Table 2. Effect of nitrogen and potassium on the color values of rose petals. The two means connected by a curved line are not different at the 5-percent level of significance, while the third mean is different from the other two at the 1-percent level of significance. The three Hunter values have been converted mathematically to the Munsell system and the values are given in the characteristic notation (RP, red purple).

Treatment	Hunter system			Munsell system	
	L	a_L (mean)	b_L		
3N-3K	32.2	+41.9	−2.7	6.5 RP	3.7/11.8
1N-1K	39.6	+40.0	−3.1	6.0 RP	4.5/9.2
1N-3K	40.2	+32.3	−6.5	3.5 RP	4.6/8.5

Only three of the four fertilizer treatments were used in the statistical evaluation of the color effect. No flowers were produced on the plants that were treated with the 3N-1K combination.

The means of the values from the Hunter color test on the petals were compared by Duncan's multiple range test (*7*) and are presented in Table 2, along with calculated (*8*) Munsell renotations.

The lightness in color, of which L is a measure, seems to be affected more by the absolute quantity of N and K rather than the N/K ratio, since a large amount of N and K results in darker roses (that is, those having less white component). The positive a_L values indicate redness and since roses grown in 3N-3K and 1N-1K are significantly redder than those grown in 1N-3K, it can be inferred that an excess of K over N results in less red color. The negative b_L values indicate blueness and here again the significantly bluer roses resulting from growth in 1N-3K indicate that a large ratio of K over N favors the appearance of blue color while it reduces redness. This result agrees with Twigg's observation that "blue" roses contained more potassium than red roses (*5*).

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Additive Inheritance of Serum Cholesterol Level in Mice

Abstract. *Serum cholesterol level (SCL) was measured in 600 mice belonging to five inbred strains of mice (DBA/1J, C57BL/10J, A/J, C3H/HeJ, and BALB/cJ) and all 20 F_1 hybrids resulting from the systematic crossing of the inbreds. Diet was kept constant and its effect on SCL was not evaluated. The data show that in mice, the mode of inheritance of SCL is neither dominant nor recessive, but is intermediate. A simple additive model accounts for the results: the SCL is a linear function of the SCL of the mother, the SCL of the sire, and the sex of the subject; the three factors do not interact.*

In 1962 Bruell, Daroczy, and Hellerstein (*1*) reported that inbred strains of mice differ in their serum cholesterol level (SCL), and that the SCL is higher in males than in females. Here I present data which show that the mode of inheritance of SCL in mice is neither dominant nor recessive, but intermediate and additive. The SCL of F_1 hybrids can be predicted accurately by adding half the SCL of one inbred parent to half the SCL of the other; and the correlation between these midparent values and observed F_1 values is highly significant.

A genetic diallel design was used. In this approach, data are obtained for N inbred strains and all $N(N\text{-}1)$ possible F_1 hybrids resulting from the systematic intercrossing of the inbreds (see Table 1). In the present investigation, five inbred strains of mice (DBA/1J, C57BL/10J, A/J, C3H/HeJ, and BALB/cJ) and all 20 F_1 hybrids resulting from their intercrossing were used. The five inbred strains were chosen at random from those available in our colony. Twelve mice per genotype and sex were used (total $N = 12 \times 25 \times 2 = 600$). All animals were fed Purina mouse breeder chow, a diet containing 11 percent fat. When each mouse was sexually mature and about 4 months old, its blood was analyzed. Blood was drawn from the tail vein and centrifuged. Two microliters of serum were then pipetted and analyzed for cholesterol according to the fluorometric ultramicro-method developed by Webster (*2*).

