The thickness and extent of Pleistocene deposits overlying reflections interpreted as basement has long been a problem in understanding seismic records from the Cape Cod and Cape Cod Bay area (*2*).

Note added in proof: After this report was submitted for publication, a third well at the westernmost tip of the hook (Race Point) also penetrated Eocene, at 213 feet.

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Specific Inhibition of $RH_o(D)$ Antibody by Sialic Acids

Abstract. Agglutination of $Rh_o(D)$ erythrocytes by specific antiserum was inhibited by crude and crystalline N-acetylneuraminic acid, less inhibited by its glycoyl derivative, and weakly inhibited by its degradation product, N-acetyl-mannosamine, and by D-mannose. A brain ganglioside containing neuraminic acid and a *Pseudomonas* polysaccharide were even more inhibitory. Inhibition was specific for anti-D sera.

Inhibition of agglutination of $Rh_o(D)$ human red blood cells by specific antiserum has been noted with nucleotides (*1*). Weak inhibition has been observed also with certain monosaccharides, streptomycin, and rutinose, the latter being most active (*2*). Since it was shown that eluates from $Rh_o(D)$ cells treated with mumps virus contained a specific inhibitor (*3*), the sialic acids were investigated as possible anti-Rh inhibitors.

Inhibition tests were performed as described previously (*3*), with trypsinized $Rh_o(D)$-positive red cells and either maximal (4+) or partial (2+) agglutinating dilutions of specific anti-Rh human serum (*4*). Complete inhibition of maximal agglutination occurred with both crude and crystalline preparations of N-acetyl neuraminic acid (NANA) (*5*), and when less antibody was used it was 500 times as effective (Table 1). The *p*H of the initial concentration of crystalline NANA in phosphate-buffered saline (*p*H 7.4) was 3.0, but inhibition by samples adjusted to *p*H values of 6.8 to 7.2 with 1.0*N* NaOH was unaffected. Higher concentrations of such samples were required for complete inhibition after storage for 5 weeks in the refrigerator, possibly due to degradation with the formation of N-acetylmannosamine. A mixture containing equal parts of the latter substance and N-acetyl-glucosamine (*6*) was inhibitory.

Table 1. Effective concentrations of anti-Rh inhibitors. 4+, maximal agglutinating dilution; 2+, partial agglutinating dilution.

Inhibitor	Anti-D control	Inhibition	
		Complete conc.	Partial (μg/0.1 ml)
NANA (75%)*	4+	500	2
NANA (75%)	2+	1–2	
NANA (3 × crystallized)*			
*p*H 3.0	4+	125	60
*p*H 6.4	4+	150	60
*p*H 6.4†	4+	1000	
1:1 N-gluc–N-mann‡	4+	500	5
D-Mannose	4+	1000	60
Beef-brain ganglioside	4+	150	20
Pseudomonas polysaccharide	4+	500	125
	2+	60	1

* Prepared from bovine submaxillary gland. † Stored 5 weeks in refrigerator. ‡ N-gluc, N-acetyl-glucosamine; N-mann, N-acetyl-mannosamine.

The chemically related N-glycoylneuraminic acid also inhibited anti-$Rh_o(D)$ agglutination, but very weakly compared to NANA. Weak inhibition occurred with D-mannose. Two substances of larger molecular-weight, a beef-brain ganglioside containing 17 percent NANA (*5*) and a polysaccharide obtained from a species of *Pseudomonas* (*7*) and suspected of containing NANA, were also effective inhibitors. In fact, these substances were practically as effective as NANA. A ganglioside obtained from human brain (*5*) and the bacterial polysaccharide formed a visible precipitate with anti-$Rh_o(D)$ serum but not with anti-C nor anti-E. Solutions of D-glucose, D-galactose, glucuronic acid, and alpha-D-galacturonic acid were not inhibitory. D-Glucose and D-galactose blocked the inhibition of anti-Rh by partially inhibitory concentrations of NANA, but not by maximal inhibitory concentrations.

The inhibition by purified NANA, N-glycoylneuraminic acid, the ganglioside, and polysaccharide was specific for anti-D, no inhibition being observed with anti-C, anti-E, anti-c, or anti-e sera. The crude NANA, however, demonstrated some inhibition of anti-C and anti-E. Further evidence of specificity was indicated by the production of a positive skin test obtained by the intradermal injection of a dilute solution of the bacterial polysaccharide into the skin of a rabbit passively sensitized 24 hours earlier with anti-$Rh_o(D)$ serum. The reaction varied markedly in severity and time from the reaction produced in a normal rabbit.

Boyd *et al.* (*2*) suggested that the terminal unit of the $Rh_o(D)$ antigen may be one of the sugars belonging to group 4 of Mäkelä's classification (*8*), and noted that streptomycin (a natural glycoside of N-methyl-L-glucosamine) also was inhibitory, while rutinose (6-O (β-L-rhamnosyl)-D-glucose) was five times more effective. Likewise, the larger molecules containing NANA used in our work were more effective inhibitors, and two formed precipitates with anti-Rh serum. It has been suggested (*9*) that sialic (neuraminic) acid occupies a terminal position in gangliosides and mucoproteins. Faillard (*10*) observed that a human brain ganglioside containing 13.2 percent neuraminic acid was resistant to neuramidase, while Gottschalk (*11*) considered that such resistance indicated a ketosidic linkage different from that in compounds susceptible to the enzyme, such as the myxovirus receptor on human erythrocytes.

