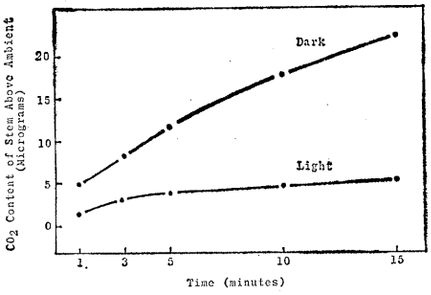

Fig. 2. Relative accumulation of carbon dioxide in the stem of *Mertensia ciliata* in the dark and light, as measured for 15 minutes. Zero, ambient carbon dioxide concentration (0.585 mg per liter).

dark by increasing the temperature of the root environment while holding stem temperature constant at 20°C. In the dark, the flux showed a straight-line relation with root temperature from 3.8 μg per minute at 19°C to 10.6 μg per minute at 38°C. Lowering the root temperature decreased the flux. These data indicate that a fair proportion of the carbon dioxide flux in a mature plant is coming from the roots or rhizomes, or both. To determine this proportion in a mature plant of *Mertensia*, the plant was sealed in the chamber (Fig. 1), and the flux through the intact stem was measured in the dark at 18°C as 6.2 μg per minute at a flow-rate of 0.2 liter per minute. The stem was then cut near the base and connected directly to the input air line, and the joint was sealed with silicone rubber. The flux originating in the stem proper was measured as 2.8 μg per minute. Input and output air lines were then connected to the stump and sealed, and flux measurements were made under the same conditions. The flux from the stump (therefore from the roots) was 3.4 μg per minute. These two figures add up to 6.2 μg per minute, the flux through the intact plant at the start. In this experiment, the root system contributed 55 percent of the flux, whereas the shoot contributed 45 percent. The proportions of the internal carbon dioxide flux undoubtedly vary with age of plant, temperatures, partial pressure of ambient carbon dioxide, and soil aeration.

During early growth conditions after snowmelt at high altitudes, stems of wet-meadow plants may be 1 or 2 dm high before leaf expansion. At this time, respiration rates are very high, and external photosynthesis is very low (*6*). Under these circumstances, most of the plant's photosynthesis is probably within the stem where the partial pressure of CO_2 is considerably above ambient and where temperatures are more favorable for photosynthesis. To measure internal stem temperatures under field conditions, a hypodermic-needle thermistor probe was inserted in the hollow stem of a young plant of *Delphinium barbeyi* emerging from a snowbank at 3100 m. For several days and nights, these internal temperatures were compared with those of the external air as measured by a shielded thermistor. In full sunlight, internal stem temperatures between 10 a.m. and 3 p.m. ranged from 30° to 37°C, while ambient air temperatures ranged from 13° to 16°C. When the shadow of a tree crossed the plant, the internal temperature dropped to that of the ambient air; at night, both temperatures dropped to freezing. In the laboratory, uptake of carbon dioxide within the stem decreased when the temperature of the air passing either through the stem or through the chamber was lowered. Since our field results indicate relatively high internal temperatures and an internal atmosphere rich in carbon dioxide, hollow stems apparently provide a favorable environment, similar to that of a greenhouse, for photosynthesis under low ambient temperatures and the low partial pressures of carbon dioxide at high altitudes.

The hollow stem has a structural advantage in rapidly growing plants of cold, high-altitude, wet meadows where the growing season is short. Apparently, it also has a physiological advantage in that some of the respiratory carbon dioxide may be used in photosynthesis within the stem, thus making more efficient use of carbon dioxide at high altitudes where the partial pressure of ambient carbon dioxide is relatively low (*5*). During early stages of growth after release from snow cover, root and stem respiration rates are relatively high even at low ambient temperatures. Under these conditions, internal stem photosynthesis may be the major photosynthetic system of the plant until the leaves are fully expanded and the weather becomes warm.

