It may be no accident that the only infrared-excess object in the field, object 29, with J − R = 2.69, R − I = 2.92, and I = 18.10 magnitude, is close to a probable lens position. It is clearly seen in Figs. 1 and 2. Its colors are less consistent with an elliptical galaxy (z = 0.8 to 1) than with an M6 (16th absolute magnitude at 550 nm) red dwarf star, and its R image looks stellar. The presence of an 18th I magnitude dwarf star in this field of 6 × 8 arc min is moderately unlikely: over the range of stellar luminosity functions compatible with current survey data, we expect between 0.01 and 0.1 M6 or later star in this field brighter than 18th I magnitude. If it were an Infrared Astronomical Satellite (IRAS) galaxy like Arp 220 (10^{12} solar luminosities), at z = 0.8 to 1, with a mass-to-luminosity ratio of 100, it would escape detection in the IRAS catalog. No IRAS source is seen at that location. Spectroscopic studies of object 29 in the red, to examine the possibility that it is an accidental foreground M dwarf star, are warranted. Recent JHK infrared photometry (*12*) confirms it as an M dwarf.

Given that 1146+111B,C are dual images of the same QSO, we reach the following conclusions about the gravitational light-deflecting object. Overall, our data on the 1146+111 field are less consistent with simple string and single massive black hole models than with the idea of an isothermal or slightly smoother mass distribution in a foreground lensing object. It is possible that this dark matter is lumpy, that is, not spherically symmetric as assumed in the lens models, giving rise to multiple images, some demagnified, of many background QSO's in the field. This idea would be supported by the discovery that objects 14, 15, and 31 are QSO's but not images of BC, D, or E.

However, the possibility must be considered that B and C are spectrally similar but physically separate QSO's. By far the simplest explanation for our data would be the seemingly unlikely possibility that B and C are separate QSO's. Recent blue spectra of B and C (*13*) show differing CIII line emission in the two QSO images. Although this difference might be attributable to rather contrived changes in the parent QSO emission over perhaps hundreds of years, together with corresponding differences in the travel time of light in the lens or string models, it will be difficult to make such models fit all the present color, intensity, position, and spectroscopic data.

REFERENCES AND NOTES

1. E. L. Turner *et al.*, *Nature (London)* **321**, 142 (1986).
2. B. Paczynski, *ibid.* **319**, 567 (1986).
3. C. Hazard, H. Arp, D. C. Morton, *ibid.* **282**, 271 (1979).
4. P. Seitzer, J. A. Tyson, H. Butcher, in preparation.
5. J. A. Tyson, P. Seitzer, R. J. Weymann, C. Foltz, *Astron. J.* **91**, 1274 (1986).
6. H. Arp and C. Hazard, *Astrophys. J.* **240**, 726 (1980).
7. A. Vilenkin, *ibid.* **282**, L51 (1984).
8. J. R. Gott, *ibid.* **288**, 422 (1985).
9. ———, *Nature (London)* **321**, 420 (1986).
10. A. A. Stark, M. Dragovan, R. W. Wilson, J. R. Gott, *ibid.*, in press.
11. J. P. Ostriker and E. T. Vishniac, *ibid.*, in press.
12. H. Thronson, *Int. Astron. Union Circ. 4227* (1986).
13. J. P. Huchra, *Nature (London)*, in press.
14. P. C. Boeshaar and J. A. Tyson, *Astrophys. J.* **90**, 817 (1985).

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Females' Choice of "Good Genotypes" as Mates Is Promoted by an Insect Mating System

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Can animal mating systems result in the choice of mates carrying genotypes that are otherwise favored by natural selection? This question is addressed by studying, in natural populations of *Colias* butterflies, how the phosphoglucose isomerase (PGI) enzyme genotype of males mating *Colias* females varies with degree of female mate discrimination. Certain PGI genotypes (as predicted from their biochemical properties) have been found previously to have an advantage in diverse fitness-related properties: flight capacity, survivorship, and overall mating success. It is shown here that males of these same genotypes have even greater advantage in remating older, more discriminating females than they do in mating previously unmated, less discriminating females. Assortative mating is not found and thus cannot explain this effect. The mating system of these insects does, at least in this case, result in active female choice of generally favorable male genotypes as mates.