Since the gangliosides were better inhibitors it may be postulated that the terminal neuraminic acid which is bound in a ketoside linkage conforms more closely to the determinant groups of the $Rh_o(D)$ antigen and exists also in cells containing the antigen. The inhibitory capacity of eluates of human red cells treated with mumps virus or periodate (*3*) might then be due to (i) related products, such as NANA, obtained by the neuramidase action of the virus on the sialic acid of the mucoprotein receptor or its degradation products,

N-acetyl-mannosamine or mannose, or (ii) to the action of periodate on the glycol bonds of more complex carbohydrate structures in carbohydrates similar to these or to those reported by Boyd.

The failure of similar eluates from cells treated with the viruses of influenza and Newcastle disease to inhibit may be due to their more vigorous enzymatic action. The importance of linkage is emphasized by the unpublished results (*12*) obtained in this laboratory, in which human urinary mucoprotein inhibited Rh antibody slightly after incubation with mumps virus, but not after incubation with influenza, NDV, or RDE; stronger inhibition occurred after incubation with trypsin.

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Venation Polymorphism and Genetic Variability in Drosophila melanogaster Loew

Abstract. Experimental evidence indicates that phenocopy production may provide an inflated estimate of the importance of genetic variability and recombination in the production of venation phenodeviants.

Some phenotypic characteristics may be misleading indicators of genetic variability. Wing venation variants in *Drosophila melanogaster* Loew are particularly suspect. Milkman (*1*) has recently reported data which he interprets as supporting the notion that a pair of individuals carries "to a large degree the potential variability of the population." He used the first 1000 F_2 progeny of wild inseminated females as samples, and surmises that the appearance of a phenodeviant not present in the female grandparent is an indication of genetic recombination and production of "rare" genotypes.

The presence of four alleles for each locus among the offspring of a mating pair obviously leads to a great number of possible genotypes in the F_2 progeny. This number is not necessarily a large fraction of the total available to the species if many large multiple allelic series exist. This also seems trivial, since with even four alleles at each of five loci (*1*) there are 10^5 possible genotypes, a number far greater than the size of most F_2's examined.

Recombination within and between loci need not be the only source of phenodeviants. Indeed, the possibility exists that among near-homozygous inbred lines environmental variations, even within one culture, could produce such effects.

Thus we have at least two possible explanations for phenodeviants: (i) rare recombinants from highly heterozygous populations, or (ii) theshold effects common to many genotypes, leading to production of phenodeviants in some environments.

An experiment was performed with highly inbred sublines of Canton-S and Oregon-R. The flies were raised at 25 ± 1°C on agar-yeast-sugar–propionic acid food media. The parent stocks were examined each generation during the experiment, as were reciprocal F_1's and F_2's. The wing venation anomalies were recorded and in most cases sketched, and the flies were preserved.

The experiment was repeated by three individuals. Reported variants included extra or missing anterior and posterior cross veins, bifurcations of vein tips, extra venation from the center of cross veins, and other extra venation. Table 1 indicates the overall frequency of venation phenodeviants in each class. Chi-square analysis yielded probabilities between .99 and .30 that the observed differences were due to chance alone.

Neither the combined results nor any of the three replications gave indication of a significant difference in frequency between classes. The most surprising (though a nonsignificant) finding was the low rate of phenodeviants among the F_2's as compared to the other classes. This could indicate that flies in a recombinant population may be less variable in this respect than inbred flies.

In addition it was noted that phenodeviants tended to come in groups from individual culture vials, in any of the classes. This lends some support to the notion that this is a threshold phenomenon requiring particular environmental conditions in addition to genetic conditions.

Table 1. Frequency of venation phenodeviants.

Stock Tested	*N*	Abnormal wing venation (*N*)	Frequency (%)
Canton-S	6,322	30	0.475
Oregon-R	5,676	24	0.423
Canton-S × Oregon-R sum reciprocal F_1's	3,557	17	0.478
Canton-S × Oregon-R sum reciprocal F_2's	12,734	37	0.2905
Sum Oregon-R and Canton-S	11,998	54	0.4501

The data presented indicate that the incidence of venation phenodeviants is not significantly different in the inbred lines, the hybrids, and the F_2 recombinants. If this is the case, then we fail to see how incidence of this type of variation in F_2's of wild inseminated females provides evidence either for or against the existence of "a large degree" of variability in the individuals Milkman (*1*) tested. In a number of our cases the venation phenodeviants were inbred, 50 to 100 offspring were observed, and no phenodeviants were noted. Thus it is unlikely that one or two loci were particularly involved, and it seems very likely that each phenodeviant represented a developmental accident.

Our experiments were carried out under conditions which Milkman (*1*) has indicated are unfavorable to the production of the cross-veinless phenotype. The incidence was actually very low: 0.00017 in the Oregon-R and Canton-S stocks (one sample in each stock). No cross-veinless phenotypes were found among 16,291 F_1's and F_2's examined. Thus, although the cross-veinless phenodeviants could and did appear in the inbred lines, they did not appear among the recombinants. If recombination were to produce this sort of variability, then we would have expected to find more cross-veinless phenotypes among the F_2's than among the parental stocks.

Studies by Dubinin, Dobzhansky, Spencer, and many others have already clearly shown the amazing variability of wild populations. We feel that studies based on such labile characteristics as wing venation anomalies are unlikely to provide more reliable estimates unless an effort is made to distinguish the variability assignable to developmental accidents. The experiment reported