Table 1 (a 5 × 5 diallel table) contains data for each of the 25 genotypes. Data for a given genotype are entered at the intersection of the maternal strain row and the paternal strain column. The leading diagonal of the table contains data for the five inbred strains. As the first step in determining the mode of inheritance of SCL, midparent values for each F_1 hybrid were computed and entered in Table 1. For example, the midparent value for F_1 (DBA♀ × C57♂) was arrived at by averaging the SCL of DBA/1♀♀ and C57Bl/10♂♂: (91 + 133)/2 = 112 mg percent. The mean absolute discrepancy between the thus computed midparent values and F_1 averages, (♀ + ♂)/2, was 5.7 mg percent (standard deviation, 4.01 mg percent), and the correlation between theoretical and observed values was 0.837 (18 degrees of freedom) and highly significant. If reciprocal crosses are pooled, midparent values and F_1 values become even more alike. For example, for F_1 (DBA♀ × C57 ♂) and F_1 (C57♀ × DBA♂) the pooled midparent value becomes 111.25 mg percent and the F_1 value becomes 112.75 mg percent. The second step was to compute the average for all parental scores and that for all F_1 hybrid scores. These two averages were 129.10 mg percent and 132.05 mg percent. The difference of 2.95 mg percent was clearly not significant. These preliminary analyses indicate intermediate rather than dominant or recessive inheritance of SCL. The F_1 hybrids resemble neither the parent with the higher nor the parent with the lower SCL, but fall midway between them; the parental average and the F_1 average do not differ. Such intermediate inheritance often is called "additive"

because, as will be shown, a simple additive model of the underlying genetic mechanisms can be constructed.

In essence, diallel studies are factorial experiments. As shown in Table 1, in this diallel study the interactions between three main factors were investigated: (i) factor *F*—hereditary material received from the female parent, represented by five "levels," that is, strains; (ii) factor *M*—hereditary material received from the male parent, also represented by five strains; and (iii) factor *S*—the sex of the subject, with two levels. Thus this study was analogous to a 5 × 5 × 2 factorial experiment, and an analysis of variance was applicable. Results of such an analysis are shown in Table 2. The three main factors, *F*, *M*, and *S*, were highly significant, but none of the interactions between factors was. This suggests an additive model for the composition of phenotypic measures, namely

$$X = G + F + M + S + E$$

in which X stands for a single observation or a population mean; G represents a constant; F, M, and S indicate effects of the main factors, and E denotes the experimental error. To illustrate, I will use this model to compute a theoretical mean for female F_1(BALB♀ × C57♂). Constant G is estimated by the overall mean of Table 1: 131.46 mg percent. The effect of a BALB/c mother on SCL, $F_{BALB/c}$, is estimated by the deviation of the overall mean for offspring of BALB/c dams (see "Mean for dam" column) from G: 141.60 − G = 10.14 mg percent (see "Dam effect" column). Similarly, $M_{C57BL/10}$, the effect of a C57BL/10 sire on SCL, is estimated by 125.90 − G = −5.56 mg percent (see "Sire effect" row). Also S♀, the effect on SCL of being female, is estimated by 116.96 − G = −14.50 mg percent (see bottom of last column). Thus, the theoretical mean for F_1 (BALB♀ × C57♂)♀♀ = 131.46 + 10.14 + (−5.56) + (−14.50) + E = 121.54 mg percent + E, where E denotes the standard error (S.E.) of means based on 12 observations. To compute S.E., an estimate of the standard deviation of measurements (S.D.) is obtained from the error variance of the analysis of variance: S.D. = $\sqrt{807.47}$ = 28.4 mg percent. Thus, S.E. = $28.4/\sqrt{12}$ = 8.2 mg percent, and theoretical mean for F_1 (BALB♀ × C57♂)♀♀ = 121.54 ± 8.2 mg percent. The observed mean of 126.0 mg percent and this theoretical mean do not differ significantly. If the theoretical values for males and females of all 25 genotypes were computed, one would find in each case similarly close agreement between computed and observed mean. In fact, this is what the analysis of variance indicates: the $F \times M \times S$ interaction was not significant. Thus, the 50 means for females and males in Table 1 can be assumed to be sums of factor effects, and each can be obtained by adding F, M, S, and E to G. An analogous interpretation applies to the other not significant interactions. For example, the not significant $F \times M$ interaction indicates that genotype averages, (♀ × ♂)/2 (see Table 1), can be obtained by simply adding F, M, and E to G; in this case factor S cancels out.

Table 1. Serum cholesterol level (mg/100 ml) in 25 genotypes of mice.

