the induction process. How osmotic shock blocks nuclear transfer is far from clear. *Tetrahymena* cells respond to a hyperosmotic shock in two stages (*18*): a quick shrinking lasting a few minutes, followed by a return to normal volume. The second phase (which takes about 1 hour at room temperature) is accompanied by an increase in the intracellular concentration of certain ions and small molecules (mainly amino acids), resulting in an increase in the intracellular osmolarity, so that the cell regains its hyperosmolarity to the medium. The detailed cellular and molecular mechanism involved in this osmoregulation has not been elucidated. Also unknown is the mechanism of reciprocal transfer of migratory gametic nuclei across the partially fused membrane separating the two conjugating cells (*19*). A variety of compounds that affect microtubule assembly also caused cytogamy in *T. thermophila* at concentrations far too low to cause osmotic shock (*7*). This suggests an essential role for microtubules in the mechanism of gametic nuclear transfer.

Conjugation among ciliates is a remarkable developmental program that determines, within a common cytoplasm, the differential location, movement, expression, DNA replication, division, and ultimate fate of two (and later three) distinct types of nuclei. Furthermore, this program possesses enough regulatory potential to bypass some of the major events, for example, nuclear exchange and establishment of new macronuclei, without lethal consequences. The present work suggests the feasibility of exploiting experimental interference and genetic blocks to dissect the interesting cellular mechanisms involved.

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14. A mutagen treatment step (*2*) is inserted just prior to the cross. Because of variability in the preparation of 2 percent proteose peptone medium, a 1-hour treatment with 1.5 percent (83 mos*M*) glucose in conjugation buffer is preferable for the osmotic shock, followed by a tenfold dilution in buffer, as in Fig. 2.
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Obesity Genes: Beneficial Effects in Heterozygous Mice

Abstract. *The mouse mutant genes obese (*ob*) and diabetes (*db*) cause similar obesity-diabetes states in homozygotes. These obesity syndromes are characterized by a more efficient conversion of food to lipid and, once stored, a slower rate of catabolism on fasting. Heterozygous mice, either* ob/+ *or* db/+*, survived a prolonged fast significantly longer than normal homozygotes (+/+); this suggests that the heterozygotes exhibited increased metabolic efficiency, a feature normally associated with both homozygous mutants. The existence of this thriftiness trait, if manifested by heterozygous carriers in wild populations, would lend credence to the thrifty gene concept of diabetes. Beneficial effects of normally deleterious genes may have played a role in the development of diabetes-susceptible human populations, as well as having provided the survival advantage that has allowed both the development and successful establishment of species in desert and other less affluent regions.*

Diabetes has been suggested to be the result of a "thrifty genotype rendered detrimental by progress" (*1*). In undeveloped countries humans foraged for a limited food supply and were subjected to periods of abundance alternating with periods of food deprivation and even famine. Those individuals (thrifty) with a predisposition to diabetes were able to utilize a limited food supply more efficiently and thereby maintained a selective advantage when food was scarce. However, as such countries developed and the food supply increased, or as representatives of such selected cultures moved to more affluent societies and became urbanized, the thrifty genotype became a liability rather than an asset. In situations of affluence, hyperinsulinemia occurred, obesity developed, the insulin synthesizing and secreting capacity of the pancreas was stressed, and diabetes often ensued. Persistence of this thrifty genotype may have provided the survival advantage that has allowed both the successful establishment of species inhabiting warm and arid climates and the persistence of the diabetes genotype in animal and human populations despite strong negative selective pressures.

The factors involved in conferring the thrifty genotype on diabetes-susceptible cultures are probably multiple and cannot be assessed readily in genetically diverse populations. The varying degrees of diabetes susceptibility are probably the results of a variety of deleterious and even beneficial genes acting in conjunction with each other. The chance of getting diabetes by any mechanism would depend on the interaction of several deleterious genes with the entire genome. If the thrifty genotype concept is to have any validity, there should be some selective advantage to the heterozygote populations under reasonably affluent or typical living conditions. However, no metabolic advantage of diabetes-like genes has been demonstrated for heterozygotes living in affluence, and it

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may be that the thrifty genotype has no advantage in normal situations but is only maintained in the population because of continual mixing of the gene pools from mutants living in adverse circumstances with those from normal populations living in affluence.

