uously harvested; (ii) recycle of partially spent nutrient. In a similar system in which we have grown *Lactobacillus casei* we have found that partially spent nutrient could be recycled, thereby improving the efficiency of nutrient utilization.

For chemotherapy studies it is of course possible to add or remove drug by other modes. To provide a model of in vivo perfusion, drug could be added with the nutrient so that the concentration in the culture would asymptotically approach the incoming level. For continuous perfusion at a constant drug level in such an experiment an initial dose of drug may be added directly to the culture. By control of flow rates, the decline of drug concentration could follow other kinetics besides the simple exponential model used so far. Further work is necessary to determine whether initial drug concentration or integrated $C \times T$ is more closely related to degree of cell kill, since for a series of initial concentrations a constant $C \times T$ can be produced by appropriate regulation of the flow rate.

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Gene Dosage at the Lactate Dehydrogenase b Locus in Triploid and Diploid Teiid Lizards

Abstract. *Triploid parthenogenetic lizards which are heterozygous at the lactate dehydrogenase* b *locus show approximately two doses of the* b *allele and approximately one dose of the* b′ *allele. This finding agrees with the triploid karyotype composed of two haploid complements of chromosomes indistinguishable from those of species carrying the* b *allele and one complement indistinguishable from that of the species which carries the* b′ *allele. Densitometry of electrophoretic patterns of lactate dehydrogenase from heart muscle and from kidney indicates approximately equal expression of each of the* b *alleles in the triploid lizards. Heterozygous diploid lizards from the same parthenogenetic species, in agreement with their karyotype, show equal doses of* b *and* b′ *alleles.*

An electrophoretic study of lactate dehydrogenase (LDH, E.C. 1.1.1.27) isozymes from seven *Cnemidophorus* species of the American southwest has revealed heterozygosity at the LDH *b* locus in two diploid parthenogenetic forms, *C. neomexicanus* and *C. tesselatus* class E (*1*). The distribution of isozyme activity in diploid heterozygotes corresponded to the presence of approximately equal amounts of two different B-type subunits. We now report quantitative corroboration of our earlier impression that each of the two *b*-type alleles is equally active in the diploid heterozygote, and more significantly, the demonstration of approximately equal activity of each of three *b*-type alleles in triploid heterozygotes also belonging to the species *C. tesselatus*.

Cnemidophorus tesselatus is a unisexual, all-female species composed of at least six defined morphotypes designated by the letters A through F (*2*). Individuals from four of these morphotypes (C through F) are diploid and possess two distinct haploid chromosome complements, one resembling that found in the *C. sexlineatus* group and the other resembling that of *C. tigris*. Individuals from the other two morphotypes (A and B) are triploid and possess two *sexlineatus*-like complements and one *tigris*-like complement (*3*). Species of the *C. sexlineatus* group, such as *C. inornatus* and *C. gularis*, carry one allele at the LDH *b* locus whereas *C. tigris* carries a different allele (*1*). These two alleles have been designated as *b* and *b′*, respectively, and their products as B and B′. There is thus a definite correlation between karyotype and the type of LDH *b* allele in these lizards. One might predict on the basis of the karyotype that triploid *C. tesselatus* would carry two *b* alleles and only one *b′* allele and would thus produce twice as many B subunits as B′ subunits, if each allele were equally active. We have tested this prediction by studying the distribution of LDH isozyme activity in both triploid and diploid *C. tesselatus*.

Electrophoresis of homogenates of heart muscle and kidney, either freshly prepared or after storage in a freezer, was analyzed. Homogenates, ground in glass, containing either 25 mg of heart or 100 mg of kidney per milliliter of gel buffer, were centrifuged at 40,000*g* for 1 hour. Samples of supernatant, either full strength or diluted with buffer, were subjected to electrophoresis on horizontal agarose gel (*4*) or vertical acrylamide gel (*5*). Gels were stained for LDH according to reported techniques (*6*). Quantitation of the dis-

Table 1. Values in the $\bar{B}$ column represent the mean proportion for each animal of electrophoretic pattern density attributable to the B subunit. The probability that diploid and triploid values have been drawn from the same population with regard to $\bar{B}$ is less than .001. The probability that diploid values have been drawn from a population where $\bar{B}$ equals 50.00 percent is between .80 and .70. The probability that triploid values have been drawn from a population where $\bar{B} = 66.67$ percent is between .10 and .05. Animals listed in the table are all *C. tesselatus* and are assigned to the following morphotypes and localities: Diploids—MCZ 101992, class E, Socorro County, New Mexico; MCZ 111861 and MCZ 111862, class C, San Miguel County, New Mexico; MCZ 111866, class F, Hidalgo County, New Mexico. Triploids—MCZ 101990, MCZ 111864, and MCZ 111865, class A, Pueblo County, Colorado; MCZ 111863, class B, Otero County, Colorado.

