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Effect of Oxygen Pressure During Culture on Survival of Mouse Thyroid Allografts

Abstract. *A marked increase in the percentage of mouse thyroids that retained function 20 days after transplantation across a major histocompatibility barrier and the percentage that lacked generalized infiltration was observed when the grafts received hyperbaric oxygen during a 4-day culture period. Perfusion of the donor animal before thyroidotomy and the addition of fetal calf serum to the culture medium did not have a significant effect on graft survival, but the percentage of grafts lacking generalized infiltration was slightly increased by the addition of hydrocortisone to the culture medium.*

Culturing an organ before it is transplanted has been reported to enhance survival of the allograft (*1*–*5*). Lafferty and co-workers described a technique for culturing mouse thyroids and grafting them under the kidney capsules of allogeneic mice (*6*–*8*). The most important aspect of this culture technique was placing the 1- to 2-mm organs on a raft at the surface of the culture medium in an atmosphere of 95 percent O_2 and 5 percent CO_2. Using the identical technique, Sollinger, *et al.* (*9*) found that rat thyroids had an enhanced survival when transplanted into mice. With both xenografts and allografts, prolonged or indefinite survival required a culture period of nearly 4 weeks.

Because of the difficulties in maintaining any organ in culture for long periods of time, we have varied conditions to find an effective culture method that requires less time. We report here the effect of perfusing the organ with culture medium before it is removed from the donor, and the effects of oxygen pressure, serum, and hydrocortisone during culture on the survival of mouse thyroid allografts.

Individual lobes of mouse thyroids were placed on microscope lens paper supported by a stainless steel wire mesh in 60-mm plastic petri dishes. Culture medium (10 ml) was added so that the lens paper was saturated and the paper was at the interface of the liquid and the gaseous atmosphere. The medium used for organ culture was Eagle's minimum essential medium (MEM) (F-15, Grand Island) supplemented with pyruvate (1 m*M*), sodium bicarbonate (2.2 g/liter), penicillin (100 unit/ml), and streptomycin (100 μg/ml). Cultures were maintained for 4 days at 37°C in sealed pressure chambers filled with 95 percent O_2 and 5 percent CO_2. Hydrocortisone acetate (10 mg/ml) was added to some cultures by diluting sterile hydrocortisone acetate suspension (50 mg/ml, Invernex) in culture medium. Before dilution, this preparation contained sodium carboxymethylcellulose (0.03 percent), polysorbate (0.026 percent), and benzyl alcohol (0.09 percent). To other cultures, fetal calf serum (FCS) (10 percent by volume) was added.

Individual BALB/c mouse thyroid lobes were placed under the kidney capsules of thyroidotomized C57BL/6 ($H\text{-}2_b$) or BALB/c ($H\text{-}2_d$) recipients. Thyroidotomy of the recipient was performed at the time of transplantation with the aid of a dissecting microscope. Particular care was taken to avoid damage to the recurrent laryngeal nerve that is located close to the left thyroid lobe. Total thyroidectomy of the recipient was not achieved.

In some cases, the donor was perfused with MEM before organ removal. The donor was anesthetized with sodium seconal, and the heart was approached through the abdomen by cutting the diaphragm. An injection of 6 ml of MEM was given in the left ventricle through a 25-gauge needle. Immediately after beginning the perfusion, the right ventricle

Table 1. Effect of perfusion before culture, and oxygen pressure, serum, and hydrocortisone acetate during a 4-day culture on ^{125}I uptake and histology of BALB/c thyroids after 19 to 21 days in a C57BL/6 host.

Group	pO_2 (mm)	Donor perfused	FCS	HC*	^{125}I† uptake	Histology‡				S + F (%)
						S	F	G	D	
1	600	+	−	−	0/11	0	0	0	11	0
2	1000	+	−	−	4/11	1	1	2	7	18
3	1300	+	−	−	8/13	4	3	1	5	53
4	1300	−	−	+	15/16	5	8	2	1	81§
5	1300	+	+	−	6/9	0	3	3	3	33
6	1300	−	−	−	10/15	2	4	4	5	40

*Hydrocortisone acetate, 10 μg/ml. †The numerator is the number of recipient mice with ten times or more ^{125}I counts in the grafted kidney than the control kidney. The lowest ratio found in ten syngeneic grafts was 12. ‡S, indistinguishable from syngeneic grafts, no infiltration; F, focal infiltration; G, generalized infiltration; D, destroyed, no visible follicles. §Significantly different from group 6, $P = .05$.

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was cut, resulting in a completely colorless thyroid gland.