Heterozygotes are not identifiable in human populations but they are readily available in the various obesity-diabetes syndromes in mice (*2, 3*). At least five mutations are available for study. Their obvious obesity, ability to withstand prolonged fasting (*4*), and tendency to gain weight and accumulate excess fat, even when pair-fed with normal controls (*3, 5, 6*), suggest that many of these mutants fit the definition of thrifty. Since each syndrome represents a separate mutation, each should have a different primary defect, possibly in different metabolic pathways. Being able to deal with the effects of only one single gene, rather than the multiple actions of many genes interacting with a wide variety of host genomes, should simplify the attempt to define these thrifty mechanisms. This report demonstrates thrifty mechanisms in mice heterozygous for two specific diabetes-producing genes, obese (*ob*) and diabetes (*db*).

Male retired breeders (+/+) of the C57BL/6J (BL/6) and C57BL/KsJ (BL/Ks) strains were obtained at 7 to 9 months of age. Known heterozygotes for diabetes (*db*) or obese (*ob*) (BL/Ks *db*/+ or BL/6 *ob*/+) mice were retired breeders of the same age. All of these mice were established as heterozygotes by their breeding records at Jackson Laboratory. A small number of BL/6 *db*/+ mice came from our research colony. These younger male mice were not retired breeders. This colony, like the BL/Ks diabetes colony, is maintained with the closely linked coat-color recessive gene, misty (*m*), in repulsion on the opposite chromosome from the diabetes mutation (*3*). Thus, heterozygotes are readily distinguishable, being thin and black (*db* +/+ *m*), from homozygous normals (+ *m*/+ *m*), being thin and gray. Other than this coat-color modification, mice homozygous for the misty gene are normal in all respects. Mice were housed individually and given free access to commercial mouse chow (96 W, Old Guilford Co., Guilford, Connecticut) and water for 1 week before initiation of total fast. Baseline measurements (body weight, liver glycogen, blood sugars, and plasma insulin concentrations) were determined as described (*7*) on representative individuals during this acclimatization period. Some mice were killed at specific times throughout the fasting period to establish any differences in these measures for each genotype. The remaining mice were weighed and subjected to a total fast with water freely available. These mice received no further treatment other than daily inspection to determine survival time.

Table 1. The effects of genotype on ability to survive fasting. Data are means ± standard error of mean; *N*, number of animals

Strain	Genotype	Starting body weight (g)	*N*	Mean survival time (days)
BL/6	+/+	36.7 ± 0.7	32	10.8 ± 0.4
BL/6	*ob*/+	36.6 ± 0.6	29	12.2 ± 0.4*
BL/6	+/+	33.3 ± 0.3	15	8.6 ± 0.3
BL/6	*db*/+	33.1 ± 0.4	14	10.6 ± 0.4†
BL/Ks	+/+	29.7 ± 0.3	26	7.2 ± 0.3
BL/Ks	*db*/+	29.9 ± 0.4	26	10.5 ± 0.3‡

*$P < .05$, Student's *t*-test. †$P < .01$. ‡$P < .001$.

Results of two separate experiments, one beginning in March 1978 and one in April, were similar, and the data were pooled for each genotype. No differences were evident between normal (+/+) and heterozygous (*ob*/+ or *db*/+) mice of either strain with respect to starting body weights, fed plasma insulin, blood sugar, or liver glycogen concentrations. A difference between strains in starting body weight was obvious; fed BL/Ks retired breeders seldom reached 35 g, whereas those of the BL/6 strain were routinely 35 g or heavier. Mice of the larger BL/6 strains survived longer than those of the smaller BL/Ks strain.

