of Gibrel increased the losses in all but the instances mentioned; these increases may reflect increase in either numbers or physiological efficiency of microbes.

It had been found (*7*) that addition of Mo and Bo to wheat straw increased the rate of decomposition; these results indicate that Gibrel, in the presence of ample nutrients, further enhances the decomposition of various crop residues and industrial wastes containing celluloses and lignins. Practical use of Gibrel as an activator in composting awaits field trials.

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23 May 1966

Albumin Naskapi: A New Variant of Serum Albumin

Abstract. *An apparently new variant of human serum albumin, albumin Naskapi, has been found in high frequency in the Naskapi Indians of Quebec and, in lower frequency, in other North American Indians. The family and population data of the albumin are consistent with its inheritance as a simple autosomal trait controlled by a gene designated* Al Naskapi. *This gene is allelic with the gene* Al^A *which controls the common albumin. Both homozygotes and heterozygotes have been distinguished. This is the first report of a homozygote for an albumin variant.*

Rare electrophoretic variants of serum albumin have been reported several times since the original description of bisalbumin by Scheurlen (*1*). In nearly all of the reports, the rare variant has an electrophoretic mobility less than that of the common form of albumin (*1–4*), but two investigators reported fast-moving forms (*5, 6*). Family studies are consistent with simple autosomal inheritance. There have been no reports of individuals homozygous for the rare variants, and the bis forms are apparently heterozygotes.

All of the albumin variants are rare in the populations tested (for example, one per 1015 in Norway) (*2*), and apparently disease is not associated with the trait. However, increase in concentration of cholesterol is associated with bisalbumin, but this elevation is not statistically significant (*3, 5*).

We now report an inherited, fast-moving, electrophoretic variant of albumin which is different from at least one of the other fast-moving variants (*6*). The trait is common in several North American Indian tribes, but has not been seen in other populations tested. Several homozygotes for the variant have also been identified.

The original finding of the albumin variant, and the subsequent screening, was made with whole serum with Ashton's discontinuous buffer system at *p*H 8.6 (*7*). The gels were prepared from hydrolyzed starch (Connaught) at a concentration 25 percent higher than that recommended by the manufacturers. The electrophoresis was performed in a horizontal system with a constant voltage of 9 volt/cm. The discontinuous buffer system (*p*H 8.6) described by Poulik (*8*) and Ashton's acid buffer system (*p*H 5.6) (*9*) with a constant voltage of 16 volt/cm were also used.

Cellulose acetate electrophoresis was performed with a microzone electrophoresis system in barbital buffer at *p*H 8.6. Immunoelectrophoresis was done in 1 percent special agar-noble gel with a modified barbital buffer (*10*) and horse antiserum to human albumin. Barbital buffer (*p*H 8.6; ionic strength, 0.75) was used in the paper electrophoresis experiments.

The populations studied are shown in Table 1. In several cases, the blood specimens were used in other studies, and the populations are described in the indicated references. Blood was collected by venepuncture, and the serum or plasma was separated and stored at −20°C until tested. The dates of collection vary from 1958 for the Athabascans to 1962 for the Naskapi and Montagnais Indians.

The fast-moving variant can be distinguished by electrophoresis in starch gel with Ashton's discontinuous buffer, Ashton's acid buffer, or cellulose acetate. It can also be seen with Poulik's buffer, but only when the serum is diluted. Good separations were not obtained with barbiturate buffer with paper as a supporting medium. The three phenotypes can be easily distinguished (Fig. 1). In immunoelectrophoresis the heterozygote has a slightly elongated albumin band, and the new fast homozygote has a greater mobility than the common slow homozygote. However, antigenic differences between the two albumins are not seen. In Fig. 1, the new variant is compared to that described by Wieme (*6*); it is clearly different. The fast-moving variant described by Tarnoky and Lestas (*5*) was not available for comparison.

The distribution of the trait in several populations and the expected frequencies as calculated by the Hardy-Weinberg formula are shown in Table 1. Serums from 365 Haida Indians from Canada (*12*), 100 Quechua and 92 Cashinahua Indians from Peru (*13*). 443 Eskimos from Alaska (*12*), and 114 Americans of European descent were tested. None of these had albumin Naskapi. The results correlate well with the expected distribution. The gene frequency of the new variant in the Indian populations ranges from 0.13 in the Naskapi to 0 in the Haida and South American groups. None was seen

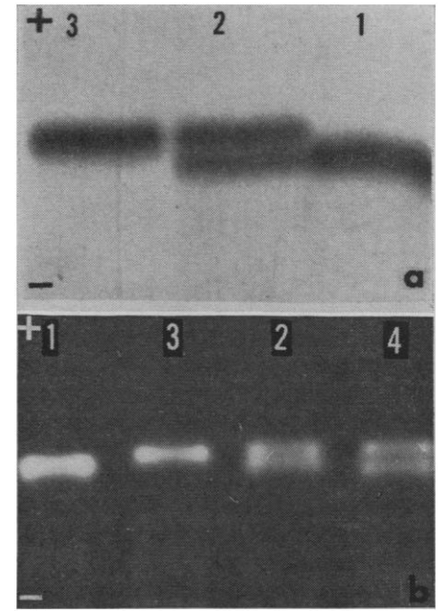

Fig. 1. Albumin variants. (a) Starch-gel electrophoresis in Ashton buffer. (b) Cellulose acetate electrophoresis (negative), Only the albumin areas are shown. (1) A/A; (2) Naskapi/A; (3) Naskapi/Naskapi, (4) Bisalbumin variant described by Wieme (*6*).