The function of the transplanted thyroid was monitored after injecting the recipient mice intraperitoneally with approximately 0.25 μCi of ^{125}I (carrier-free) 19 to 21 days after transplantation. The recipient was killed 24 hours later, and both kidneys were removed and placed in counting tubes containing buffered formalin. Radioactivity in the kidney containing the thyroid transplant and that in the unoperated contralateral kidney were counted in a gamma counter. Test kidneys that showed ten times or more counts than did the control kidneys were considered to be carrying a functional transplant. All transplants were sectioned and examined histologically after counting. The histological appearance of the transplants was evaluated as described (*8*).

Survival on day 19 to day 21 of BALB/c allografts in C57BL/6 mice was increased by partial pressures of O_2 above 600 mm during the 4-day culture (Table 1, groups 2 and 3). No significant effects resulted from perfusion before culture (group 4) or the addition of serum during the culture (group 5). The addition of 10 μg of hydrocortisone acetate per milliliter of culture medium increased slightly but significantly the percentage of grafts lacking generalized infiltration (group 6).

These results indicate that mouse thyroid allografts survived as well after a 4-day culture period in hyperbaric oxygen and hydrocortisone as they did after a 4-week culture in 95 percent oxygen at atmospheric pressure (*8*). Both radioactive iodine uptake and histological appearance after 21 days correlated with a long-term survival graft (*8*).

The above results probably explain the failure of Raff *et al.* (*10*) to obtain prolonged survival of cultured rat thyroids when atmospheric O_2 (150 mm) was used. They also explain our failure (*11*) to obtain prolonged survival of thyroid grafts after retransplantation from a thymectomized, irradiated, allogeneic recipient to a normal animal of the same strain. The effect of hydrocortisone on these experiments may be similar to the prolongation of allogeneic rat skin graft survival after treatment of the grafts with fluocinolone acetonide (*12*).

The mechanism by which oxygen prolongs graft survival in an allogeneic host is not known. An attractive explanation is that oxygen selectively kills passenger leukocytes, which are efficient stimulators of allograft immunity (*8*). If this is the mechanism, further selective modifications of the culture conditions may further decrease the time of culture required, and should facilitate the application of the culture technique to the transplantation of other organs.

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The Essentiality of Vitamin D Metabolites for Embryonic Chick Development

Abstract. *Laying hens maintained on 1,25-dihydroxyvitamin D_3 as their sole source of vitamin D produce eggs which appear normal but which produce embryos having a defective upper mandible and which die at 18 to 19 days of embryonic life. Hens maintained on 25-hydroxyvitamin D_3, on the other hand, produce normal embryos. Hens fed a vitamin D deficient diet produce eggs which develop the same embryonic defect. Injection of the affected eggs from the 1,25-dihydroxyvitamin D_3 fed hens with vitamin D_3, 25-hydroxyvitamin D_3, or 1,25-dihydroxyvitamin D_3 greatly increases the percentage of normal embryos. It therefore appears that 1,25-dihydroxyvitamin D_3 is not transferred from hen to egg in sufficient amounts to support embryonic development and that vitamin D or its metabolites, or both, are necessary for normal chick embryo development.*

Although it is well accepted that vitamin D must undergo 25-hydroxylation followed by 1α-hydroxylation to be effective at physiologic doses, there is still considerable discussion of whether the resultant hormonal form of vitamin D_3, namely, 1,25-dihydroxyvitamin D_3 [1,25-$(OH)_2D_3$] can carry out all of the vitamin's functions (*1*). During the course of a study of egg production of hens maintained on 1,25-$(OH)_2D_3$ as their sole source of vitamin D, we learned that the embryos from these eggs failed to hatch and died prior to emergence from the shell. Examination of the affected embryos (Fig. 1) showed that the upper mandible failed to develop properly. In other respects the embryos appeared normal. An experiment was therefore carried out to determine if higher levels of 1,25-$(OH)_2D_3$ given to the hens could support normal embryonic emergence.

Forty-eight White Leghorn pullets, 40 weeks of age, from the University of Wisconsin flock were fed a basal diet composed (in grams of ingredient per kilogram of diet) of ground corn (752), alfalfa meal (20), meat scraps (30), soy-

Table 1. Effect of 1,25-$(OH)_2D_3$ on egg production, eggshell quality, body weight, and bone ash of laying hens.

Form of vitamin added	Amount per kilogram of diet (μg)	Egg production (%)	Eggshells with 0.001-mm deformation (%)	Body weight gain* (g)	Bone ash (%)†
None	0	35.7	21.9	16	55
Vitamin D_3	8	76.4	16.7	189	62
Vitamin D_3	12	68.0	15.2	216	62
1,25-$(OH)_2D_3$	1	76.0	15.1	386	61
1,25-$(OH)_2D_3$	2	70.5	17.9	273	65
1,25-$(OH)_2D_3$	4	73.5	15.8	198	67
1,25-$(OH)_2D_3$	8	64.1	16.0	57	64

*The hens averaged 1637 to 1708 g at 34 weeks and the weight gain is given in grams for the 34- to 61-week period. †Percentage of dry, fat-free weight.

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