however, both a and c are larger in meionite than in marialite. If the curves of a and c versus composition for natural scapolites are extrapolated to the marialite and meionite compositions, a and c values are obtained which differ significantly from the values found for the synthetic end members. However, all of the cell dimensions obtained for the natural materials are within the range represented by synthetic marialite and meionite. When the cell volumes of scapolites are plotted as a function of increasing meionite content (Fig. 1c), it is seen that the volumes of the natural scapolites lie on or just below a line connecting the mean values for synthetic marialite and meionite. Apparently, the cell volume can be used to determine the composition of natural and synthetic scapolites. However, better calibration of the curve is desirable. Deviations of Shaw's natural scapolites from the straight line (Fig. 1c) do not appear to be related to departures in composition from the ideal marialite-meionite join as calculated by Shaw (*5*). Large deviations might be possible for scapolites containing more sulfate, bicarbonate, potassium, and other constituents.

The behavior of the cell dimensions may reflect (i) differences in structural state dependent on temperature, (ii) differences in structural state dependent on composition, or (iii) a combination of (i) and (ii). However, since no data are available for synthetic scapolites of intermediate composition, the possibility remains that the variations observed depend only on the composition. All of these explanations are consistent with the data presented here and with the structure proposed by Pauling (*1*). The similarities in composition between scapolite and plagioclase feldspar make it natural to suspect that differences in structural state, related to order-disorder phenomena, may also exist in scapolite. Syntheses of scapolites of intermediate composition as well as heating experiments are now under way to test this hypothesis (*7*).

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Additional Genetic Variation of Human Serum Transferrin

Abstract. A new molecular species of human transferrin, Tf $D_{Montreal}$, is described. Starch gel electrophoresis under high-voltage conditions permits the new variant to be clearly distinguished from previously described variants of closely similar mobilities. The presence of a faint iron-binding component which migrates slightly more rapidly than the principal transferrin with which it is associated is also described.

The technique of starch gel electrophoresis (*1*) has led to the discovery of an extensive genetically determined polymorphism of human and primate transferrins, the iron-binding globulins of the blood serum. Various authors have presented evidence for the inheritance of transferrin as an autosomal two-allele system without dominance. This evidence has been significantly increased by the recent report of Beckman (*2*) on the segregation of three transferrins in a single pedigree. At the present time, 13 different molecular species of human transferrin have been recognized (*3*); each of these transferrins is distinguishable by its characteristic mobility in starch gel electrophoresis. The most common molecular type is labeled transferrin C (Tf C) and is found in high frequency in all populations. Transferrins which are faster-moving electrophoretically than type C are labeled B, and slower-moving variants are named D. Subscripts are used to distinguish variants within the fast- and slow-moving categories. An interesting feature of the human transferrin polymorphism is the occurrence of particular transferrin variants in particular populations (*3*). Thus, transferrins B_{0-1} (Navajo) (*4*), B_2 (Caucasian) (*5*), D_{Chi} (Chinese) (*3*), and D_1 (Negro) (*6*) have each been observed in measurable gene frequency in their respective populations. The remaining variants have been found only in isolated individuals and their immediate families.

The purpose of the present report is to describe a new transferrin variant which has been observed in a Canadian individual of French and Irish descent. Examination of the serum by conventional vertical starch gel electrophoresis (*7*) revealed the individual to be heterozygous for transferrin C and a slower-moving transferrin variant. By means of a specially designed water-cooled apparatus, it has been possible to carry out the electrophoresis under conditions of relatively high voltage (*8*), thereby reducing the time required for a given separation of the protein components. By this method, the spread of the protein bands by diffusion is significantly reduced, and more precise comparisons of the relative mobilities of the proteins can be obtained. The increased sharpness of the bands also makes it possible to observe minor serum components whose presence is difficult to detect under conventional conditions. For example, under the high-voltage conditions, two components which migrate approximately in the ceruloplasmin position and four components unrelated to haptoglobin which migrate in the region of haptoglobin 1-1 can be clearly resolved in various sera. Differences among individual sera are also observed in the region between ceruloplasmin and albumin, where as many as five components can be identified; variations in these "post-albumins" have previously been described by Smithies (*7*), who has suggested that the improved resolution in this region may result in part from the variable potential gradient observed at the trailing edge of the albumin band (*8*).