A QUESTION OF INTEREST IN EVOLUTIONARY biology is whether sexual selection can drive the evolution of generally adaptive characteristics or only the evolution of those features directly useful in securing mates (*1–5*). Do mating systems evolve so that animals choose mates carrying the most fit genotypes, whose offspring are likely to be generally fit? Does mate selection by one sex mainly concern display or recognition characteristics of the other sex? Or, is evolutionary reality a mixture of these extremes? Selection for male display characteristics, whether mediated by male-male competition, female choice, or a mixture of these, is well documented (*5*). Mate selection for generally adaptive phenotypes has been found in several systems (*2, 3, 6*), but evidence that generally adaptive genotypes are thus favored is rare (for example, *3, 4, 7*). This rarity may stem from the fact that the causes of fitness differences, or of differences in fitness components, have seldom been traced to alternative genotypes of any specific loci (*8*).

Pierid butterflies, especially the genus *Colias* (the "Sulfurs"), are now widely used as a model system for study of evolutionary processes, including sexual selection (*9–13*). *Colias'* mating is governed by a males-find-and-court, females-choose system (*11–13*). Courtship signals used are visual patterns, short-range pheromones, and male courtship flight and "wing-flicking" (*12–15*). Discrimination in acceptance or rejection of potential mates, on the basis of these signals, by an unmated female increases dramatically during the first hour of adult life (*11*), and in the laboratory (*16*) continues to do so for 24 hours. This choice does not depend on male-male competition but is exercised with respect to individual males. Once mated, females become, for several days, refractory to further courtship. In *Colias'* relative *Pieris*, this results from distension of the female bursa copulatrix, caused by the spermatophore that the male deposits there during copulation; hormonal cues may also be involved (*17*). The spermatophore carries both sperm and nutrients that may be used by the female (*18, 19*). Resistance to remating later declines, and females remate up to three times in the wild (*14*). While a male may need as little as 5 seconds to court a young unmated female successfully (*11*), a male's persistence in courtship flight does on average increase his chance of mating an unmated female in the field (*20*). Male persistence is crucial to successful remating

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of a previously mated female in the laboratory (*16, 19, 21*).

Adult fitness-related activities, including finding and courting of mates, depend on effective flight (*10, 22, 23*). Flight in turn depends on a high and narrow range of body temperatures; much attention has therefore been paid to the thermoregulation of these insects (*10, 23*). This embedding of measures of fitness in a known physiological-ecological context has led to understanding of natural genetic polymorphisms in *Colias*' glycolytic enzymes (*22, 24*), and thus to our present study of mating success in one such polymorphism.

Glycolysis begins the reaction sequence supplying carbohydrate-derived adenosine 5′-triphosphate (ATP) fuel to these insects' flight muscles. In lowland *Colias* populations, four alleles (labeled 2, 3, 4, and 5) are common (have frequencies >0.05) at the phosphoglucose isomerase (PGI) enzyme locus in glycolysis (*22*). Among the ten genotypes made up of these alleles, there are major differences in enzyme kinetics and thermal stability (*22*). These data, combined with information on the thermal ecology of flight, led us to predict which genotypes would maintain fuel supply to flight most effectively under natural thermal and environmental conditions, and thus to predict genotypic differences in the wild in flight capacity, allele frequency differences as functions of habitat temperature, and genotypic differences in survivorship and fecundity (*22*). Several of the predicted effects had already been found in natural populations (*22*). Others were confirmed by new study of existing data (*22*) or by de novo field experiments (*25, 26*). All eight predictions tested thus far have been confirmed.