W. D. BILLINGS
P. J. GODFREY
Department of Botany, Duke University, Durham, North Carolina

References and Notes

1. A. Barthelemy, *Ann. Sci. Natur. Bot.* [5] **19**, 131 (1874); V. M. Conway, *New Phytol.* **36**, 64 (1937); M. H. van Raalte, *Ann Jard. Bot. Buitenzorg* **50**, 99 (1940); H. E. Laing, *Amer. J. Bot.* **27**, 861 (1940); D. A. Barber, M. Ebert, N. T. S. Evans, *J. Exp. Bot.* **13**, 397 (1962); J. M. Teal and J. W. Kanwisher, *ibid.* **17**, 355 (1966); W. T. Williams and D. A. Barber, *Symp. Soc. Exp. Biol.* **15**, 132 (1961); D. A. Barber, *J. Exp. Bot.* **12**, 243 (1961); K. B. Vallance and D. A. Coult, *ibid.* **2**, 212 (1951); D. A. Coult and K. B. Vallance, *ibid.* **9**, 384 (1958); H. van der Heide, B. M. de Boer-Bolt, M. H. van Raalte, *Acta Bot. Neerl.* **12**, 231 (1963); W. Armstrong, *Nature* **204**, 801 (1964); J. M. Teal and J. W. Kanwisher, *Limnol. Oceanogr.* **6**, 388 (1961); ———, *J. Exp. Bot.* **17**, 355 (1966).
2. R. T. Hartman and D. L. Brown, *Ecology* **48**, 252 (1967).
3. The infrared analyzer used for both laboratory and field measurements was a Beckman Instruments model 15, calibrated for 0 to 600 ppm of carbon dioxide. We thank J. J. Godfrey for assistance in the construction of the assimilation chamber.
4. Gross photosynthesis of whole plant represents net photosynthesis and dark respiration.
5. W. D. Billings, E. E. C. Clebsch, H. A. Mooney, *Science* **133**, 1834 (1961).
6. E. B. Hadley and L. C. Bliss, *Ecol. Monogr.* **34**, 331 (1964).
7. Supported by NSF grants GB-1219 and G-5501.

14 August 1967

Autosomal Linkage between the Albumin and Gc Loci in Humans

Abstract. *Naskapi and Montagnais families segregating for albumin Naskapi give evidence for close linkage of the* Gc *and albumin loci with a high probability. One possible case of crossover is included in the data.*

We have recently reported a "new" variant of serum albumin that occurs in North American Indians (*1*). This protein, which has been called albumin Naskapi, is inherited as a simple autosomal trait, and its presence is determined by the gene Al^{Na} that appears to be allelic with the gene Al^{A} that determines the presence of the common albumin (albumin A). The group specific substance system (Gc) was discovered by Hirschfeld (*2*); Gc is a protein that migrates in the alpha-globulin region on agar-gel and paper electrophoresis and in the post-albumin region on starch-gel electrophoresis. There are three common phenotypes (Gc 1-1, Gc 2-2, and Gc 1-2) that are controlled by two genes, Gc^1 and Gc^2, segregating at an autosomal locus. Other rare phenotypes are also known.

In this report, we present evidence for close linkage between the loci that determine these two inherited traits, using family material from the Naskapi and Montagnais Indians of the Labrador peninsula. A brief preliminary report has been published (*3*).

Naskapi and Montagnais Indians belong to the Algonquin language group.

Table 1. Studies on the linkage between the albumin and *Gc* loci and the albumin and *ABO* loci.

Recombination fraction θ	Albumin and *Gc* loci				Albumin and *ABO* loci	
	Families N1, N27, and M8		Families N1, N25, N27, and M8		Families N5, N58, N61, N63, N67, and N77	
	Total lod score	Antilog*	Total lod score	Antilog*	Total lod score	Antilog*
0.00	+3.9134	8193	$-\infty$	0	$-\infty$	0
.02	+3.7730	5929	+2.6673	464.8		
.04	+3.6298	4264	+2.8162	654.8		
.05	+3.5572	3608	+2.8360	685.5	−0.8408	0.1443
.06	+3.4865	3066	+2.8399	691.6		
.07	+3.4098	2569	+2.8255	668.8		
.10	+3.1820	1521	+2.7383	547.7	+ .0946	1.244
.15	+2.7870	612.4	+2.4950	312.6	+ .5220	3.327
.20	+2.3713	235.2	+2.1775	150.5	+ .6906	4.905
.25	+1.9357	86.24	+1.8107	64.68	+ .7296	5.365
.30	+1.4827	30.39	+1.4070	25.53	+ .6937	4.940
.35	+1.0182	10.42	+0.9772	9.488	+ .4340	2.716
.40	+0.5659	3.685	+ .5482	3.534	+ .4111	2.577
.45	+ .1706	1.505	+ .1666	1.468	+ .1867	1.537

* Relative probabilities of θ.

