compared to the CBA strain over the dose range of administered goldthioglucose. Mice of the latter strain were unique in that they showed a uniform response with respect to these 2 parameters. The F_1 hybrid mice showed a weight gain response and a LD/100 dose comparable to that of the CBA strain.

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A Pneumotropic Line of Lymphocytic Leukemia from Inbred Princeton Mice. (25954)

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Lymphocytic leukemia (lymphosarcoma) occurs naturally in adult Princeton mice (Pr, PRI, or P) from the random-bred colony maintained at the Rockefeller Institute. The disease is sporadic with a morbidity rate of 3.1% in 1050 discarded breeders, 10 to 12 months old, killed and autopsied during the past 7 years. Six of the 33 cases of leukemia (2.7%) were recorded in 215 males and 27 (3.2%) in 835 females. A branch of the Princeton strain with a high rate of leukemia, 80 to 90% in old adults, was established by Dr. Clara Lynch in 1946(1). This branch, designated PL or PrL, was inbred for more than 20 generations. In connection with other studies on murine leukemia it was of interest to determine the behavior of the PrL line in random-bred Princeton mice. Differences in activity of neoplastic cells from the 2 lines, one with low morbidity under natural conditions and the other high, were brought out in transmission experiments and are presented here. The naturally acquired leukemia of adult mice from the random-bred Princeton colony is characterized chiefly by marked enlargement of regional lymph nodes and spleens. The disease is reproducible in weanlings from the same colony by intraperitoneal injection of organ suspensions from naturally affected adults and is transmissible by serial passage. On repeated passage in recently weaned mice hepatitis virus may appear and alter the leukemic reaction.

Materials and methods. Random-bred female weanlings under 15 g in weight, usually 10 to 12 g, were used in most of the following experiments. The PrL line was discontinued by Dr. Lynch in 1951 but a subline was maintained by Dr. Wilhelmina F. Dunning at Univ. of Miami, Fla. We are indebted to her for a supply of young PrL mice with transplanted leukemia. Suspensions for intraperitoneal injection were prepared from pooled mesenteric lymph nodes and spleens of 2 or more leukemic mice. A bulky but unmeasured tissue mince, finely cut with scissors, was added to 2 or 3 ml of saline. After standing for a brief period the turbid supernatant was removed and constituted the inoculum. Similar suspensions were prepared from lungs and thymus for nasal instillation. Lymphocytes were verified by phase microscopy. In the presence of hepatitis virus liver minces were homogenized in a glass tissue grinder with sufficient saline to make a concentration of about 10%. Testing of suspensions was carried out in groups of 5 or 6 mice. Intraperitoneal injections were made with an inoculum of .1 to .15 ml and nasal instillation, following ether anesthesia, with about .05 ml. The injected mice were usually held under observation until one or more deaths had occurred. At this time all survivors that showed signs of illness were killed with ether and autopsied.

Results. A passage series begun in weanlings with an organ suspension from a random-bred leukemic adult was maintained for 40 transfers in 240 mice. The interval between injection and onset of external signs varied from 25 days in the first passage to 7 days after continued transfer. Mortality rate