Table 1. Overall comparison of PGI genotypes between males flying with females in samples and males successfully mating with those females of *Colias* genus, *C. eurytheme* and *C. philodice eriphyle*, at two locations near Tracy, California, and two in Colorado. x* = Goldstein's approximate normal deviate for testing differences of two percentages (*31*). The tests are one-tailed, as they test a specific prediction of genotypic overrepresentation among successfully mating males (*26*).

Species and sample	Frequency of kinetically favored males: Flying	Frequency of kinetically favored males: Mating	x*	*P*
C. eurytheme				
Tracy-MB, Sept. 1984	0.404 (19 of 47)	0.774 (48 of 62)	−3.93	<0.0001
Tracy-PP, Sept. 1985	0.520 (52 of 100)	0.673 (33 of 49)	−1.78	0.038
C. p. eriphyle				
Olathe totals,* July–Aug. 1984	0.520 (167 of 321)	0.738 (62 of 84)	−3.59	0.0002
Gunnison, July 1985	0.555 (65 of 117)	0.852 (52 of 61)	−3.96	<0.0001

*A series of subsamples of the Olathe population were taken, all at the same times of day under optimal thermal conditions (*30*) in late July and early August 1984. This population showed overlapping-generation demography throughout this period; all subsamples show similar allele and genotype frequencies, with no significant heterogeneity by Goldstein's or *G* tests (*31*). Hence, they have been pooled for this analysis.

Table 2. Analysis of mating success of male *Colias* PGI genotypes versus female mating number (number of times mated) and population location (two locations near Tracy). The multiway *G* test (*31*) shows that kinetically favored males are, compared to other males, even more successful in mating experienced females than inexperienced females. There is no significant difference in this effect among populations sampled, though there are differences among samples in extent of multiple mating.

Species and sample	Male genotype	Male mating success for mating of female: 1	2	3	4	All
C. eurytheme						
Tracy-MB, Sept. 1984	Kinetically favored	26	18	4	0	48
	Other	12	1	1	0	14
Tracy-PP, Sept. 1985	Kinetically favored	21	7	4	1	33
	Other	15	1	0	0	16
C. p. eriphyle						
Olathe, July–Aug. 1984	Kinetically favored	51	8	2	1	62
	Other	21	0	1	0	22
Gunnison, July 1985	Kinetically favored	41	11	0	0	52
	Other	8	1	0	0	9

Three-way *G* test

	G	df	*P*
Overall homogeneity	47.55	24	<0.005
♂ genotype by mating number	14.16	3	<0.005
♂ genotype by location	6.29	3	~0.15
Mating number by location	22.82	9	<0.01
Three-way interaction	5.23	9	>0.80

We now study the role of female choice in PGI genotype–specific male mating success, contrasting the genotypes of males that mate females first, when females are young and exercising minimal discrimination, with those of males that remate females, when females are older and more choosy, as already discussed. Since *Colias* females never fully absorb spermatophores as do some other butterflies (*14, 18*), the number of spermatophores carried by a female accurately reflects the number of times she has mated (*14, 27*). Sperm precedence is absolute in *Colias*' remating; eggs laid by a female, though she has mated repeatedly, all have the most recent mate as father (*19*). PGI genotypes were scored by electrophoresis (*22*). The genotypes of the males in each population sample estimate PGI genotype frequencies among the viable males available to females as potential mates. We collected eggs from each female in each population sample, then dissected each female and counted her spermatophores to determine the number of times she had mated, then scored her PGI genotype. We then raised and scored enough of each female's progeny to determine her most recent mate's genotype (*26*). These data estimate PGI genotype frequencies among successfully mating males, for comparison to estimated frequencies among other viable males.

Males of kinetically favored PGI genotypes show greater flight capacity [(*22, 25*); for brief discussion of which genotypes are kinetically favored, and why, see (*28*)]. If, as we predict, males with favored PGI genotypes can therefore sustain courtship more effectively, then (except in very low density populations not studied here) we should see not only overall mating advantage of favored male genotypes as before, but a further increase in mating success of those genotypes in females' second and later matings. We can also distinguish active female choice from general assortative mating, by testing for association between females' PGI genotypes and those of their mates.