The population included in the study reside in adjacent communities near Schefferville, Province of Quebec, and have been described in other publications (*1*, *4*).

Albumin phenotypes were determined by starch-gel electrophoresis with the discontinuous buffer system (*1*); Gc types were determined by the method of Hirschfeld (*2*) with the use of agar-gel electrophoresis. Antiserums supplied by the Pasteur Institute were used in the preliminary typings, and questionable results were retyped with Behringwerke (Marburg) horse antiserum to Gc. Data were analyzed for linkage by the application of the lod scores of Morton (*5*) with the use of Smith's (*6*) method; data were also analyzed by Finney's method (*7*) and Penrose's sib-pair method as suggested by Mohr (*8*). The pedigrees used in the calculations are shown in Figs. 1 and 2, in which the albumin and Gc phenotypes are indicated. Families N1, N25, N27, and M47 ("complete" families) were analyzed by Smith's and Finney's methods. In the remaining families ("incomplete" families M10, M251, M11, N10, N21, and N55) only Penrose's sib-pair method was used. The results of the sib-pair method were combined with those of the Finney method for the complete families, as suggested by Mohr (*8*).

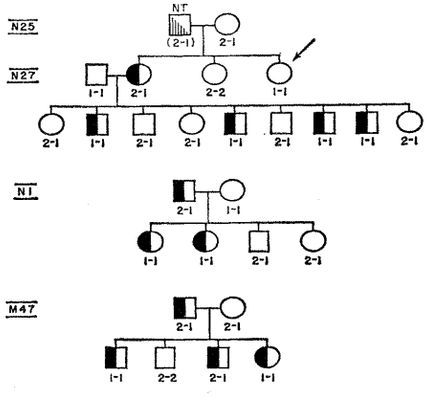

Fig. 1. Pedigrees of the "complete" families (N25, N27, N1, and M47). Males are represented by squares, females by circles. Filled squares or circles indicate albumin Naskapi homozygotes; half-filled squares or circles, albumin Naskapi/albumin A heterozygotes; and empty symbols, albumin A homozygotes. The Gc phenotypes are given under the symbols. Phenotype of the father in family N25 (NT) is inferred from the phenotypes of his offspring.

Phenotypes for the following systems were obtained for most of the family members: ABO, MNS, Rh, Lewis, Lutheran, P, Kell, Kidd, Duffy, haptoglobin, transferrin, Gc, Gm, phosphatase, Ag, and Lp. These were used to rule out illegitimacy. All the relations shown on the pedigree are consistent with the inheritance of these traits.

Inspection of the pedigrees indicates a high probability of linkage between the albumin and *Gc* loci. The phenotypes are all consistent with the segregation of Gc^1 with Al^{Na}. There is one possible crossover (Fig. 1, arrow, family N25) in which the Gc^1 gene apparently segregates with the Al^A gene of the father rather than his Al^{Na} gene. In this family, phenotypes of the dead father had to be assumed from the phenotypes of his offspring, including the daughter with the apparent crossover, which precludes the possibility of ruling out illegitimacy for this child. However, all phenotypes for the systems listed above for the child, its sibs, and mother are consistent with legitimacy. Linkage calculations were performed on the assumptions both that the crossover did occur (that is, including family N25) and that it did not occur (that is, excluding this family).

The results with Smith's method are shown in Table 1. When only three of the four complete families are included (that is, excluding family N25), the probability of linkage is $\Lambda/21+ = 0.9788$, and the probability of free recombination is this value subtracted from 1, that is, $21/21+\Lambda = 0.0212$. When all four of the complete families, including N25, are used, the probability of linkage is 0.9012, and the probability of free recombination is 0.0988. Figure 3 shows the probabilities of the recombination fractions (probability $\lambda\theta/S$). From this, the recombination frequency is less than 7 percent.

