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 ($H\text{-}2^d/H\text{-}2^b$; $H\text{-}2^b/H\text{-}2^a$; $H\text{-}2^d/H\text{-}2^k$) (*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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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.

Table 2. Effect of antiserum and complement on the development of embryos in vitro. Day 1 (2-cell) or day 2 (8- to 16-cell) embryos were obtained from Swiss Webster females in which ovulation was induced at the time of mating to Swiss Webster males and cultured in a modified Whitten's medium (*6*) with the indicated concentrations of antiserums and 1 percent fresh-frozen rabbit complement. They were observed 1 day later for their developmental status. The numbers in parentheses indicate the number of embryos.

Equivalent antiserum (%)	Percentage of embryos		
	Normal serum	Anti-LDH-1	Anti-LDH-X
	From 2-cell to 8- to 16-cell stage		
0	91 (56)	91 (56)	91 (56)
6	0 (50)	88 (51)	96 (50)
12	96 (51)	92 (51)	61 (49)
24	88 (50)	16 (44)	98 (50)
	From 8- to 16-cell to blastocyst		
0	77 (39)	77 (39)	77 (39)
6	76 (25)	82 (22)	90 (20)
12	71 (24)	79 (24)	71 (24)
24	83 (24)	79 (24)	83 (24)

day 0. Our antiserum to LDH-X had a lower inactivation titer than they reported (an average 100 percent inactivation titer of 0.15 ml/unit compared to 0.0059 ml/unit), and 0.1 ml was given subcutaneously daily (Table 1). Although Goldberg and Lerum (*1*) reported their result in terms of the number of treated mice that had litters, we would expect a direct embryotoxic action of the antiserum to affect the size of the litter as well as the presence or absence of litters. Therefore, the average number of pups born per treated female and the percentage of females delivering litters are both shown in Table 1, rows 1 to 3. These injections of antiserum to LDH-X, either before, during, or after implantation, had no effect on the presence or size of litters.

Despite these negative effects, we were concerned that antiserum to LDH-X might have a teratogenic effect or an effect on the developing male gonad. Several dozen offspring of females passively immunized with antiserum to LDH-X were given physical examinations for external abnormalities, and none were found. Four male offspring from such females were killed and dissected and found to have normal appearing testicles, epididymides, and ducti deferentes. More important, seven out of ten males whose mothers received antiserum to LDH-X during days 1 to 4 and seven out of seven control males (antiserum to LDH-1 given on days 1 to 4) were fertile when tested for > 2 months with two females of known fertility. All males born to females treated with antiserum to LDH-X prior to fertilization or treated with normal serum were fertile (four tested in each case). The sex ratios (the number of males to the total number) of the offspring born to passively immunized females prior to fertilization were also determined since Bennett and Boyce (*6*) have shown mild effects on the ratio with a different antiserum. We found 0.49 (61 mice) with antiserum to LDH-1 (controls), 0.38 (58 mice) with antiserum to LDH-X ($\chi^2 = 1.11$ against first ratio, not significant), and 0.52 (21 mice) with normal serums.

To investigate further the possible embryotoxicity of rabbit antiserum to LDH-X under conditions in which much higher concentrations of antiserum could be administered, in vitro cultures of embryos were set up. Day 1 (2-cell) and day 2 (8- to 16-cell) embryos were obtained and incubated in Whitten's medium with minor modifications (*7*). Various additions of filtered gamma globulin fractions [precipitable in 33 percent $(NH_4)_2SO_4$], which were concentrated threefold compared to the original serums, were made, and filtered fresh-frozen rabbit complement was also included (Table 2). Although concentrations of antiserum to LDH-X in these in vitro experiments are very much greater than would have been present in vivo, there were no deleterious effects of antiserum to LDH-X and rabbit complement on 2-cell or 8- to 16-cell embryos. Menge (*8*) has shown that uterine secretory immunoglobulin A (IgA) has embryopathic effects, but that serum IgG has none. However, passive immunization in vivo could not affect secretory IgA but could result in immune IgG in the uterine fluids.

We also studied the effects of the antiserum on in vitro fertilization, another condition in which very high concentrations of antiserums could be used. In vitro fertilization was effected by the method of Hoppe and Pitts (*9*), and the number of eggs fertilized were counted. Although heat-treated antiserum to LDH-X depressed fertilization in vitro, a similar effect was found at the next higher concentration with antiserum to LDH-1 (Table 3). Much larger equivalent concentrations of partially purified gamma globulins were required to inhibit fertilization, but antiserum to LDH-X did show a greater inhibitory effect than did the control serums. These levels of antibody are much greater than could be achieved in vivo. Experiments in which sperm and eggs were exposed separately prior to incubation and then washed before being brought together demonstrated that this effect was on sperm rather than on the oocyte.

There is no evidence for the presence of LDH-X in mouse embryos prior to implantation (*10, 11*), or for the location of embryonic LDH on the surface of intact viable embryos where

Table 3. Effects of antiserums on in vitro fertilization. In vitro fertilization was performed with epididymal sperm and hybrid ova in embryological watch glasses (with mechanical agitation) (*8*). The ova were observed 1 day later for cleavage. The numbers in parentheses indicate the numbers of ova.