Animal	Analyses (No.)		Total subunit production	
	Heart	Kidney	$\bar{B}$ (%)	S.D.
	Diploid			
MCZ 101992	4		50.65	1.99
MCZ 111861		3	49.05	0.21
MCZ 111862	7	4	50.49	0.99
MCZ 111866	6	2	50.30	1.52
		Mean	50.12	0.63
	Triploid			
MCZ 101990	4		66.55	2.26
MCZ 111864		3	64.82	0.73
MCZ 111865	8		61.53	1.18
MCZ 111863		3	62.95	1.16
		Mean	63.96	1.90

Table 2. Comparison of the measured relative amount of enzyme activity in each isozyme band, as determined by densitometry, with the amount predicted by the binomial expansion, given the assumptions discussed in the text. Measured values represent averages from the analyses listed in Table 1. Values for *P* represent the probability that the measured value has been taken from a population whose mean is that value predicted by the binomial expansion.

Isozymes	Diploid					Triploid				
	Binomial prediction (%)	Measured value	S.D.	*N*	*P*	Binomial prediction (%)	Measured value	S.D.	*N*	*P*
$B_4B'_0$	6.3	10.37	0.96	3	.005>*P*>.001	19.8	20.94	1.20	3	.20>*P*>.10
$B_3B'_1$	25.0	24.69	0.57	3	.40>*P*>.30	39.6	32.89	3.61	3	.05>*P*>.025
$B_2B'_2$	37.5	30.06	2.04	3	.01>*P*>.005	29.6	30.14	1.89	3	.70>*P*>.60
$B_1B'_3$	25.0	24.77	0.46	3	.40>*P*>.30	9.9	13.14	1.96	3	.05>*P*>.025
$B_0B'_4$	6.3	10.12	1.29	3	.01>*P*>.005	1.2	2.89	1.55	3	.20>*P*>.1

tribution of enzyme activity was accomplished by densitometry (*7*) of the stained gels.

In heart muscle and kidney from *Cnemidophorus* heterozygous at the LDH *b* locus, five isozyme bands are resolved by electrophoresis, as compared to only a single band for homozygous lizards (*1*). The multiple-band pattern reflects the presence of two B-type subunits and their expected association to form five discrete tetrameric isozymes. These isozymes are assumed to possess the following combinations of subunits and are listed in order of increasing electrophoretic mobility toward the anode: $B_4B'_0$, $B_3B'_1$, $B_2B'_2$, $B_1B'_3$, and $B_0B'_4$ (Fig. 1).

Both diploid and triploid *C. tesselatus* possess five LDH isozymes in heart muscle and kidney; they differ strikingly, however, in the relative proportions of these five isozymes (Fig. 1). Unlike the symmetrical distribution of densities in the isozyme pattern of the diploid, the triploid pattern is asymmetric, with the bands near the cathode appearing more dense. These bands contain a higher proportion of B subunit, which suggests that the total amount of B subunit exceeds that of the B′ subunit in the triploid.

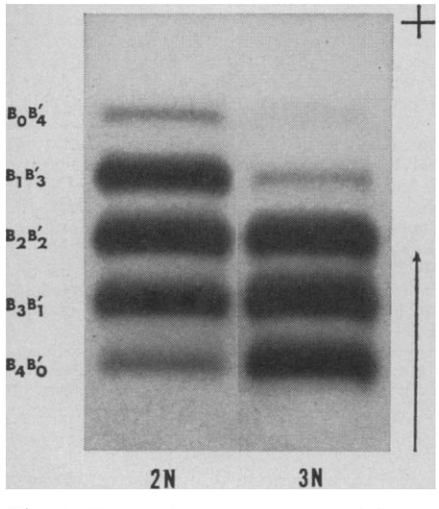

Fig. 1. Electrophoretic patterns of LDH isozymes from kidney as revealed by the acrylamide technique described in the text. Bands are labeled according to their expected subunit composition; 2N, *C. tesselatus* class C, diploid (Harvard University Museum of Comparative Zoology (MCZ) 111862, San Miguel County, New Mexico); 3N, *C. tesselatus* class A, triploid (MCZ 111865, Pueblo County, Colorado).