When Finney's method is used for families N1, N27, and M8 there is a linkage value of $1-4X = +1$ with a standard error of 0.1491, and the amount of information, $\kappa c = 45$. If

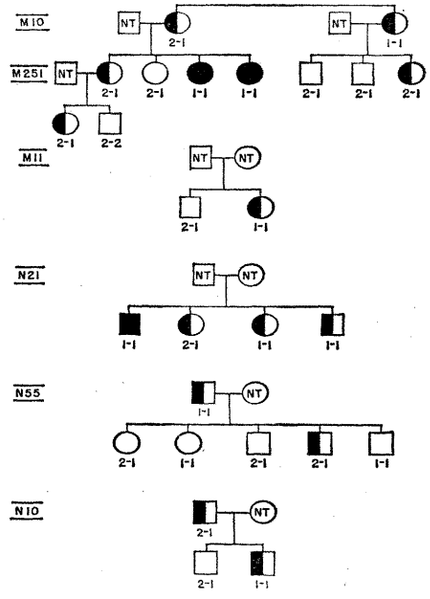

Fig. 2. Pedigrees of the "incomplete" families (M10, M251, M11, N21, N55, and N10). Males are represented by squares, females by circles. Filled squares or circles indicate albumin Naskapi homozygotes; half-filled squares or circles, albumin Naskapi/albumin A heterozygotes; and empty symbols, albumin A homozygotes. The Gc phenotypes are given under the symbols.

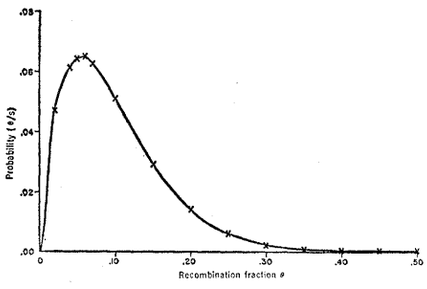

Fig. 3. Probalilities (θ/S) for the occurrence of values of the recombination fraction θ.

family N25 is included, the linkage value is +0.9565 with a standard error of 0.1474, and amount of information, $\kappa c = 46$. These values give a frequency of crossing-over of $c = 1.1$ percent.

When incomplete families M10, M251, M11, N10, N21, and N55 are treated by Penrose's sib-pair method and the results combined with those obtained by the Finney method, the results do not add to the probability estimate.

The probability of linkage between the albumin locus and the *ABO, MNS, Rh*, Lewis, *P*, Kidd, Duffy, haptoglobin, and transferrin loci in the appropriate families, including some not shown in Figs. 1 and 2, were tested by Smith's method. For the *ABO* locus there was a probability of free recombination of 0.8788. The lod scores are shown in Table 1. For all the other loci, the probability of free recombination was greater than 0.9545, except for Duffy, which was 0.9283.

Inspection of the pedigrees and the application of Smith's and Finney's methods give evidence for close linkage of the *Gc* and albumin loci with a very high probability. Weitkamp and Rucknagel (*9*) reported a high probability of close linkage between the *Gc* and albumin loci in three families that segregate for the slow-moving albumin (albumin B), which occurs as a rare trait in Europeans and Americans. They found a recombination frequency of less than 7 percent, which agrees closely with our results when family N25 was included. Even if family N25 is not included in our data, the possibility remains that the recombination frequencies for these two loci may be different in the Naskapi and Montagnais populations from those in the population studied by Weitkamp and Rucknagel.

In the populations we studied, the Gc^1 variant is always found with albumin Naskapi, whereas for many linked traits such association may not occur (*8*). In the Naskapi and Montagnais, the Gc 2-2 phenotype is very rare. There were 12 occurrences of the Gc 2-2 phenotype and 75 of the albumin Naskapi among the 330 individuals tested. The probability of these two phenotypes occurring together is less than 1 to 120, if one assumes that there is a random segregation of alleles at the two loci and that 12/330 and 75/330 are unbiased estimates of the frequency of genotypes *Gc 2-2* and albumin Naskapi, respectively. Most of the members of these Indian populations are closely or remotely related. More complete pedigrees will be published elsewhere (*10*).