Female parent	Sex	Male parent					Mean for dam	Dam effect
		DBA/1	C57BL	A/J	C3H	BALB/c		
DBA/1J	♀	91.0	109.0	117.0	108.0	126.0	110.2	
	♂	115.0	126.0	148.0	150.0	159.0	139.6	
	(♀+♂)/2	103.0	117.5	132.5	129.0	142.5	124.9	− 6.56
	MP*		112.0	117.5	128.5	127.0		
C57BL/10J	♀	100.0	106.0	115.0	119.0	126.0	113.2	
	♂	116.0	133.0	121.0	138.0	156.0	132.8	
	(♀+♂)/2	108.0	119.5	118.0	128.5	141.0	123.0	− 8.46
	MP	110.5		125.0	136.0	134.5		
A/J	♀	116.0	104.0	113.0	117.0	120.0	114.0	
	♂	130.0	136.0	144.0	166.0	159.0	147.0	
	(♀+♂)/2	123.0	120.0	128.5	141.5	139.5	130.5	− 0.96
	MP	114.0	123.0		139.5	138.0		
C3H/HeJ	♀	100.0	114.0	138.0	126.0	125.0	120.6	
	♂	146.0	158.0	146.0	166.0	154.0	154.0	
	(♀+♂)/2	123.0	136.0	142.0	146.0	139.5	137.3	+ 5.84
	MP	120.5	129.5	135.0		144.5		
BALB/cJ	♀	116.0	126.0	125.0	133.0	134.0	126.8	
	♂	146.0	147.0	164.0	162.0	163.0	156.4	
	(♀+♂)/2	131.0	136.5	144.5	147.5	148.5	141.6	+10.14
	MP	124.5	133.5	139.0	150.0			
Mean for sire	♀	104.6	111.8	121.6	120.6	126.2	116.96	−14.50
	♂	130.6	140.0	144.6	156.4	158.2	145.96	+14.50
	(♀+♂)/2	117.6	125.9	133.1	138.5	142.2	131.46	0.00
Sire effect		−13.86	−5.56	+1.64	+7.04	+10.74		

* Midparent.

Table 2. Summary of analysis of variance of 5 strain × 5 strain diallel study of serum cholesterol level (mg/100 ml) in 600 mice.

Source of variation	df	Mean square	F
Female parent (*F*)	4	7,584.36	9.39*
Male parent (*M*)	4	11,671.51	14.45*
Sex (*S*)	1	127,108.81	157.42*
$F \times M$	16	742.38	0.92†
$F \times S$	4	958.10	1.19†
$M \times S$	4	758.19	0.94†
$F \times M \times S$	16	774.87	0.96†
Error	550	807.47	
Total	599		

* $P < .001$. † Not significant.

What do these findings suggest concerning the genetic mechanisms underlying SCL in mice? This study was not designed to provide information regarding the number of genes controlling SCL. The data are compatible with explanations hypothesizing control by one gene pair or many such pairs. For simplicity of exposition I will adopt a one-gene pair model. Table 1 shows that the five inbred strains of mice fell into three distinct SCL groups, namely, DBA/1; C57BL/10 and A/J; and C3H and BALB/c. Thus we must hypothesize that SCL is controlled by an allelic series of genes consisting of at least three alleles, A_1, A_2, and A_3. Each allele controls the accumulation of differing amounts of cholesterol in the blood stream. The SCL in homozygotes is controlled by the separate but equipotential action of each member of the homozygous gene pair AA. In heterozygotes SCL is also controlled by the separate action of genes, for example, A_1 and A_2; in this case, however, the action of the two genes is not equipotential but depends on their differential potencies. The action of these SCL-controlling genes is modified by factors associated with the sex of the animal: in males this action is enhanced on any genic background, in females it is suppressed. The SCL genes and factors associated with sex act independently; here, too, the action is additive (*3*).

