Analysis of products of other T1 oligonucleotides digested with ribonuclease under these conditions indicates that cleavage occurs almost exclusively at pyrimidines followed by A (or G). There is some cleavage between two C's and almost none between C and U and two U's. This is consistent with work of Witzel (*11*); he showed that the relative velocity constants for ribonuclease digestion were: CpA, 270; UpA, 110; CpG, 45; CpC, 22; UpC, 3.6; CpU, 2.5; and UpU, 1.

Partial digestion with spleen phosphodiesterase (Worthington) showed that the 5′ terminus of oligonucleotide 14-6 is Cp. The only sequence compatible with the analyses, above, the genetic code and the known coat protein sequence is:

CpUpUpCpUp ApApCpUpUpUp
ApCpUpCp ApGp

To our knowledge this is the first instance of the isolation of an RNA fragment which encompasses a site of mutation and a known protein sequence (*12*).

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5. Abbreviations used are Gln, glutaminyl residue; C, cytidine; U, uridine; A, adenosine; G, guanosine; p, 2′ or 3′ phosphates of the above four nucleosides; DEAE, diethylaminoethyl; tris, tris(hydroxymethyl)aminomethane.
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12. There is a recent conference review [Correspondent, *Nature* **223**, 133 (1969)] of a remarkable experiment by J. A. Steitz (M.R.C. Laboratory of Molecular Biology, Cambridge, England) who reportedly has separated and sequenced R17 fragments (obtained by pancreatic ribonuclease digestion) corresponding to ribosome attachment sites on R17 RNA. One of her sequences, believed to be the coat-protein binding site, terminates in . . . GpCpUpUpCpUpApApCpUpUpUp identical with a portion of the sequence, above.
13. We thank Dr. J. J. Kelley for advice. This work was supported by NIH grants AI 01466 and AI 21,942.

4 August 1969

Physical and Chemical Studies on Ceruloplasmin: Crystallization of Desialized Human Ceruloplasmin Asialoceruloplasmin

Abstract. *Completely or partially desialized human ceruloplasmin crystallizes as blue hexagonal prisms terminating at both ends in shallow pyramids.*

When the terminal sialic acid residues from several of the carbohydrate chains of the plasma copper-protein, ceruloplasmin, are removed, there is an almost instantaneous disappearance of the infused desialized protein (*1*). Yet the blue color, copper content, oxidase activity, and immunochemical characteristics of asialoceruloplasmin and ceruloplasmin are identical, although such physical properties as electrophoretic mobility and solubility are altered by the loss of the sialic acid and its carboxyl groups. We now report that asialoceruloplasmin crystallizes as hexagonal prisms in contrast to the tetragonal prisms of ceruloplasmin (*2*).

Human ceruloplasmin (*2*) and neuraminidase from *Clostridium perfringens* (*3*), at respective concentrations of 7 percent and 0.06 unit/ml, or of 3 percent and 0.03 unit/ml, were incubated for 20 hours at 25°C and for 24 hours at 4°C, in an 0.10*M* sodium acetate buffer, *p*H 5.45, containing 1 percent NaCl (buffer A). The blue crystalline

Table 1. Quantitative comparison of some characteristics of ceruloplasmin and of asialoceruloplasmin. Analyses for protein concentration, sialic acid, copper, and enzymatic activity were carried out as in (*6*), except that the assay medium for enzymatic activity contained $1.5 \times 10^{-3}M$ $CaCl_2$ in addition to the other components. C, crystal. S, supernatant. Sialic acid is expressed as the percentage in protein. Purity is expressed as the ratios of absorbances (*A*) at 610 and 280 nm and of absorbance at 610 nm to copper (*6*).