Equivalent antiserum (%)	Percent fertilization		
	Normal serum	Anti-LDH-1	Anti-LDH-X
	Serum		
0	97 (33)	97 (33)	97 (33)
0.5	92 (36)	97 (32)	82 (33)
1	92 (37)	94 (35)	3 (33)
5	94 (37)	28 (40)	0 (31)
	Gamma globulin		
0	85 (192)	85 (192)	85 (192)
3	100 (33)	87 (31)	97 (32)
7.5	100 (28)	100 (41)	52 (30)
15	89 (37)	86 (86)	16 (86)
30	27 (27)	43 (30)	7 (27)
60	0 (27)	4 (26)	0 (27)

it would be accessible to antibody (*11*). However, we have been able to detect LDH-X on the surface of maturing spermatozoa (*12*), and this could provide a basis for an inhibitory effect of antiserum to LDH-X on fertilization in vitro. The antiserum may have blocked sperm penetration by enhancing sperm aggregation and subsequently preventing sperm-egg interaction. Spermatozoal toxicity was unlikely because the antiserum used for the fertilization experiments in vitro was heated to inactivate complement, and the gamma globulin fractions lack some of the components of complement. Nevertheless, although the plasma membrane of sperm fuses with the plasma membrane of the egg and some LDH-X might be brought to the egg membrane by the sperm, there was no effect on development when the early embryo was exposed to high concentrations of antiserum to LDH-X (Tables 2 and 3), again indicating that the effect was on sperm and not on the egg or zygote.

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Arsenic Tolerance in a Population of the Grass Andropogon scoparius Michx.

Abstract. *Samples of* Andropogon scoparius *Michx. collected on an arsenic mine exhibited a wide range of tolerance to arsenic in solution, whereas plants of the same species growing in uncontaminated soil showed no tolerance. Arsenic tolerance must be an evolved character under genetic control. Furthermore, the degree of tolerance is related to the amount of arsenic in which the plant was growing.*

The study of heavy metal tolerance in plants provides one of the best available examples of evolution in action (*1*). Mine populations of the common bent grass, *Agrostis tenuis* Sibth., have evolved edaphic races which exhibit tolerance to lead, zinc, nickel, or copper (*2*). Plant populations growing on an abandoned arsenic mine in Floyd County, Virginia, provide an ideal opportunity to study the effects of arsenic, which is not a heavy metal and which is not known to be an essential mineral nutrient. In fact, arsenic compounds are known to be toxic to higher plants (*3*). It has been reported that tolerance in man and some other mammals can be induced through gradual habituation (*4*). Therefore, the presence of several plant species on the Floyd County mine immediately raises the question of whether these populations belong to species inherently tolerant to arsenic or whether they have become habituated or have actually evolved a tolerance to arsenic.

Plants of *Andropogon scoparius* Michx., little bluestem, the dominant mine species, were collected along several transects at 5-meter intervals. Each plant was individually potted in normal soil and placed in a cold greenhouse. Control plants from two populations of *A. scoparius* found in uncontaminated soil were also tested. Samples of the control-Floyd population, C-F, were collected within a half a mile (1 mile = 1.6 kilometers) of the mine, while plants from the control-Montgomery population, C-M, came from 25 miles away. Using the method of Wilkins (*5*), we compared root growth of individual tillers in Na_2HAsO_4 solutions of various concentrations with root growth in the absence of arsenic, taking the length of the longest root. Testing was done in all-glass aquariums, and Pyrex or stainless steel stirrers were used to prevent concentration gradients and to ensure aeration. Arsenic concentrations chosen for this test were 1, 3, 5, 10, 25, and 50 ppm as elemental arsenic. We did not test at less than 1 ppm because of the difficulty of maintaining such low concentrations (Table 1).

Soil samples were collected from the root zone of each plant. After oven drying, each sample was analyzed for total arsenic content by means of x-ray emission spectrometry. The soil arsenic concentration in parts per million (dry weight) is shown for each plant in Table 1. Although the total amount of arsenic in the soil is not necessarily the amount available to the plant, it is an indication of the relative amounts potentially able to affect plant growth (*6*).

The results show that the mine population possesses a tolerance to 1 ppm arsenic which the control plants lack. As the concentration was increased, the mine plants showed a steady decrease in root growth. The mine population

Table 1. Root growth of *A. scoparius* Michx. in arsenic solution as a percentage of control growth.

Plant	Soil arsenic total (ppm)	Plants (No./m²)	Growth (%) in arsenic solutions at*					
			1 ppm	3 ppm	5 ppm	10 ppm	25 ppm	50 ppm
D-5	41,200	2	95	82	64	64	22	10
D-1	25,300	5	90	69	46	29	9	0
D-8	20,500	4	85	58	43	25	10	0
D-9	17,000	2	91	79	49	40	23	10
D-2	14,600	6	91	75	36	25	9	0
D-4	6,100	7	85	41	19	8	0	0
D-3	1,320	9	91	50	30	20	0	0
E-1	310	6	86	13	2	0	0	0
E-2	100	11	67	11	0	0	0	0
C-F	10		0	0	0	0	0	0
C-M	10		0	0	0	0	0	0

* Except for control populations, each figure represents the mean of five tillers from each plant. The figures given for the two control populations (C-F and C-M) represent the averages of five tillers from each of five plants.