Smith (*11*) noted that if linked genes are very close together, as these seem to be, there may be association because the mixing action of recombinations will not have sufficient opportunity to take effect. Boyer *et al.* (*12*) have pointed out that the nonequilibrium between coupling and repulsion phases can also be used to reckon the duration of coexistence of linked genes, if one knows the recombination frequency between loci, but the values could be misleading in the absence of extensive data.

There is a slight possibility that there might be linkage between albumin and *ABO* loci. If they are linked, the best estimate of the recombination frequency is 25 percent. The possible linkage of the *ABO* locus with that for xeroderma pigmentosum (*13*) and with the *Gc*-albumin linkage, and its definite linkage with the locus for the nail-patella syndrome (*14*), would make the chromosome involved the best-mapped human autosome.

E. KAARSALO
LIISA MELARTIN
B. S. BLUMBERG

Institute for Cancer Research, Fox Chase, Philadelphia, Pennsylvania 19111

References and Notes

1. L. Melartin and B. S. Blumberg, *Science* **153**, 1664 (1966).
2. J. Hirshchfeld, *Sci. Tools* **7**, 18 (1960).
3. B. S. Blumberg, E. Kaarsalo, L. Melartin, *Clin. Res.* **14**, 482 (1966).
4. B. S. Blumberg, J. R. Martin, F. M. Allen, J. L. Weiner, E. M. Vitagliano, A. Cooke, *Human Biol.* **36**, 263 (1964).
5. N. E. Morton, *Amer. J. Human Genet.* **7**, 277 (1955).
6. C. A. B. Smith, *ibid.* **11**, 289 (1959).
7. D. J. Finney, *Ann. Eugen.* **10**, 171 (1940).
8. J. Mohr, *A Study of Linkage in Man* (Munksgaard, Copenhagen, 1954).
9. L. R. Weitkamp, D. L. Rucknagel, H. Gershowitz, *Amer. J. Human Genet.* **18**, 599 (1966).
10. L. Melartin, in preparation.
11. C. A. B. Smith, *Royal Stat. Soc. Ser. B* **15**, 153 (1953).
12. S. H. Boyer, D. L. Rucknagel, D. J. Weatherall, E. J. Watson-Williams, *Amer. J. Human Genet.* **15**, 438 (1963).
13. H. El-Hefnawr, S. Maynard Smith, L. Penrose, *Ann. Human Genet.* **28**, 273 (1965).
14. J. H. Renwick and S. D. Lawler, *ibid.* **19**, 312 (1955).
15. Supported by PHS grants CA 06551 and CA 08069 and by a special grant from the M. Dorrance Strawbridge Foundation. We are indebted to R. J. Martin, A. Cooke, J. Cooke, the Early Sites Foundation, and M. Savoie for assistance with our initial field study.

20 July 1967

Histocompatibility Antigen Transfer in Utero: Tolerance in Progeny and Sensitization in Mother

Abstract. *Subcellular antigens obtained from donor spleen and kidney were administered to pregnant rabbits treated with hyaluronidase. Partial immunological tolerance to the donor was thus induced in the fetuses during the adaptive phase. The duration of tolerance was proportional to the total dose of antigen administered to the mother. Maternal sensitization to the offspring was noted in all rabbits giving birth to partially tolerant progeny.*

Hyaluronidase has been shown to alter the barrier between mother and fetus, permit entry of maternal antigen into the immunologically immature fetus, and thus induce varying degrees of tolerance (*1*). In these studies, the effects of early exposure of the fetus to foreign (nonmaternal) antigens were studied. In the New Zealand albino rabbit, the adaptive period lasts until the 22nd day of gestation (*2*); therefore, the donor antigen was administered to the mother prior to this time. Six doses were given intravenously (representing a total of 175 mg dry weight of tissue), with 10,000 turbidity-reducing units of hyaluronidase each time. Injections were administered three times a week, during the 2nd and 3rd weeks of gestation. The antigen was prepared from disrupted spleen and kidney by methods previously described (*3*). The offspring were grafted at 3 weeks of age with full-thickness skin (1 by 1