Populations of two "semispecies" (*11, 29*) were studied: *C. eurytheme* Boisduval at two locations near Tracy, California (elevation, 50 m), and *C. philodice eriphyle* Edwards at Olathe, Colorado (elevation, 1700 m), and

Table 3. Test of assortative mating among *Colias* PGI genotypes. Goldstein's test (x*) (*31*) was used to test percentages of kinetically favored males mating all females versus those mating only females of kinetically favored genotype. There is no predicted direction of assortative mating, so tests are two-tailed. Assortative mating is not found, and thus cannot account for observed mating success differences among male PGI genotypes.

Species and sample	Female genotype	Kinetically favored males		x*	P
		Counts, total	%		
C. eurytheme					
Tracy-MB,	All	48 of 62	77.4		
Sept. 1984	Kinetically favored	22 of 28	78.6	−0.122	0.90
Tracy-PP,	All	32 of 49	65.3		
Sept. 1985	Kinetically favored	15 of 24	62.5	+0.235	0.81
C. p. eriphyle					
Olathe, July–Aug. 1984	All	62 of 84	73.8		
	Kinetically favored	39 of 57	68.4	+0.697	0.38
Gunnison,	All	52 of 61	85.2		
July 1985	Kinetically favored	30 of 34	88.2	−0.406	0.68
Pooled samples					
All females	All	194 of 256	75.8		
	Kinetically favored	106 of 143	74.1	+0.367	0.72
Females with	All	138 of 195	70.8		
one mating	Kinetically favored	79 of 113	69.9	+0.159	0.87
Females with	All	56 of 61	91.8		
>1 mating	Kinetically favored	27 of 30	90.0	+0.286	0.77

Gunnison, Colorado (elevation, 2350 m). Samples were taken at peak flight density, with the insects flying in their thermal optimum (compare *25*). The use of two related taxa allowed us to study the same PGI genotypes on different, naturally variable genetic backgrounds, but in otherwise similar conditions (*30*). All standard techniques were carried out as before (*26*).

As before (*26*), males of kinetically favored PGI genotypes succeed in mating females at a higher frequency than do males of the other PGI genotypes flying with those females (Table 1).

Among the four samples, there is a highly significant increase in frequency of favored male genotypes among males that remate older females (Table 2). This increase does not differ significantly among samples (and therefore not between taxa), though samples vary in the proportion of multiple matings. Thus the samples can be pooled for further analysis. By Goldstein's test for differences between two percentages by approximate normal deviate (x*) [(*31*); one-tailed, since we are testing specific predictions], over all samples, the female's second and later matings (mating numbers >1) show an even higher proportion of kinetically favored genotypes as mates (56 out of 61, 91.8%) than does her first mating, mating 1 (138 out of 195, 70.8%; x* = −3.35, $P = 0.0004$). Results for matings 2, 3, and 4 are similar: proportions of kinetically favored male mates for second matings (44 out of 47, 93.6%) do not differ significantly from third and fourth matings combined (12 out of 14, 85.7%; x* = +0.95, $P = 0.34$). Such a threshold effect is expected from the pattern of increase of discrimination with female age.

There is no assortative mating detectable in these data (Table 3). Thus, Table 2 shows the action of female choice, rather than just a higher probability of association of favored genotypes of both sexes.

More detailed analysis of the pooled samples, for the three most common PGI genotypes (3/3, 3/4, and 4/4), extends predictions and results further. The rankings of these three genotypes with respect to kinetic effectiveness are, in descending order, 3/4 > 3/3 > 4/4 (*22*). These three genotypes differ in the same descending order in flight capacity (*25*), survivorship (*22*), and overall male mating success (*26*). Among these three male genotypes, females at their first mating chose 89 3/4, 51 3/3, and 5 4/4 (61.4% 3/4) as mates, whereas remated females chose 31 3/4, 4 3/3, and 0 4/4 (88.6% 3/4) as mates. This is significant [x* (one-tailed) = −3.063, $P = 0.0011$]. The ranking by genotype of males successful in mating with experienced females is as expected.