in the Eskimos or Caucasian Americans tested. Many of the smaller populations of Indians included related individuals, a fact which may affect the calculation of the gene frequency. Therefore, the frequencies given in Table 1 are only approximate. Three pedigrees in which the trait is segregating are shown in Fig. 2. The first (Naskapi) shows the segregation of the trait in three generations; the albumin types of all parents are known. In the second (Montagnais), a mother with both types of albumin has three children, of which one has only the common albumin, one has only the new variant, and the third has both forms. The third (Naskapi) shows a large family where the variant apparently has segregated to five of seven children in the oldest generation. A man in the second generation has only the new type, while his three children from two marriages have both types. An analysis of eleven Naskapi and Montagnais families (including some not in the pedigrees of Fig. 1) is shown in Table 2.

The albumin is different from the slow-moving variant and from the fast-moving albumin described by Wieme (*6*). It is proposed that this new variant be called albumin Naskapi, after the Indian population in which it was first discovered, and that the gene determining the trait be designated *Al Naskapi* (abbreviated Al^{Na}). The common albumin variant is designated albumin A (gene notation Al^{A}) and the originally described slow-moving variant, albumin B (gene notation Al^{B}). As new variants are discovered, it would be convenient to give them geographic, population, or other specific designations.

The segregation of *Al Naskapi* in the families is consistent with simple autosomal inheritance, with full expression of *Al Naskapi* and the alternate gene Al^{A} in the heterozygote. On the basis of the populations tested, albumin Naskapi appears to be restricted to Northern American Indians. The high frequency in the Naskapi could be associated with the inclusion of many family members in the sample tested. However, the Montagnais and other groups included mainly nonrelated individuals. This gene may serve as a useful marker for Northern Indian groups, as does the red blood cell Diego factor for other Indians.

None of the albumin variants described previously occurred with such high frequency in the general population. The high frequency of albumin Naskapi could be due to the operation of selective forces in the environment of these populations, or to genetic drift.

The Naskapi and Montagnais have a high frequency of two transferrin variants which are rare in most other populations (*14*). The transferrins are not found in any of the other Northern Indian groups, with the exception of the Tlingit, where they are found in very low frequency. If the high frequencies of albumin Naskapi in the Indians were due to simple gene mixture, and if selection were not operating on either of these protein polymorphic traits, then it would be expected that the transferrin variants would occur in the same Indian groups in which the albumin variant occurs. This was not found to be the case.

There appears to be a broad north-south cline for the frequency of albumin Naskapi which cuts across known ethnic relations of the Indians. This is the reverse of the cline for the genes which determine the Diego factor and haptoglobin-1, both of which occur with low frequency in North American tribes but in high frequency in Central and South American tribes.

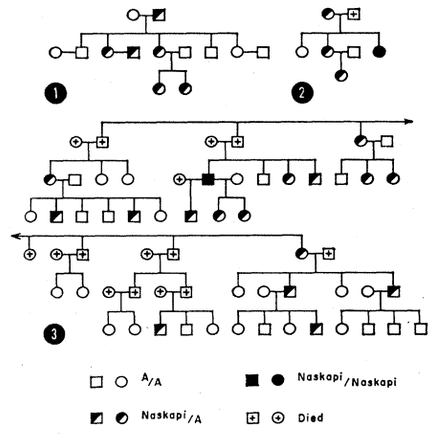

Fig. 2. Segregation of albumin Naskapi in two Naskapi families (genealogies 1 and 3) and one Montagnais family (genealogy 2.

In addition to the albumin Naskapi, we have found an albumin variant which occurs with high frequency in the Eskimo population of a small Indian-Eskimo village in Alaska. This variant may be different from albumin Naskapi, but additional specimens will be needed to complete the comparison. A slow-moving variant occurs with

Table 1. Distribution of albumin types in different populations.

Occurrence of phenotypes	Naskapi/ Naskapi (No.)	A/Naskapi (No.)	A/A (No.)	Gene frequency	
				Al^{Na}	Al^{A}
		Naskapi, Canada (11)			
Observed	1	37	113	0.130	0.870
Expected	2.6	34.1	114.3		
		Montagnais, Canada (11)			
Observed	2	14	96	.080	.920
Expected	0.6	16.5	94.8		
		Sioux, United States			
Observed	0	2	158	.007	.993
Expected	0	2.2	157.8		
		Athabascan, Alaska and Canada (12)			
Observed	1	11	218	.028	.972
Expected	0.2	12.4	217.4		
		Tlingit, Alaska (12)			
Observed	0	1	99	.005	.995
Expected	0	1	99		

Table 2. Segregation of albumin types in families.