In the present experiments vertical starch gel electrophoresis was carried out at 0°C in the borate buffer system of Smithies (*7*) for 5 hours at 20 volt/cm in a gel of 6 mm thickness; to facilitate heat transfer, the surface of the gel was covered with a thin cellophane film instead of petroleum jelly. Under these conditions transferrin C migrated approximately 5 inches from the origin. Iron-binding components were identified by autoradiography (*9*) and protein components were detected by the amido black stain.

Examination of the serum of the Canadian individual under these conditions revealed that the electrophoretic mobility of the slower-moving transferrin was slightly more rapid than

that of transferrin D_{Chi} (Fig. 1). The mobility of the transferrin variant was confirmed in three serum samples obtained at different times from the proband; the same variant was also identified in the serum of both the father and the sister of the individual. Incubation of the variant with various concentrations of the enzyme neuraminidase produced the characteristic stepwise pattern (*10*) in starch gel electrophoresis of four additional slower-moving, iron-binding components; at high enzyme concentrations, only the slowest-moving component was present. From this result it appears that the variant, like the nine other transferrins which have been examined (*11*), contains four sialic acid residues accessible to the enzyme.

The description by Harris *et al.* (*12*) of transferrin D_4 indicates that the present variant is distinctly slower-moving than D_4, since transferrins D_1, D_{Chi}, and the present variant are not easily resolved under conventional conditions of electrophoresis, whereas transferrin D_4 clearly migrates with a mobility approximately intermediate between transferrins C and D_1. It is therefore concluded that the slow-moving transferrin of the Canadian individual represents a new genetically determined transferrin variant. Unfortunately, no satisfactory nomenclature has yet been formulated for transferrin. The generally consistent practice (see Fig. 2) has been to assign subscripts to characterize the electrophoretic mobility of the variants; thus D_{0-1} was tentatively suggested as a possible designation for D_4. It is difficult to select a convenient descriptive symbol for the mobility of the variant in the Canadian individual, and it is therefore suggested that the new variant be named transferrin $D_{Montreal}$.

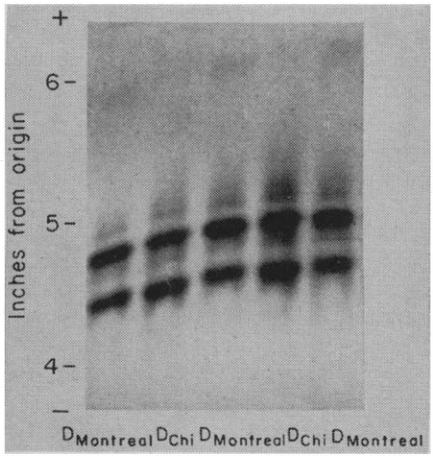

Fig. 1. Comparison of transferrins $CD_{Montreal}$ and CD_{Chi} by autoradiography after vertical starch gel electrophoresis. As shown, serum from the Canadian individual with the new variant $D_{Montreal}$ and serum from a Chinese individual heterozygous for the variant D_{Chi} occupied adjacent slots in the gel. Tf C is the faster-migrating and the D-type variant the slower-migrating of the two principal components. The faint iron-binding components are described in the text. The faint component migrating in the position of ceruloplasmin (6 inches from the origin) is not clearly seen in this photograph.

