gen resulting from biological nitrogen fixation on adjacent reef algal flats undoubtedly contributed to the increased productivity in both of these situtations (*16*).

Calothrix is distributed abundantly and ubiquitously in Pacific coral reef communities and in other shallow tropical marine environments (*17*). Other nitrogen-fixing blue-green algae such as *Hormothamnion* (*5*) are also abundant in some reef communities. These often drab-looking communities have been accorded a much lower conservation priority than the adjacent colorful coral communities. The research described here indicates that these algal reef flats are indeed of considerable importance as a source of fixed nitrogen for adjacent communities and consequently should be accorded a higher priority for conservation where siting of dredging, construction, and industrial outfalls are concerned.

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4. The algae were identified at Enewetak by Dr. Roy Tsuda, University of Guam.
5. In contrast, we found that vigorous stirring resulted in an increase of about 50 percent in fixation rates for *Hormothamnion enteromorphoides*, another Enewetak blue-green alga (R. E. Johannes and W. J. Wiebe, in preparation).
6. Our estimate for the daily nitrogen fixation rate on the reef flat was based on an average of the day and night rates, and on the estimate that the ratio of algal-covered surface per horizontal square meter of algal pavement zone is 1.9 for a similar type of reef flat [A. L. Dahl, *Mar. Biol. (Berl.)* **23**, 239 (1973)].
7. The highest measured terrestrial rates of nitrogen fixation appear to be from 130 to 330 kg ha^{-1} $year^{-1}$ for alfalfa fields [M. Alexander, *Microbial Ecology* (Wiley, New York, 1971)]. Highest recorded marine rates of about 3 kg ha^{-1} day^{-1} are for shallow tropical seagrass communities [J. J. Goering and P. L. Parker, *Limnol. Oceanogr.* **17**, 320 (1972)].
8. Because the surface contours of these communities are quite variable and complex, we did not attempt to calculate fixation rates per unit horizontal area; however, there is undoubtedly at least 4 m^2 of algal-covered surface per horizontal square meter of reef, and thus it is reasonable to assume at least equal rates.
9. R. Tsuda (personal communication) found that about 60 percent of the gut contents of five specimens of the surgeonfish *Acanthurus guttatus*, which he examined at Enewetak, consisted of *Calothrix crustacea*. Although *Acanthurus triostegus* and *Scarus jonsi* profusely graze the *Calothrix* turf on the reef flat at high tide, the bulk of the tooth marks probably result from *Acanthurus guttatus*; G. J. Bakus, *Micronesia* **3**, 135 (1967).
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16. Fixed nitrogen levels are generally even lower in the Caribbean than in the tropical Pacific. Nitrogen fixation might thus provide even greater stimulus for marine productivity in the Caribbean. Bunt *et al.* were unable to find much nitrogen fixation in Florida, the Bahamas, or the Virgin Islands [J. S. Bunt, K. E. Cooksey, M. A. Heeb, C. C. Lee, B. F. Taylor, *Nature (Lond.)* **227**, 1163 (1970); ———, in *Scientists-in-the-Sea*, J. W. Miller, J. G. Van Derwalker, R. A. Waller, Eds. (U.S. Department of the Interior, Washington, D.C., 1969), p. VI-247]. Their observations were at depths of 20 m or more. At Enewetak, we also found much lower nitrogen fixation rates at depths of 3 to 6 m on the outer reef slope (Table 1) than in shallower water. This possibly is a result of the spectral distribution of light at these depths. There is an indication that blue light does not support nitrogen fixation in some blue-green algae [W. M. Pulich, Jr., and C. Van Baalen, *Arch. Microbiol.* **97**, 303 (1974)].
17. Species of the genus *Calothrix* also appear to be important in marine environments of the temperate zone. Nitrogn fixation, mainly by *Calothrix contarenii*, could account for the nitrogen biomass of the marsh grasses in a New England salt marsh [C. Van Raalte, I. Valliela, E. K. Carpenter, J. M. Teal, *Estuarine Coastal Mar. Sci.* **2**, 301 (1974)]. Nitrogen fixation by *C. scopulorum* has been reported to be significant in the supralittoral zone in Norway [P. Wärmling, *Bot. Mar.* **16**, 237 (1973)].
18. Contribution 666 from the Virginia Institute of Marine Science. Contribution from the Mid-Pacific Marine Laboratory. Supported in part by the Oceanography Section, National Science Foundation (grants GA 35866 and 35806), and by the Atomic Energy Commission [grant AT-(20-2)-226] for the operation of the Mid-Pacific Marine Laboratory. We thank M. Gresham, J. Olmon, P. Postal, and A. Thompson for technical assistance.