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Isotope Ratios in Marine Mollusk Shells after Prolonged Contact with Flowing Fresh Water

Abstract. *The ratios C^{13}/C^{12} and O^{18}/O^{16} in the calcium carbonate of shells of the marine mollusk,* Macoma calcarea, *appear unaltered after exposure for 4500 years to flowing fresh water of higher O^{16}/O^{18} ratio.*

The widespread use of carbon and oxygen isotopes as paleoenvironmental indicators in sedimentary geochemistry (*1*), for radiocarbon dating, and for paleotemperature determinations (*2*), depends to a large extent on the assumption that the original isotopic composition has not been altered by postdepositional, diagenetic processes (*3*). While oxygen isotope exchange in the calcium carbonate–water system proceeds relatively rapidly at high temperatures (*4*), it appears that original carbon isotope ratios may be preserved in calcitic limestones over very long periods of time, as indicated by consistent changes in the values of δC^{13} found in a regional study of isotope ratios in the Vanport limestone of Pennsylvanian age (*5*). Furthermore, in a study of over 500 marine and fresh water carbonates of Phanerozoic age (*6*), the original carbon isotope ratios appear to have remained unchanged since the Devonian—the earliest fresh-water limestone known to us is Devonian—because the expected difference in mean isotopic composition of marine and fresh-water limestones was confirmed. These samples, however, were mostly compact, dense limestones which were apparently not subjected to a significant flow of intrastratal solutions around grain boundaries.

Table 1. Carbon and oxygen isotope ratios of six individual shells of the marine mollusk, *Macoma calcarea*. The values, per mill, are relative to Chicago PDB-standard carbon dioxide.

δC^{13}	δO^{18}
−1.56	+.72
−1.64	−.58
−1.77	+.40
−1.47	+.98
−1.51	+.58
−1.37	−.43
Mean	
−1.55	+.28
S.D.	
.141	.636

* Collected by J. F. Schwietering, from north bank of Rivière du Sud, 1.6 Rm east of St. François, Montmagny county, Quebec, now 45 m above sea level.

Gross (*7*) reports that marine Pleistocene limestone from Bermuda and from the atolls, Bikini and Eniwetok, has been altered by the Ghyben-Herzberg lens of fresh water and by the precipitation of secondary calcite in a relatively short period of time so that C^{13}/C^{12} and O^{18}/O^{16} ratios approach those of fresh-water limestones. Unpublished data from this laboratory suggest that, if certain corals have contributed calcium carbonate to the calcareous sediments, some of these anomalous isotope ratios may, in fact, be the original ratios which obtained at the time of deposition.

Because of the importance of the preservation of the original isotopic record in carbonates, a collection of marine shells of the mollusk *Macoma calcarea* which were exposed to percolating freshwater for a known period of time, was made and analyzed for isotope ratios by standard techniques (*8*). After deposition in the bottom sediments of the Champlain Sea between 9500 and 9900 years ago (*9*), the mollusk shells have been elevated and subjected to the effects of percolating fresh water in the banks of Rivière du Sud, Quebec, for at least 4500 years. The isotopic composition, expressed as the difference in C^{13}/C^{12} or O^{18}/O^{16} ratio of the sample and the Chicago PDB standard carbon dioxide, in per mill, by the formula

$$\delta C^{13} = \left(\frac{C^{13}/C^{12}_{\text{sample}}}{C^{13}/C^{12}_{\text{std.}}} - 1 \right) 1000,$$

has been corrected for errors of measurement, such as capillary leaks and the presence of O^{17} (*10*), and is presented in Table 1. The results are well within the accepted range of marine carbonates (*11*), and indicate that under some conditions original isotope ratios may be unaltered or altered to an indetectable extent, after contact with flowing fresh water for as long as 4500 years.

The statement of Rubin and Taylor (*3*), that the degree of alteration can be determined by mass spectrometric studies of isotopes is not necessarily true, especially in the case of fresh-water mollusk shells, some of which originally exhibit a wide range in isotopic composition (*12*; *13*).

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Zinc Activation of a Coordinated Response in *Hydra*

Abstract. *In* Hydra littoralis, *the feeding response normally activated by reduced glutathione can be elicited by zinc ions under special experimental conditions: some calcium is necessary for the activation to be realized, but a high concentration of calcium inhibits the zinc-activated response. Zinc inhibits the normal feeding response induced by reduced glutathione.*

Zinc ions are known to have immediate physiological effects at several levels of biological organization. Vallee *et al.* showed that the addition of zinc to the apoenzyme of carboxypeptidase activates that enzyme (*1*). Isaacson and Sandow found that zinc potentiates the twitch of muscle (*2*). In this report we describe the activation by zinc of the feeding response in *Hydra littoralis*.

The feeding response of *Hydra* is normally activated by the ubiquitous