We conclude that greater mate discrimination by older females increases the overrepresentation, among males successfully mating those females, of PGI genotypes superior in other fitness measures. This was predicted to result from greater male persistence in courtship, stemming from the known greater flight capacity of these genotypes (*22, 25*). Other contributing factors might also be possible (though now purely hypothetical); for example, the PGI genotype–specific differences in glycolytic function might affect composition of the short-range pheromones crucial to final courtship stages (*15*).

There is no evidence that these effects represent females' choice of heterozygosity per se among their potential mates. Here, the favored male genotypes happen to be heterozygotes—but at other loci, whether major genes or polygenes, particular homozygotes or homozygous combinations might well be favored by female choice, depending on their specific properties. The *Colias* mating system is now seen to be structured so that females that exercise greater discrimination can choose males whose genotypes are more favored by other evolutionary pressures. The evolution of such a mating system is itself a focus of much interest (*1–5, 32*).

REFERENCES AND NOTES

1. C. R. Darwin, *The Origin of the Species and The Descent of Man and Selection in Relation to Sex* (Modern Library edition, Random House, New York, 1936); R. A. Fisher, *The Genetical Theory of Natural Selection* (Dover, New York, ed. 2, 1958); M. Kirkpatrick, *Evolution* **36**, 1 (1982); R. Thornhill and J. Alcock, *The Evolution of Insect Mating Systems* (Harvard Univ. Press, Cambridge, MA, 1983).
2. A. Kodric-Brown, *Behav. Ecol. Sociobiol.* **17**, 199 (1985).
3. R. Trivers, *Social Evolution* (Benjamin/Cummings, Menlo Park, CA, 1985).
4. P. O'Donald, *Genetic Models of Sexual Selection* (Cambridge Univ. Press, Cambridge, 1980).
5. T. R. Halliday, in *Mate Choice*, P. Bateson, Ed. (Cambridge Univ. Press, Cambridge, 1983), pp. 3–32.
6. L. G. Mason, *Evolution* **23**, 55 (1969).
7. A. H. Sturtevant, *J. Anim. Behav.* **5**, 351 (1915).
8. E. B. Ford, *Ecological Genetics* (Wiley, New York, ed. 4, 1975); D. A. Powers, L. DiMichele, A. R. Place, *Isozymes Curr. Top. Biol. Med. Res.* **10**, 147 (1983); W. B. Watt, *ibid.* **12**, 89 (1985).
9. C. L. Remington, *Adv. Genet.* **6**, 403 (1954).
10. W. B. Watt, *Evolution* **22**, 437 (1968).
11. O. R. Taylor, Jr., *ibid.* **26**, 344 (1972).
12. R. Silbergleid and O. R. Taylor, Jr., *Behav. Ecol. Sociobiol.* **3**, 203 (1978).
13. R. L. Rutowski, *J. Res. Lepid.* **23**, 125 (1984).
14. S. M. Graham, W. B. Watt, L. F. Gall, *Proc. Natl. Acad. Sci. U.S.A.* **77**, 3615 (1980).
15. J. W. Grula, J. D. McChesney, O. R. Taylor, Jr., *J. Chem. Ecol.* **6**, 241 (1980).
16. W. B. Watt, in preparation.
17. T. Suguwara, *J. Comp. Physiol.* **130**, 191 (1979); Y. Obara, *Naturwissenschaften* **69**, 551 (1982).
18. C. L. Boggs, *Evolution* **35**, 931 (1981).
19. ——— and W. B. Watt, *Oecologia (Berlin)* **50**, 320 (1981).
20. R. L. Rutowski, *Z. Tierpsychol.* **70**, 103 (1986).
21. This is consistent with the gradual return of female mating receptivity as spermatophore resources are depleted; R. L. Rutowski, *Behav. Ecol. Sociobiol.* **3**, 163 (1978).
22. W. B. Watt, *Genetics* **103**, 691 (1983); *ibid.* **87**, 177 (1977).
23. J. G. Kingsolver, *Ecology* **64**, 546 (1983); ——— and W. B. Watt, *ibid.* **65**, 1835 (1984).
24. W. B. Watt, *Am. Nat.* **125**, 118 (1985).
25. ———, R. C. Cassin, M. S. Swan, *Genetics* **103**, 725 (1983).
26. W. B. Watt, P. A. Carter, S. M. Blower, *ibid.* **109**, 157 (1985).
27. J. M. Burns, *Proc. Natl. Acad. Sci. U.S.A.* **61**, 852 (1968); A. H. Ehrlich and P. R. Ehrlich, *J. Kans. Entomol. Soc.* **51**, 666 (1979).
28. Adaptive advantage at this locus consists of having, and maintaining a high V_{max}/K_m ratio [the ratio of maximum velocity to the Michaelis constant (which equals the substrate concentration giving half-maximal velocity)] to maximize glycolytic transient response and steady-state flux capacity (*22, 24*). Most heterozygotes are kinetically advantaged as compared to most homozygotes, especially at low to moderate body temperatures; the 3/4 heterozygote is kinetically advantaged over its "component" ho-