9 December 1974; revised 31 January 1975

Thyroid Allograft Immunogenicity Is Reduced after a Period in Organ Culture

Abstract. *The survival time of mouse thyroid, transplanted under the kidney capsule of an H-2 incompatible recipient, is extended by holding the thyroid in organ culture for 12 days prior to transplantation.*

The generally accepted view of allograft rejection postulates that histocompatibility antigen (H antigens) are strong immunogens which can elicit a vigorous response to T (thymus derived) cells in the allogeneic host, and that this response is responsible for the initiation of graft rejection. Because H antigens form an integral part of the cell membrane and are widely distributed throughout the tissues of the body (*1*), there would appear to be little point in attempting to reduce the immunogenicity of a tissue transplant by treatments other than those directed at suppressing the host's immune system.

However, our studies of allograft reactions (*2–4*) indicate that the conventional view is inadequate. In our opinion, H antigens by themselves are not strongly immunogenic, and the generation of a vigorous T cell response specific for H antigens requires the simultaneous presentation of both H antigen and an allogeneic stimulus to the potentially responsive T cell population (*4*). The allogeneic stimulus is an inductive stimulus provided by metabolically active lymphoid cells (*5*) (the term lymphoid includes both phagocytic and nonphagocytic lymph-borne cells). According to this view of allograft rejection the fate of an allotransplant might sometimes be enhanced by treatments that remove from the graft those lymphoid cells which provide the postulated allogeneic stimulus. These latter cells play a major role in the sensitization of the host to foreign antigens (*3, 4*).

The reports of Jacobs and Huseby (*6*) and those of Summerlin (*7*) and Summerlin *et al.* (*8*), that allograft survival could be enhanced by the cultivation of tissue in organ culture prior to transplantation, are consistent with our view of graft rejection. The period in organ culture might deplete the tissue of viable hematogenous elements and lymphoid cells that could take part in allogeneic interactions with host lymphoid cells. We were unable to repeat the experiments of Summerlin *et al.* (*8*) with mouse skin allografts. In our experience, both isografts and allografts failed to take on recipient mice after being held in organ culture. We attributed this result to a failure of the cultured skin (either isogeneic or allogeneic) to revascularize before

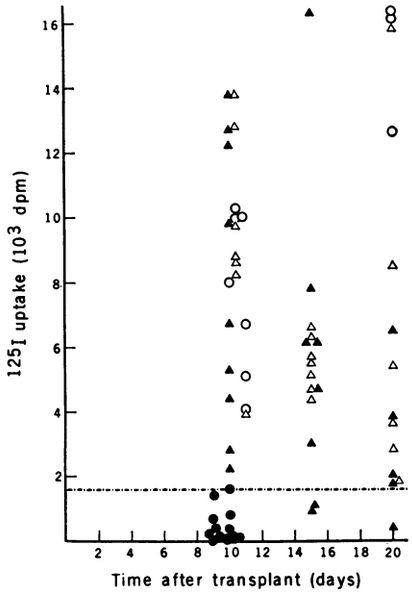

Fig. 1. Function of BALB/c thyroid at various times after transplantation beneath the kidney capsule of BALB/c or C57B1 recipient mice. ^{125}I uptake is expressed as the activity (10^3 disintegrations per minute) of the grafted kidney minus that of the contralateral unoperated kidney. The abscissa shows the time after transplantation at which ^{125}I was injected. The kidneys were removed and radioactivity was counted 24 hours after the injection of iodine. Points shown above the broken line indicate a functioning thyroid transplant; ○, uncultured isograft; △, cultured isograft; ●, uncultured allograft; ▲, cultured allograft.

this tissue became dehydrated. Ninnemann *et al.* (*9*) have been unable to reproduce the findings of Summerlin *et al.* (*8*), using a system in which a proportion of cultured skin isografts were shown to survive.

Mouse thyroid is a much more suitable tissue for such studies. Thyroid tissue survives well in organ culture, and the cultured organ is rapidly revascularized when transplanted under the kidney capsule of recipient mice. The following studies show that thyroid allograft survival is markedly enhanced if the transplant is held in organ culture for 12 days before transplantation.

Individual lobes of mouse thyroid were placed on rafts in 60-mm organ culture dishes (Falcon) containing Eagle's minimum essential medium (Grand Island Biological, F-15) supplemented with 10 percent fetal calf serum. The cultures were maintained for 12 days at 37°C in an atmosphere of 95 percent O_2 and 5 percent CO_2. The organ culture medium was changed three times each week. Our initial studies showed that there were strain differences in survival of mouse thyroid. Thyroids from BALB/c mice were histologically intact after a culture period of 12 days, while organs from C57B1 mice showed partial necrosis after this treatment.