The densitometric estimation of subunit production in triploid and diploid lizards (Table 1) was obtained as follows. The proportion of total pattern density comprised by each isozyme band was calculated from the densitometric curve of that pattern. The presumed subunit composition of each isozyme permitted the assignment of an isozyme's relative density to either B or B′ subunit, or both subunits, on a fractional basis. For example, subunit B was assigned the entire relative density of isozyme $B_4B'_0$, three-fourths of $B_3B'_1$, one-half of $B_2B'_2$, one-fourth of $B_1B'_3$, and none of $B_0B'_4$. The degree to which this procedure reflects the actual subunit production by *b* and *b′* alleles depends on the following assumptions: (i) all subunits produced by these alleles have subsequently associated to form enzymatically active tetramers; (ii) tetrameric isozymes have equal activity per molecule under the conditions used regardless of their subunit composition; and (iii) the measured density of isozyme patterns is linearly proportional to enzyme activity. The last assumption has been tested by determining the distribution of pattern density after electrophoresis of full-strength and two- and fourfold dilutions of the same sample. No significant difference in the distribution of pattern density was found under these conditions, thus rendering acceptable the last assumption.

The evident difference between diploid and triploid electrophoretic patterns is supported by our estimation of subunit production (Table 1). Equal amounts of the two subunits, B and B′, are found in the diploid animals, in agreement with the karyotypically predicted genotype of *b*/*b′*. In triploid lizards, the amount of B subunit significantly exceeds 50 percent and approximates the two-thirds expected for the karotypically predicted genotype of *b*/*b*/*b′*. These findings suggest that each allele at the LDH *b* locus within an individual genotype produces nearly equal amounts of subunits in both the diploid and triploid animals.

Close agreement was not found between measured relative density of all individual isozymes within a pattern and the corresponding values predicted by the binomial expansion when the predicted genotypes, equal activity of alleles, and random combination of the subunits to form tetramers are assumed (Table 2). It is possible that the combination of subunits to form tetramers may not proceed entirely at random in these tissues.

Qualitative evidence consistent with equal contribution of all three alleles to a triploid phenotype has been obtained from the studies of catalase in maize endosperm (*9*) and serum proteins in triploid salamanders (*10*). Our data are consistent with the presence of two doses of the LDH *b* allele and one dose of the *b′* allele in triploid *C. tesselatus*. In the case of LDH at least, all three alleles at a particular locus in a triploid individual actively influence the individual's phenotype, and each allele appears to exert an equivalent influence. This finding suggests that the expression of one allele may be constant in its relation to its other alleles in the genotype, regardless of whether the locus concerned is in the diploid or triploid state.

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Assembly of Protein and Nucleoprotein Particles from Extracted Tobacco Rattle Virus Protein and RNA

Abstract. *Protein extracted from tobacco rattle virus by treatment with formic or acetic acid reassociated in vitro to form rings and tubular structures of indeterminate lengths in 0.25 molar glycine at* p*H 7.5 to 9.5. When tobacco rattle virus RNA that had been extracted with phenol was incubated with the protein at 9°C, particles with more regular lengths formed; these particles correspond in size and sedimentation properties to the short particles that normally accompany infection by tobacco rattle virus. The biological activity of the reconstituted short particles was identical to that of native short particles.*

Reconstitution of plant viruses characterized by helical symmetry was first accomplished with tobacco mosaic virus (*1*) and with barley stripe mosaic virus (*2*). Tobacco rattle virus (TRV) has a similar cylindrical construction; however, repolymerization of extracted protein resulted in only extremely short particles (*3*).

An unusual property of infection by TRV is the recovery of nucleoprotein particles of two distinct lengths, both of which are necessary for production of complete progeny particles (*4*). The longer rod (190 nm) presumably regulates production of the viral nucleic acid while the shorter rod, 50 to 115 nm (depending on the isolate), codes for production of the protein coat. Infection with only long rods results in the production of infectious RNA, or the unstable form of TRV, whereas typical nucleoprotein particles, or the stable form of TRV, are recovered only after infection by both long and short particles. We report the extraction of protein from purified TRV and the reassociation of protein subunits into elongated tubular structures and biologically active nucleoprotein particles, 80 to 90 nm, with the addition of TRV-RNA.