mozygotes 3/3 and 4/4 at all temperature and pH conditions studied (22). The one kinetically effective homozygote, 2/2, is roughly equal in initial V_{max}/K_m ratio to 3/4, but much less stable to high body temperature than any other common genotype, so that it will not maintain its initial kinetic capacity with exposure to high body temperature (22). Thus, the genotypes considered to be kinetically favored are: 3/4, 2/3, 2/4, 2/5, and 3/5. The heterozygote 4/5 is kinetically disadvantaged.
29. We use the term "semispecies" in the sense of E. Mayr [*Animal Species and Evolution* (Harvard Univ. Press, Cambridge, MA, 1963)]: closely related allopatric populations that are not yet fully reproductively isolated, though they differ in many heritable characteristics.
30. Adjustment of solar absorptivity and insulating "fur" in relation to local thermal niche structure (10, 23) renders these populations very similar in their thermally dependent flight profiles through the day at the seasonal times in which sampling took place (see also 25).
31. A. Goldstein, *Biostatistics* (Macmillan, New York, 1964); R. R. Sokal and F. J. Rohlf, *Biometry* (Freeman, San Francisco, 1969); F. J. Rohlf and R. R. Sokal, *Statistical Tables* (Freeman, San Francisco, CA, 1969).
32. We thank S. Borish, A. Head, P. Hecht, P. Nambu, and L. Watt for superior technical assistance; C. Boggs, M. Feldman, K. Holsinger, and J. Peck for stimulating discussions; and R. Watt and L. Watt, the National Science Foundation (BSR 84-00299), and the National Institutes of Health (GM 26758) for research support.

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Replacement of Liver Function in Rats by Transplantation of Microcarrier-Attached Hepatocytes

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Isolated hepatocytes, harvested from normal rat livers by portal vein collagenase perfusion, can be attached to collagen-coated dextran microcarriers and transplanted by intraperitoneal injection into rats. Survival and function of the transplanted hepatocytes have been demonstrated in mutant rats lacking bilirubin–uridine diphosphate glucuronosyltransferase activity (Gunn strain) and rats with inherited lack of plasma albumin (Nagase analbuminemia rat strain). This simple technique promises to be useful in the treatment of acute liver failure in humans.