Thyroid transplantation was carried out by placing individual lobes under the kidney capsule of thyroidectomized recipients. Thyroidectomy was carried out at the time of transplantation with the aid of a dissecting microscope. Particular care must be exercised at this stage to avoid damage to the recurrent laryngeal nerve that runs very close to the left thyroid lobe. The function of the transplanted thyroid was followed by injecting recipient mice with 0.25 μc of carrier-free ^{125}I (IMS 30, Amersham/Searle). The recipient was killed 24 hours later, and both kidneys were removed and placed in counting tubes containing Formol saline. The kidney containing the thyroid transplant and the unoperated contralateral kidney were then counted in a gamma counter. Under these conditions, radioactivity of the control kidney was approximately 400 disintegrations per minute; a count of 1600 dpm or more, exceeding that of the control kidney, was taken to indicate a functional transplant in the test kidney (Fig. 1). All transplants were sectioned and examined histologically after counting.

Figure 1 shows the data on iodine uptake obtained when cultured or uncultured BALB/c thyroids were transplanted into normal syngeneic or allogeneic (C57B1) recipients. At 9 to 10 days after transplantation all uncultured allografts were found to be nonfunctional, and histological examination showed that the transplants were replaced by scar tissue (Fig. 2A). At this stage, cultured allografts showed a similar pattern of ^{125}I uptake to either cultured or uncultured isografts. Histologically three of the ten allografts showed little or no mononuclear cell infiltration (Fig. 2B), while the remaining transplants showed some mononuclear cell infiltration around the transplanted organ. Fifteen days after transplantation, six of eight cultured allografts showed similar ^{125}I uptake to cultured isografts, and by 20 days four of five cultured allografts were still functional. Again cultured and uncultured isografts showed similar patterns of ^{125}I uptake. By 20 days all cultured allografts showed some mononuclear cell infiltration around the transplanted tissue, but the major portion of the organ appeared histologically intact (Fig. 2D).

There is considerable variation in the degree of ^{125}I uptake by functioning transplants. The amounts of ^{125}I uptake should be considered only as a qualitative indication that a transplant has survived. The actual amount of iodine incorporated into the kidney carrying a transplanted thyroid depends on the

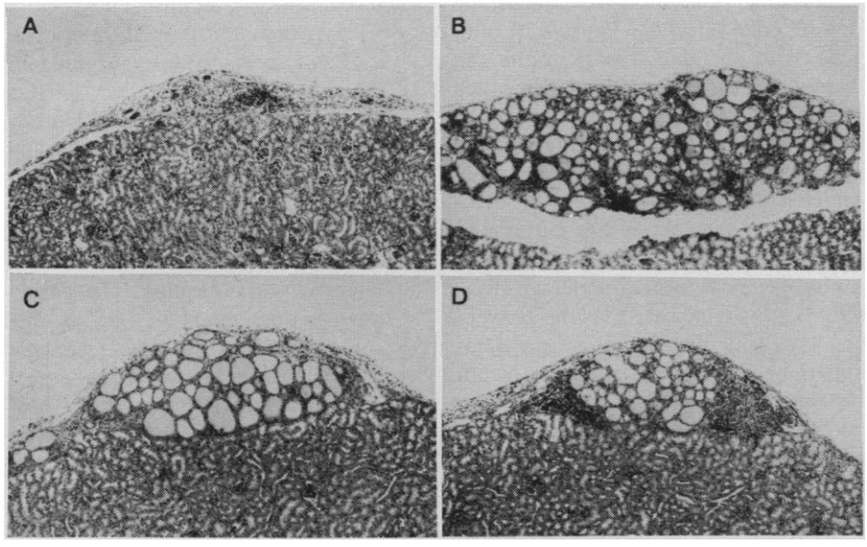

Fig. 2. Histological appearance of BALB/c thyroid allograft, transplanted beneath the kidney capsule of C57B1 recipient mice. (A) Uncultured allograft, 11 days after transplantation. (B) Cultured allograft, 11 days after transplantation. (C) Cultured allograft, 16 days after transplantation. (D) Cultured allograft, 21 days after transplantation (×50).

functional integrity of the thyroid transplant at the time iodine was injected, and on variables that were not controlled in these experiments such as the size of the thyroid lobe transplanted and the level of thyroid-stimulating hormone circulating in the blood of the thyroidectomized recipient.

The results of these studies are clear-cut. When thyroid tissue is held in organ culture for 12 days prior to allotransplantation, its survival in the allogeneic host in enhanced. The mechanism involved in this prolongation of graft survival is not understood. However, these findings are consistent with our views (summarized above) of the way allogeneic interactions affect the process of allograft rejection (*2–4*).