Occurrence of phenotype	Albumin type of children		
	A/A (No.)	A/Naskapi (No.)	Naskapi/ Naskapi (No.)
	One family, A/A – Naskapi/Naskapi		
Observed	0	2	0
Expected	0	2	0
	Ten families, A/A – A/Naskapi		
Observed	17	15	0
Expected	16	16	0

relatively high frequency in Mexican Indians. Therefore, our findings indicate that albumin variants occur with greater frequency than previous evidence had indicated.

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Antibodies to Photoproducts of Deoxyribonucleic Acids Irradiated with Ultraviolet Light

Abstract. *A rabbit immunized with complexes of methylated bovine serum albumin and ultraviolet-irradiated DNA from calf thymus produced antibodies directed toward the photoproducts in the DNA. Serologic activity appeared after irradiation of DNA at 270 mμ and decreased upon irradiation at 235 mμ. The antigenic determinants of the ultraviolet-treated DNA appear to be photoproducts associated primarily with thymine, as measured by direct dependence of serologic activity on the adenine-thymine content of the DNA, and by inhibition of the serologic reaction by the irradiated di-, tri-, and tetra-(thymidine-5'-phosphate) nucleotides.*

Deoxyribonucleic acid (DNA) can be rendered immunogenic by formation of complexes with methylated bovine serum albumin (MBSA) (*1*). Rabbits, immunized with DNA that has been irradiated in the presence of methylene blue, oxygen, and tris buffer and complexed with MBSA, produce antibodies not only to the normal bases, but also to a specific product of photooxidation (*2*).

The characterization of the specific reaction of photoproduct with antibody to photoproduct was possible since the immune response to the photooxidation product was so strong that the activity of the antibodies to normal constituents of DNA could be eliminated by dilution of the antiserum. We now describe the preparation of antibodies directed toward lesions in DNA induced by ultraviolet light. Evidence is presented that the antigenic determinants, resulting from ultraviolet irradiation, are composed of thymine photoproducts.

For preparation of the immunogen, calf thymus DNA (Calbiochem; used without further purification) was denatured at a concentration of 125 μg/ml in a mixture of 0.15*M* NaCl and 0.015*M* sodium citrate, *p*H 7.4, for 10 minutes at 100°C, and quickly chilled. The denatured DNA was irradiated with 1×10^5 erg/mm^2 of monochromatic light at 270 mμ. A single rabbit was injected with MBSA complexes of the irradiated denatured DNA by the method of Plescia *et al.* (*1*). Native DNA from *Proteus vulgaris* was exposed to various dosages of ultraviolet light from a germicidal lamp and assayed by complement (C′) fixation (*3*) at a 1/2000 dilution of the antiserum (Fig. 1). No serologic activity was observed with either native or denatured unirradiated DNA at this dilution of antiserum, although the antibodies to the normal DNA bases could be measured with five to ten times more antiserum (depending on the DNA used). Serologic activity appeared after 15 to 30 seconds of irradiation at a distance of 5 cm and continued to increase with time of irradiation. There is neither an increase nor decrease of serologic activity after 105 seconds of exposure. After varying doses of ultraviolet irradiation, denatured DNA from *P. vulgaris* showed a somewhat faster increase in complement-fixing activity. The photoproducts, therefore, are formed in both double- and single-strand DNA although the rate of their formation appears to be influenced by the conformation of the DNA.

There is some controversy concerning the exact chemical nature of the photoproduct or products responsible for the biological effects of ultraviolet irradiation. Much evidence exists that thymine and cytosine may be sites of damage in the irradiated DNA (*4*). Since thymine dimers are formed more readily than cytosine dimers, and are more stable under physiological conditions than are cytosine hydrates, it was anticipated that antibodies would be directed primarily to the thymine photoproducts. Thus, the serologic activity of DNA exposed to ultraviolet irradiation would reflect the thymine content of the DNA, much as the ultraviolet sensitivity of bacteria reflects the thymine content of their DNA (*5*). When DNA's that varied in A+T (*6*) content from 62 to 28 percent were exposed to 1×10^5 erg/mm^2 of monochromatic light (270 mμ), the resulting serologic activity was highest with *P. vulgaris* DNA (62 percent of A+T) and lowest with *Micrococcus lysodeikticus* DNA (28 percent of A+T). DNA's with intermediate A+T contents (*7*) were intermediate with respect to serologic activities. Thus it appears that thymine photoproducts are part of the antigenic determinants of the irradiated DNA.

The dimerization of pyrimidines by ultraviolet irradiation is a reversible reaction, and the equilibrium between dimer formation and dimer splitting depends, in part, on the wavelength of irradiation (*4, 8*), monomerization being favored at short wavelengths, while dimerization is favored at longer wavelengths. Thus irradiation of DNA at 270 mμ should lead to increased serologic activity, while exposure of the