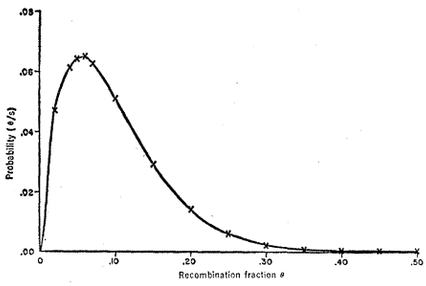

Fig. 3. Probalilities (θ/S) for the occurrence of values of the recombination fraction θ.

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.

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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).
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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

Table 1. Comparison of skin graft survival (in days) from control, mother, and donor.

Family	Litter size	Days of survival				
		Control	Mother	*P*	Donor	*P*
1	8	6.1 ± 1.0	9.0 ± 1.8	< .01	10.8 ± 2.8	< .01
8	9	7.4 ± 1.8	11.4 ± 2.4	< .05	11.7 ± 2.6	< .01
17	3	11.0	12.0		11.0	
55	5	11.0	12.2		14.4	< .02
60	7	9.7 ± 0.5	10.3 ± 1.3	< .1	15.0 ± 2.4	< .01
67	7	8.8 ± 1.0	11.2 ± 1.3	< .01	16.6 ± 2.1	< .01

cm) on the dorsal aspect of the ear. Grafts were considered to have been rejected when capillary flow stopped and when induration and more than 75 percent epidermal necrosis appeared. Prolongation of the survival of skin homografts taken from the antigen donor was taken to indicate the maternal-fetal transfer of antigen under the influence of hyaluronidase. In view of the fact that the hyaluronidase was administered during the adaptive phase, maternal antigen might also be expected to be transmitted to the fetus. Therefore, skin homografts from both the mother and the donor of the antigen were placed on the progeny of treated mothers, along with unrelated, control grafts. Antigen nitrogen was measured by the micro-Kjeldahl technique, based on wet weight. Large discrepancies in graft survival, presumably due to different histocompatibility relationships, made it necessary to evaluate each litter individually and to correlate the altered graft survival by means of an index. This expresses the ratio of the length of survival of experimental to control grafts.

Table 2. Dose effect on skin graft survival.

Antigen source (4 litters per group)	Amount given (as dry weight of tissue) (mg)	(D - C)/(M - C)*
Spleen	120–150	0–1.0
Spleen	180	1.6–3
Spleen and kidney	200–220	2.8–9

* Survival of donor skin — survival of control skin/survival of maternal skin—survival of control skin.

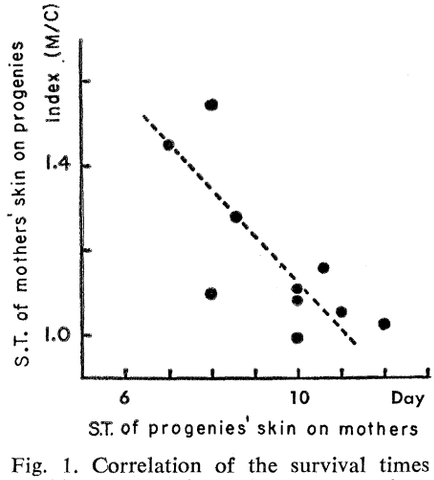

Fig. 1. Correlation of the survival times of skin grafts exchanged between mothers and progenies in ten litters in which immunological tolerance was induced by maternal route. *S.T.*, survival time. *Index (M/C)*:S.T. of mothers' skin/S.T. of controls' skin (on progeny).

Table 1 shows the survival times of skin grafts taken from the control, the mother, and the antigen donor in each litter. Comparison of the survival times of grafts in each litter reveals that the antigen donor's skin showed the longest survival, which was statistically significant in five of six families. The survival time of the mother's skin was not quite so long as that of the donor, but exceeded that of the control. We believe that this effect on the mother's skin was caused by the transmission of maternal antigens into the fetus. When donor antigen was injected without hyaluronidase in four litters, there was no difference in the survival time of skin taken from the mother, the control, or the donor. The specific prolongation of the skin taken from the antigen donor is thought to be the result of immunological tolerance brought about by maternal-fetal transmission of the administered subcellular antigen during the adaptive period.

Because only temporary tolerance was produced, the protocol was modified to administer increasing doses of antigen to the mother. In addition to spleen, one of the donor's kidneys was removed and used as an additional antigen source. Four litters were studied at each dosage. Table 2 illustrates the relation between the antigen dose given to the mother and graft survival in the offspring. The correlation coefficient is 0.83, indicating definite parallel correlation between the amount of antigen administered and the survival time of the donor graft. This is apparently a truly dose-related phenomenon.

In reciprocal grafts (from offspring to mother) in ten litters (Fig. 1), the longer the maternal graft survived on the progeny, the earlier the progeny's skin was rejected by the mother. The correlation coefficient between the survival of grafts from mother to offspring and from offspring to mother was −0.9. While maternal antigens passed into the fetus, fetal antigens also apparently crossed in the reverse direction in large enough amounts to sensitize the mother.

Bradbury *et al.* described a fibrinoid layer between maternal and fetal cells in the placentas of mice (*4*). In regard to the transmission of antigen, this layer has been credited with being the barrier which separates mother from offspring. Simmons has questioned these observations (*5*). In view of this conflict, glutaraldehyde-fixed portions of the junctional region of placentas from normal rabbits, and those treated with hyaluronidase, were studied by electron microscope. There was prominent deposition of fibrinoid substance in the intercellular area of the trophoblastic cell layer. However, in no instance could the presence of a fibrinoid layer on the trophoblastic microvilli be shown, nor were there demonstrable differences in the ultrastructure of control and treated placentas. These observations, although made in a different species, cast further doubt upon the existence of a physically isolating layer between maternal and trophoblastic cells.

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References and Notes

1. P. Nathan, E. Gonzalez, B. F. Miller, *Nature* **188**, 77 (1960); J. S. Najarian and F. J. Dixon, *Proc. Soc. Exp. Biol. Med.* **112**, 136 (1963).
2. K. A. Porter, *Nature* **185**, 789 (1960).
3. N. A. Halasz, L. N. Seifert, H. A. Rosenfield, M. J. Orloff, H. A. Stier, *Proc. Soc. Exp. Biol. Med.* **123**, 924 (1966).
4. S. Bradbury, W. D. Billington, D. R. S. Kirby, *J. Roy. Microscop. Soc.* **84**, 199 (1965).
5. R. L. Simmons, *Fed. Proc.* **25**, 940 (1966).

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