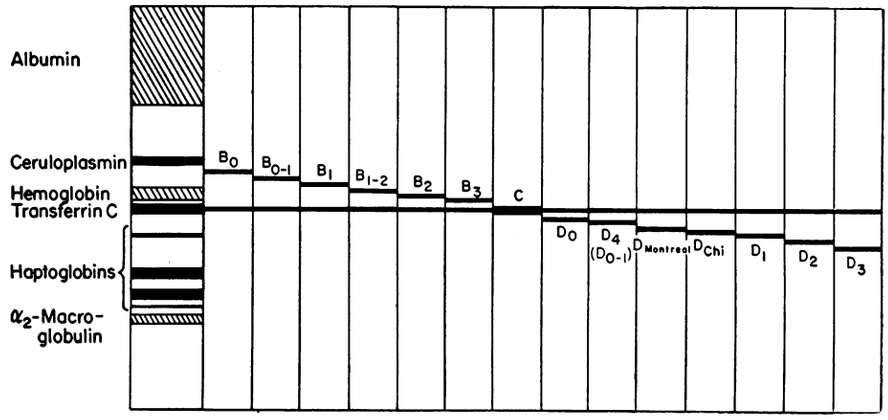

Fig. 2. Diagrammatic representation of the mobilities by starch gel electrophoresis of the 14 known human transferrin variants. Migration is toward the anode at the top of the diagram.

From Fig. 1 it is observed that in addition to the prominent bands representing transferrins C, D_{Chi}, and $D_{Montreal}$ there are also faint iron-binding components associated with each principal transferrin. These faint components have been consistently observed in all serum samples examined under high voltage and are present in both tris-citrate (*13*) and borate buffer systems; they do not appear to be related to the storage conditions of the sera or to the four faint, slower-moving components described in cord sera (*11*). The faint component which migrates slightly more rapidly than transferrin C has not previously been described. Under conventional conditions of electrophoresis, its presence is considerably blurred and difficult to detect; in the high-voltage system it can be clearly identified by autoradiography, and a corresponding protein band can be observed in the amido-black stain of the starch gel. In experiments in which the highest resolution was achieved, it was possible to distinguish by autoradiography two iron-binding components, one considerably less intense than the other, in the position of this more rapidly migrating faint component. The fast minor components have also been observed in association with transferrins B_{0-1}, B_2, and D_3; in samples in which transferrin C is absent, such as in sera from individuals of transferrin phenotypes B_2B_2 or D_1D_1, the faint components associated with Tf C are also absent.

A faint iron-binding component migrating more slowly than transferring C was first reported by Harris *et al.* (*14*) and has also been described by Boyer and Young (*15*). In certain sera in the present experiments, a second slower-moving faint component has also been observed. The significance of these faint fast and slow components is not known, although some possible interpretations have been discussed (*3*). The consistent association of these faint components with the principal transferrins suggests that they may be under the control of the transferrin locus. Multiple transferrins under the apparent control of a single allele have been described in cattle (*16*) and mice (*17*). Figure 1 also reveals the presence of a faint iron-binding component which migrates in the position of ceruloplasmin. The possibility that binding of iron to ceruloplasmin may occur in vivo is under investigation.

Figure 2 illustrates the relative electrophoretic mobilities of the 14 known human transferrin variants. On the basis of two-dimensional starch gel electrophoresis, Smithies and Connell (*18*) have suggested that the variants differ in charge rather than molecular size. Studies on the removal of sialic acid from transferrin by neuraminidase suggest that the difference in mobility between the fastest-moving variant B_0 and the slowest-moving variant D_3 may be represented by four charge units (*11*). It is therefore possible that each of the variants represents a single amino acid

substitution in the transferrin molecule. Previous experiments (*11*) have shown that the purified transferrins B_2, C, and D_1 have similar overall amino acid compositions, as determined by the method of Moore *et al.* (*19*) and Spackman *et al.* (*20*), and similar sedimentation properties in the ultracentrifuge. It has also been shown that transferrins B_{0-1}, B_1, B_2, C, D_1, and D_3 are immunologically identical (*11*). These observations, together with the similar sialic acid content of the ten variants which have been examined, are compatible with a single amino acid substitution as the basis of the variation in human transferrin. Of particular interest are the cluster of transferrin variants in the D_1 region. The five transferrins D_0, D_4, $D_{Montreal}$, D_{Chi}, and D_1 appear to differ from transferrin C by a single charge unit; the characteristic electrophoretic mobility of each variant suggests that the alteration in charge is expressed slightly differently in each case. The closely similar mobilities of these transferrins considerably strengthen the suggestion of Smithies and Connell (*18*) that starch gel electrophoresis is capable of resolving small differences in macromolecules at the level of expression of single charge units; it appears from the present experiments that under suitable conditions such resolution can be extremely fine.