Material analyzed	Incubation with neuraminidase (min)	Sialic acid (%)	A_{610}/A_{280}	$A_{610}/\mu g$ Cu	Enzymatic activity (ΔA_{530}/min × μg Cu)
		Pure crystalline ceruloplasmin			
Crystals	0	2.11	0.044	0.022	0.032
		Partially desialized ceruloplasmin			
Crystals	20	0.30	0.042	0.023	0.030
Supernatant	20	0.68	0.042	0.023	0.030
Crystals	60	0.40	0.040	0.021	0.031
Supernatant	60	0.43	0.043	0.022	0.032
		Asialoceruloplasmin			
Crystals	1200	0.00	0.042	0.022	0.034
Supernatant	1200		0.043	0.022	0.033

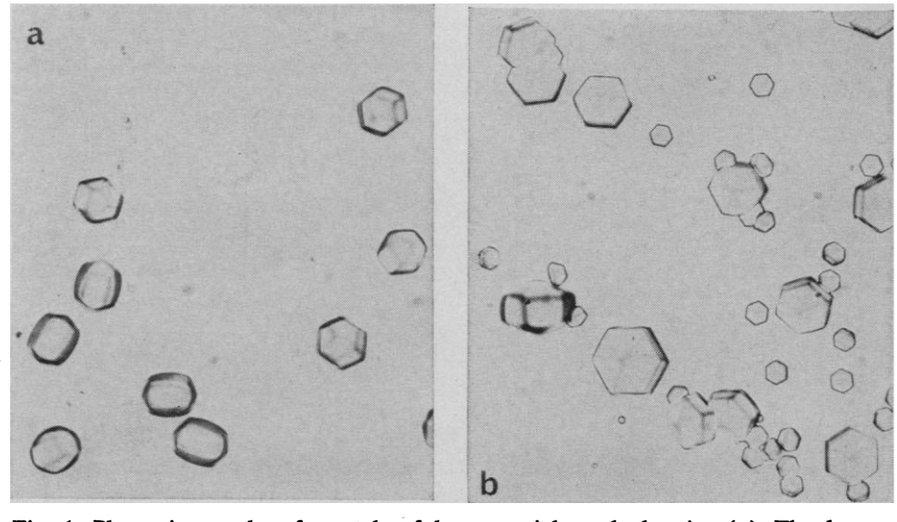

Fig. 1. Photomicrographs of crystals of human asialoceruloplasmin. (a) The longer prism sides of the crystals formed from 7 percent ceruloplasmin solution are evident when the crystals seen on edge at the left of the picture are compared to those seen on end at the right (× 280). (b) The short prism sides of the darkly outlined crystal at the left are characteristic of all crystals formed from 3 percent ceruloplasmin solution (× 250).

sediment, accounting for most of the protein present, was centrifuged and washed three times with cold buffer A. For chemical and physical analyses, the crystals were dissolved in 0.05*M* sodium phosphate buffer, *p*H 7.0, containing 1 percent NaCl.

The crystals of asialoceruloplasmin are anisotropic, blue, hexagonal prisms terminating at both ends in shallow pyramids. The prisms are longer in the crystals formed from 7 percent protein solutions than in those from 3 percent (Fig. 1). Their shape resembles that of crystals of cytochrome b_2 freed of DNA (*4*). The crystals may be reversibly decolorized by the addition of sodium ascorbate, and suspensions of the crystals in an 0.10*M* sodium acetate buffer, *p*H 5.0, catalyze the oxidation of *p*-phenylenediamine.

Crystals can also be obtained from partially desialized ceruloplasmin. After a shorter period of incubation, three volumes of an ethanol-chloroform mixture (9:1 by volume) are added to inactivate neuraminidase and to precipitate the protein which crystallizes after re-solution in buffer A (Table 1).

When neuraminidase from *Vibrio cholerae* (*5*) (250 unit/ml) and ceruloplasmin (3 percent) are incubated for 22 hours at 25°C in buffer A containing $2 \times 10^{-3}M$ $CaCl_2$, crystals are formed which are identical to those obtained after treatment with the *Cl. perfringens* enzyme. To our knowledge this is the first time a glycoprotein, freed of a portion of its carbohydrate, has been crystallized.