Although various therapies have been developed for treating severe acute liver insufficiency (1–5), none but whole liver transplantation have proven clinically useful (6, 7). During the past few years, several investigators attempted to develop techniques for transplanting hepatocytes (8–10), which would be simpler and less expensive than transplanting whole organs, would allow the use of living related donors, permit the use of a single donor organ for multiple recipients, and make possible the storage of hepatocytes for future use. However, long-term survival and function of the transplanted hepatocytes in vivo have not been unequivocally demonstrated (8–12). Mutant rats (Gunn strain) with inherited deficiency of bilirubin–uridine diphosphate glucuronosyltransferase (UDPGT) activity lack conjugated bilirubin in bile and exhibit lifelong nonhemolytic unconjugated hyperbilirubinemia (13). Gunn rats have been used to evaluate the success of hepatocyte transplantation (11, 12, 14): after intrasplenic injection of normal hepatocytes, only transient biliary excretion of relatively minor amounts of conjugated bilirubin was observed (14). Thus, so far, the results of hepatocyte transplantation have been largely disappointing.

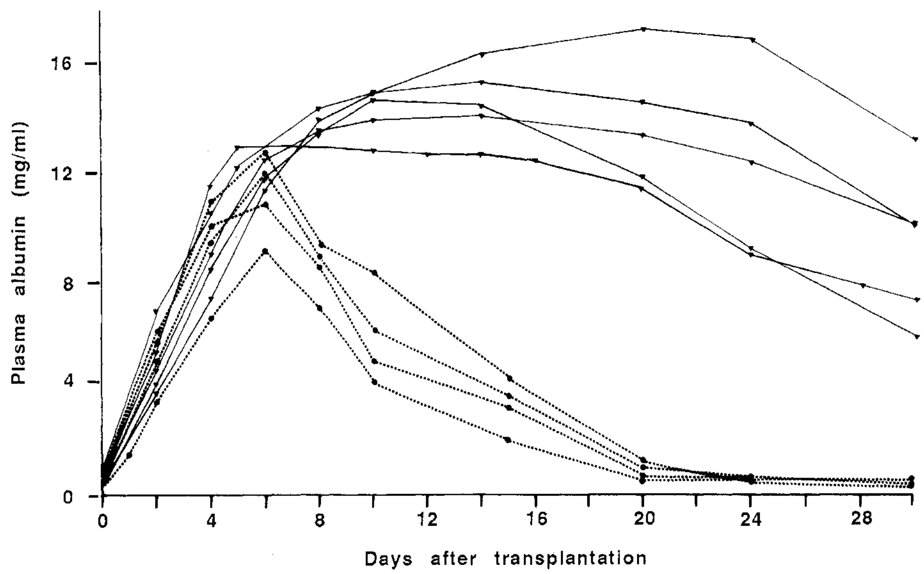

Fig. 1. Plasma albumin concentration in NAR rat recipients of transplanted allogeneic normal hepatocytes. Solid lines represent rats treated with cyclosporin A; interrupted lines represent untreated recipients.

We describe a technique in which isolated rat liver cells are attached to collagen-coated dextran beads and are intraperitoneally injected into rats. Two mutant rat strains, Gunn rats and Nagase analbuminemic rats (NAR rats), which have an inherited defect of albumin synthesis and only a trace of albumin in plasma (15), were used as recipients to evaluate the survival and function of the transplanted hepatocytes. The function of transplanted hepatocytes was demonstrated by the appearance of albumin in the plasma of analbuminemic recipients and conjugated bilirubin in the bile of recipient Gunn rats.

Rat liver cells were released by portal vein collagenase perfusion of donor rats (16); hepatocyte-enriched fractions were prepared by differential centrifugation (17). Between 80 and 95% of the harvested hepatocytes were viable, as assessed by trypan blue exclusion. Collagen-coated microcarriers (Cytodex 3, Pharmacia Fine Chemicals, Uppsala, Sweden) were hydrated in sterile phosphate-buffered saline (PBS) and incubated at 37°C for 90 minutes. The microcarriers were washed, resuspended into Dulbecco's minimal essential medium with 20% fetal calf serum, and transferred into 175-ml polystyrene flasks. Hepatocytes were inoculated into the flasks and incubated for 2.5 hours at 37°C in 5% CO_2 to allow cells to attach to the microcarriers. The number of viable cells was always greater than 90% in aliquots that had been treated with collagenase to release the hepatocytes. Subsequently, 1×10^7 cells

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