The survival time for *ob*/+ BL/6 mice was prolonged 1.4 days ($P < .05$) over that for homozygous normal BL/6 mice (Table 1). A significant increment in survival time was also observed for *db*/+ BL/6 heterozygotes when compared to a group of weight-matched normal (+/+) mice. The similarity in prolongation of life-span (1.4 days for *ob*/+ and 2.0 days for *db*/+) in the BL/6 background suggests that both mutations confer about the same amount of thriftiness to the heterozygotes.

Similar results (Table 1) were obtained with *db*/+ heterozygotes on the BL/Ks background except that the prolongation of life-span (3.3 days) when compared to normal mice was greater than for diabetes heterozygotes on the BL/6 background even though body size was significantly smaller than that of BL/6 mice. The extra increment in life-span in the BL/Ks *db*/+ heterozygotes may represent a synergistic interaction of the mutant gene with the genetic background. Both genes, diabetes and obese, in the homozygous state cause severe diabetes on the BL/Ks background and only a severe obesity with little and only transient diabetes on the BL/6 background (*8*). The survival time of BL/Ks *ob*/+ heterozygotes has not been determined.

These data demonstrate a possible beneficial effect in the heterozygous (carrier) state of two different deleterious mutant genes, obese and diabetes, with respect to rate of depletion of food stores and ability to survive prolonged fasting. The metabolic changes involved in this more efficient utilization of food reserves have not been established. Most of the physiological parameters measured in these heterozygotes (both fed and after fasting) were no different than those in normal (+/+) homozygotes. Insulin, the hormone of the fed state, besides playing a fundamental role in maintaining anabolic processes (lipogenesis, glycogenesis, or protein synthesis), is intimately involved in both of these diabetes and obese syndromes. Large increases in plasma-immunoreactive insulin are typical of both obese or diabetes homozygotes. No significant increases were observed in these studies in heterozygotes of either genotype of both inbred strains. However, in earlier studies BL/K *db*/ + heterozygotes more than 2 years of age (*9*) and noninbred obese *ob*/ + heterozygotes at 6 months of age (*10*) had increased plasma insulin concentrations approaching twice normal. Although no increase was apparent in the present, the heterozygous condition may confer extra sensitivity to normal concentrations of insulin and thereby promote increased anabolism relative to that of normal mice.

Although the effects of both obese and diabetes genes in the homozygote state have been extensively studied, the only abnormalities that have been observed in heterozygotes were those in plasma insulin concentrations and a report that fat pads from heterozygote obese (*ob*/+) mice oxidized glucose slower than those from normal mice but faster than those from homozygous mutants (*11*). This decreased rate of glucose oxidation could contribute to the increased metabolic efficiency observed in mutant and heterozygous mice. Both obese and diabetes mice have thermoregulatory defects (*12*). This failure of mutants to thermoregulate properly could result in conservation of energy normally used to maintain body temperature and thereby lead to increased metabolic efficiency. A partial manifestation of this defect by obese and diabetes heterozygotes could contribute to their prolonged survival.

In most of the obesity-diabetes syn-

dromes, animals show an increased capacity to convert even restricted amounts of food into lipid (*3, 5, 6*). Further, this food, once stored, is released much more slowly in the homozygous obese (*ob/ob*) or diabetes (*db/db*) mutants, which leads to greatly increased food efficiency and remarkable ability to withstand a fast (up to 40 days) (*9*). This report indicates that the heterozygous "normal" carriers have more efficient pathways of metabolism intermediate between those of homozygous mutant and the homozygous normal mice. This thriftiness trait, if manifested in wild populations, could provide the heterozygote with a selective advantage when food was scarce and yet not be deleterious when food was abundant. The existence of this trait in mice heterozygous for one mutant allele for either of two obesity-diabetes syndromes lends credence to the thrifty genotype etiology of diabetes (*1*).

Such mechanisms may have played a role both in the development of diabetes-susceptible human populations (various Indian and primitive cultures) and the persistence of diabetes susceptibility in the population despite negative selection pressures. The metabolic abnormalities controlling thriftiness should be more amenable to analysis in genetically defined models where the effects of one gene can be studied rather than in genetically ill-defined human populations where the interaction among many "thrifty" genes (both heterozygous and homozygous) may make interpretation impossible.