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16 June 1969

Polymorphism in an Inbreeding Population under Models Involving Underdominance

Abstract. *Models of selection favoring homozygotes over the heterozygotes and involving frequency-dependency in their competitive abilities were simulated in order to determine the conditions for maintaining stable polymorphism at a diallelic locus in large inbreeding populations. With heavier inbreeding, frequency-dependency could increasingly override the effects of underdominance in both pure stand and the competing ability components of fitness in terms of yielding stable nontrivial equilibriums. The significance of such selection models is discussed for the retention of variability in inbreeding populations with a minimum of segregational load and higher overall stability in contrast to the overdominance models.*

Many plant species undergo mixed selfing and random mating; of these the predominant selfers such as barley, lima bean, and wild oat have been investigated recently for the various factors maintaining genetic polymorphism in their populations (*1*). These studies have essentially explored the models involving overdominance (selection favoring heterozygotes) or the so-called marginal overdominance arising from various epistatic situations, genetic homeostasis, or disassortative mating. With overdominance, in particular for the case of predominant selfing, the polymorphic populations at equilibrium involve high segregational load and appear to have restrictive stability conditions in that the fate of polymorphisms is precariously tied with the fitness of a few heterozygous individuals in the population. Harding *et al.* (*2*) reported a case of frequency-dependent selection ensuring a constant hybridity level, although in this case overdominance itself was sufficient for maintaining the locus (*S, s*) polymorphic without frequency-dependency.

Lewontin (*3*) analyzed frequency-dependent selection with a general

Table 1. Notation and recurrence equations.

Genotypes	A_1A_1	A_1A_2	A_2A_2
Relative proportions of genotypes after selection in nth generation	$f_1^{(n)}$	$f_2^{(n)}$	$f_3^{(n)}$
Allelic frequencies	$p^{(n)} = f_1^{(n)} + \frac{1}{2}f_2^{(n)}$, $q^{(n)} = 1-p^{(n)}$		
Zygotic proportions prior to selection in $(n+1)$th generation	$z_1^{(n+1)} = s[f_1^{(n)} + \frac{1}{4}f_2^{(n)}] + tp^{(n)2}$ $z_2^{(n+1)} = \frac{1}{2}s[f_2^{(n)}] + 2tp^{(n)}q^{(n)}$ $z_3^{(n+1)} = s[f_3^{(n)} + \frac{1}{4}f_2^{(n)}] + tq^{(n)2}$		(1)
Relative proportions of genotypes after selection in $(n+1)$th generation	$f_1^{(n+1)} = [x + b_{12}z_2^{(n+1)} + b_{13}z_3^{(n+1)}]z_1^{(n+1)}/\overline{W}$, $f_2^{(n+1)} = [b_{21}z_1^{(n+1)} + 1 + b_{23}z_3^{(n+1)}]z_2^{(n+1)}/\overline{W}$, $f_3^{(n+1)} = [b_{31}z_1^{(n+1)} + b_{32}z_2^{(n+1)} + y]z_3^{(n+1)}/\overline{W}$		(2)

Table 2. Examples of equilibriums, and the parameters giving the genotypic composition and marginal fitnesses (W_i); (initially $p = 0.2$, $F = 0$).

s	x, y	N	Genotypic proportions			p	F	$\overline{W}_1$	$\overline{W}_2$	$\overline{W}_3$
			f_1	f_2	f_3					
0.0	0.4, 0.5	53	0.252	0.521	0.227	0.513	−0.042	0.704	0.760	0.697
	.5, .5	98	.479	.442	.079	.700	− .052	.670	.721	.601
	.6, .5	67	1.0	0	0	1.0	1.0			
	.4, .6	180	0.128	.421	.451	0.339	0.060	.843	.711	.779
	.5, .6	102	.345	.482	.174	.586	.008	.750	.741	.755
	.6, .6	46	1.0	0	0	1.0	1.0			
0.95	0.7, 1.1	66	0.692	0.016	0.292	0.700	0.962	0.923	0.508	0.935
	.8, 1.1	177	.935	.005	.060	.937	.959	.846	.502	.869
	.9, 1.1	83	1.0	0	0	1.0	1.0			
	.8, 1.3	89	0.468	.014	.518	0.475	0.972	1.192	.507	1.190
	.9, 1.3	85	.712	.012	.277	.718	.971	1.110	.506	1.128
	1.0, 1.3	288	.963	.002	.035	.964	.968	1.027	.501	1.060
	1.1, 1.3	60	1.0	0	0	1.0	1.0			