We already have some evidence that a similar effect can be obtained in three different mouse strain combinations, all of which differ at the H-2 locus (H-2^d/H-2^b; H-2^b/H-2^a; H-2^d/H-2^k) (*10*). Moreover, a proportion of thyroids, held in organ culture for approximately 4 weeks, show no histological signs of rejection up to 40 days after allotransplantation. Such transplants may survive indefinitely in the allogeneic host.

Note added in proof: Two recent reports (*11*) show that the survival of ovarian allografts is enhanced after organ culture of the transplanted tissue.

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27 November 1974

Lactate Dehydrogenase X: Effects of Antibody on Mouse Gametes but Not on Early Development

Abstract. *A rabbit antiserum specific for LDH-X, the spermatozoal form of mouse lactate dehydrogenase, was prepared. This antiserum had no effect on fertility of female mice when injected before or after insemination. Similarly, there was no toxicity to the embryo when high concentrations of the antiserum were added to cultures of 2-cell and 8- to 16-cell embryos. There was, however, a moderate inhibitory effect on fertilization in vitro, which may be attributable to a direct action of antiserum to LDH-X on sperm.*

It has been reported that immunity to mouse lactate dehydrogenase X (LDH-X) has an embryotoxic effect when impregnated female mice are actively immunized or passively immunized with a rabbit antiserum, while a decreased fertilization rate, rather than embryopathy, occurs in actively immunized rabbits (*1, 2*). We have been unable to find an embryopathic effect of a heteroantiserum against LDH-X, in vivo or in vitro, although very high concentrations of the serum did inhibit in vitro fertilization.

The LDH was extracted from mouse testes according to the procedure of Goldberg (*3*); the portion precipitating between 40 to 70 percent saturation with $(NH_4)_2SO_4$ was prepared for affinity chromatography (*4*). The LDH-X was separated from LDH 1-5 by this procedure. The LDH-X fraction was then heated at 55°C for 5 minutes, and the denatured proteins were removed by centrifugation. The supernatant was concentrated by vacuum dialysis and subjected to electrophoresis on 7.5 percent polyacrylamide gels. Protein staining (*4*) of the gels showed only one band in the LDH-X position. The portion of the gel which stained histochemically for LDH-X activity was sliced out, frozen, and stored for use in immunization.

Acrylamide slices containing an estimated 75 μg of LDH-X protein were pulverized in saline and homogenized with equal volumes of complete Freund's adjuvant for the first subcutaneous injection and with incomplete Freund's adjuvant for subsequent subcutaneous injections. The animals were bled at weekly intervals after the fourth weekly injection, and booster injections were given monthly. The antiserum was pooled from the fourth to sixth bleedings. It showed a single precipitin line when reacted with testicular extracts, and this band of precipitation fused with the band found on reacting the antiserum with purified LDH-X. There was no cross-reaction with crude extracts of other organs, and absorption with such extracts did not remove the precipitation arc. A rabbit antiserum to purified mouse LDH-1 (*5*) and pooled rabbit normal serums were used as controls.

Pregnant female mice were passively immunized according to the procedures of Goldberg and Lerum (*1*). We followed their schedules but used a different timing convention in which the day the copulation plug is found is called

Table 1. Effect of passive immunization of females with rabbit antiserums to LDH-1 (anti-LDH-1), and LDH-X (anti-LDH-X) on litter frequency and size. For the data in rows 1 to 3, virgin female Swiss Webster mice were mated with Swiss Webster males, and the day a copulation plug was found was counted as day 0. Injections (0.1 ml) of the indicated antiserums were given subcutaneously as indicated. The number of live born offspring was found by cage checks on alternate days. For the data in row 4, 0.2 ml of the indicated antiserum was injected subcutaneously into females which were then placed with the males. The injection was repeated daily until a copulation plug was found or for up to 4 days when unplugged females were discarded.

Row	Time (days)	Normal			Anti-LDH-1			Anti-LDH-X		
		Mice* (No.)	%†	Mean ± S.E.‡	Mice (No.)	%	Mean ± S.E.	Mice (No.)	%	Mean ± S.E.
1	0–3	10	60	10.2 ± 0.9	9	67	12.3 ± 1.1	13	69	10.1 ± 1.0
2	3–6	13	69	10.9 ± 0.5	12	92	9.5 ± 1.2	17	76	9.6 ± 0.8
3	6–9	6	83	8.8 ± 1.4	6	67	10.0 ± 1.2	7	71	10.0 ± 0.5
4	−1§	10	60	6.0 ± 0.6	13	61	8.1 ± 1.0	14	50	9.7 ± 0.5

* Number of fertile females treated. † Percent delivering litters. ‡ Mean number of pups ± standard error. § One day or more before fertilization.