The transferrin gene locus appears to be capable of numerous viable mutations, none of which has been associated with a clinical abnormality. It is essential that new variants, especially those occurring in relatively high frequency in particular populations, be carefully compared with known variants. In the absence of a common selective advantage or a chemical predisposition favoring a given mutation, it is unlikely that the same transferrin variant will occur in unrelated populations (*21*).

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Revision of Aleutian Prehistory

Abstract. Mongoloid skeletons of the Eskimo-Aleut stock, bone, stone and ivory artifacts, together with sea mammal, fish, bird, and invertebrate remains date to 1788 ± 180 B.C. at Chaluka, Umnak Island. Faunal composition and physical type of the human population present no appreciable changes for over 3000 years. Styles of artifacts change, but none indicate that ecological adaptation was affected.

New radiocarbon dates from the ancient village site of Chaluka, Umnak Island, in the eastern Aleutians, confirm an earlier radiocarbon date and locate the earliest recognizable appearance of Eskimo-Aleut people in association with their material culture and a large, varied series of faunal remains. Hrdlicka first recognized the importance of this site (*1*). He recovered many "pre-Aleut" skeletons here and estimated that the population arrived about the time of Christ. Three age measurements derived from charcoal specimens recovered in excavations of 1961 have been made by Isotopes, Inc. (Table 1). These figures are in excellent agreement with one determined by Libby (*2*) of 3018 ± 230 years; Libby used a sample from a nearby trench with equivalent stratigraphy less than 1 meter above the floor. The size of the Chaluka midden, some 215 m long, 61 m wide, and up to 6.8 m deep, reflects its antiquity. This depth has served to prevent contamination and disturbance. In an excavation at Krugloi Point, Agattu Island, western Aleutians, Spaulding (*3*) recovered a bottom specimen with an age of 2630 ± 300 years, suggesting that the westward migration might have taken as long as 1000 years.

Sixteen Paleo-Aleut skeletons from Chaluka (pre-Aleut of Hrdlicka) conform to Spaulding's basic description. They are Mongoloid, rather than American Indian; this is indicated by dental traits; dehiscences of the tympanic plate and mandibular and palatine tori; simple sutures; general cranial physiognomy; and short lower leg in comparison with upper leg. In contrast to the later Aleutian population (Neo-Aleuts), the Paleo-Aleuts have narrow, long heads. In some respects they resemble two groups, the much later Ipiutak people of Point Hope, described by Debetz (*4*), and the Paleo-Konyags of Kodiak. A lifespan longer than arctic Eskimos, which is evident in the later Aleuts, cannot yet be confirmed for this series, though there is presumptive evidence. One marked case of cranial hyperostosis in an adolescent establishes the presence of a hemolytic anemia. Aside from three diverse specimens in the Upper Cave, Chou Kou Tien, and the partial remains of Tzeyang man and Liu-chiang man, China, the Chaluka series appears to contain the oldest existing indubitable Mongoloids, and raises the important questions of rates of evolution, in general, and the recency of Mongoloids, in particular. Though the ultimate origin of these Mongoloids is Asiatic, there is ample biological, artifactual, and physiographic evidence that they migrated westward from the Alaska mainland. Failure to find older series on the mainland and in the Bering Strait region apparently reflects sampling deficiencies and also indicates that populations in the less favored areas were very small and widely spaced, in contrast to the Aleutians, where a higher frequency of closely spaced villages was supported by a lavish environment.

The artifactual remains consist of a variety of harpoon heads and spear heads (side prongs of the three-pronged bird spear included), stone lamps, chipped adze blades and whalebone wedges (indicative of a heavy woodworking industry essential for boats and houses), scrapers and gravers made on prismatic or lamellar blades, chipped stone semilunar knives, tanged knives, chipped stone end points for harpoon heads (one found embedded in a sea lion humerus), bolas, compound fish hooks, labrets, and bird bone and sea otter bone awls. Large stones (68 cm