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Electroencephalogram Correlates of Higher Cortical Functions

Abstract. *By means of two-stage, nonlinear multivariate pattern recognition, electroencephalograms (EEG's) were analyzed during performance of verbal and spatial tasks. Complex scalp distributions of θ-, β-, and, to a lesser extent, α-band spectral intensities discriminated between the two members of a pair of tasks, such as writing sentences and Koh's block design. Small EEG asymmetries were probably attributable to limb movements and other uncontrolled noncognitive aspects of tasks. Significant EEG differences between cognitive tasks were eliminated when controls for inter-task differences in efferent activity, stimulus characteristics, and performance-related factors were introduced. Each controlled task was associated with an approximately 10 percent reduction, as compared with visual fixation, in the magnitude of α- and β-band spectral intensity. This effect occurred bilaterally and was approximately the same over occipital, parietal, and central regions, with some minor difference over the frontal region in the β band. With these controls, no evidence for lateralization of different cognitive functions was found in the EEG.*

For several years, researchers have reported correlations between interhemispheric asymmetries of ongoing brain electrical activity (EEG) and differences between "cognitive" tasks (*1, 2*). These results have been interpreted to reflect the functional specialization (in right-handers) of the left and right cerebral hemispheres, respectively, for sequential-analytical (verbal-logical) and simultaneous-holistic (spatial) cognitive processes. Frequently, interhemispheric EEG α- band asymmetries were the only indices studied, the assumption being that such asymmetries were associated with the cognitive aspects of the tasks. In fact, it has never been established that asymmetries or other EEG features are directly related to cognitive activities. In addition to their cognitive differences, the tasks used in most of these studies have involved differences in stimulus characteristics, efferent activities (limb and eye movements), and performance-related factors (task demands and a subject's ability and effort). These noncognitive factors, which are known to affect the EEG, have not been adequately controlled in previous experiments.

Using two-stage, multivariate, nonlinear pattern recognition, we have searched for sets of EEG features, including but not limited to asymmetries, which might discriminate between commonly employed verbal-logical and spatial tasks (*1, 2*). Initially, several strongly discriminating features, not related to asymmetry, were found. While weak asymmetry effects were also found, it seemed likely that they were attributable to limb movements and other uncontrolled noncognitive aspects of the tasks. When limb movement was not required during task performance, and when stimulus characteristics and performance-related differences between tasks were relatively controlled, our analytical procedures did not uncover EEG patterns of any sort that could significantly distinguish between logical and spatial cognitive tasks. Each of the controlled tasks was associated with an approximately 10 percent reduction, as compared with visual fixation, in the magnitude of α- and β-band spectral intensity. This effect occurred bilaterally and was approximately the same over occipital, parietal, and central regions with some minor difference over the frontal region in the β band. With these controls, asymmetry differences between the tasks were all but absent.

The results reported here were derived from two experiments in which EEG's were recorded from normal, right-handed (as assessed by questionnaire; siblings and parents were also right-handed) subjects while they performed batteries of randomly ordered cognitive and control tasks.

In the first experiment, 23 adults (18 males and 5 females) performed two or three 1-minute trials of reading, writing a summary of the previously read material from memory, Koh's block design, mental paper folding (the reconstruction of a cube from a flat pattern), scribbling, undirected block manipulation, and visual fixation on a spot (*3*). Reading and writing from memory have previously been used as examples of verbal-logical tasks, and Koh's block design and mental paper folding as spatial tasks (*1, 2*). Scribbling, undirected block manipulation, and visual fixation served as control tasks.

Experiment 2 was intended to separate possible EEG patterns associated with the cognitive aspects of tasks from those associated with efferent components, stimulus characteristics, and performance-related factors, all of which are intermixed in complex tasks such as those used in experiment 1. In this experiment, 32 adults (23 males and 9 females) (*4*) each performed 30 trials of shorter, simplified tasks, including: (i) mental rotation of block structures, a spatial task;

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