The "C" isolate of TRV used in these studies was purified as reported (*5*). This isolate is composed of particles of three lengths, 50, 85, and 190 nm (Fig. 1, a and b) which sediment as distinct populations in sucrose density gradients (Fig. 2a). A 1 to 2 percent virus suspension was treated with 1 to 2 volumes of formic or acetic acid (*6*) in an ice bath for 30 minutes, cleared by low-speed centrifugation, and dialyzed (1 to 2 days) at 4°C against several changes of water. After centrifugation at 100,000*g* for 1 hour, the supernatant was brought to 0.5 saturation with $(NH_4)_2SO_4$. The resulting precipitate was recovered by centrifugation, resuspended, and dialyzed against water adjusted to *p*H 4 to 4.5 with 10 percent acetic acid.

The water-clear solution had a typi-

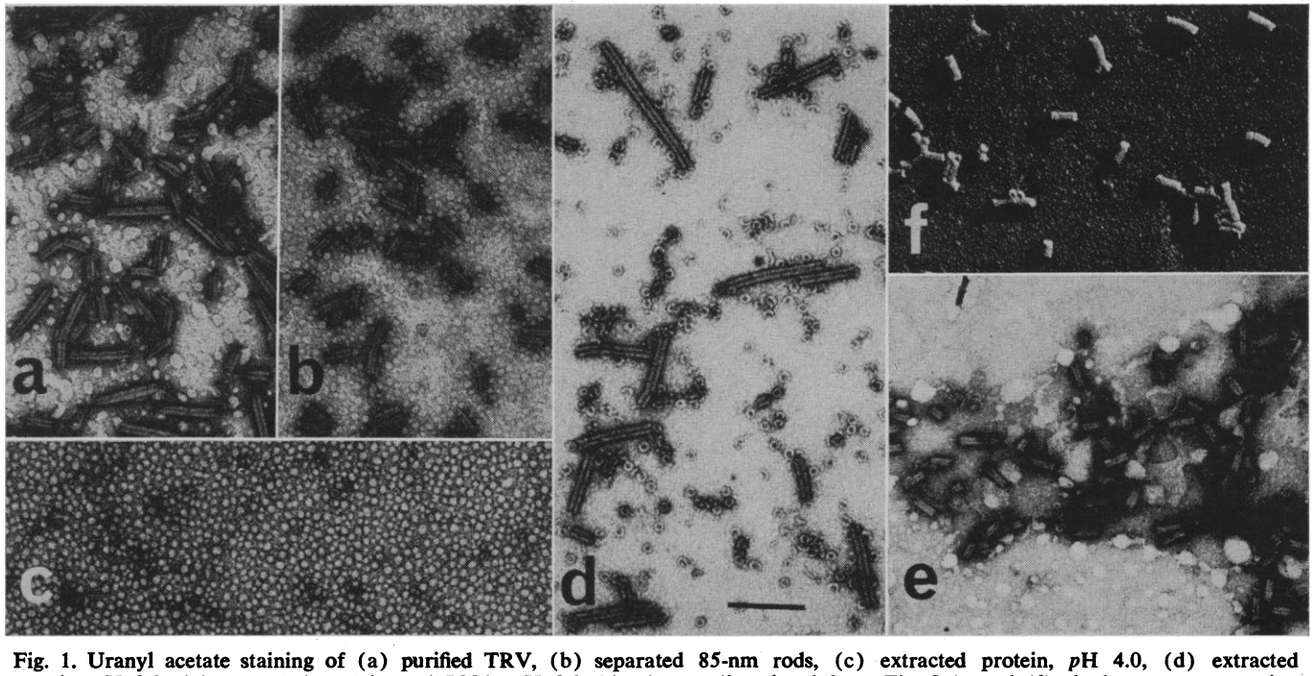

Fig. 1. Uranyl acetate staining of (a) purified TRV, (b) separated 85-nm rods, (c) extracted protein, *p*H 4.0, (d) extracted protein, *p*H 8.0, (e) extracted protein and RNA, *p*H 8.0 (density gradient band from Fig. 2c), and (f) shadow-cast preparation of (e). Scale is 0.25 μ.