Transplant Int (1991) 4: 110–115

TRANSPLANT
International

A comparison of the effects of hyperbaric oxygen culture on survival of murine and canine thyroid gland grafts

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Received September 24, 1990/Received after revision January 3, 1991/Accepted January 4, 1991

Abstract. Canine thyroid tissue (CTy) was subjected to hyperbaric oxygen culture (HOC) under conditions that affect immunoalteration in murine thyroid tissue (MTy). Survival of autografts and allografts implanted under the kidney capsule was determined after 21 days by ^{125}I uptake and histology. Unlike MTy, autograft CTy subjected to normothermic HOC (95% O_2, 5% CO_2; 1.76 kg/cm^2) for 48 h did not survive (0/8) whereas decrease of culture duration to 24 h resulted in autograft CTy survival (3/3). Under hypothermia (5°C), HOC could be extended to 7 days with autograft CTy survival (3/3 after 4 days and 3/3 after 7 days). Allograft CTy after 24 h of normothermic HOC and 7 days of hypothermic HOC was rejected. Indicators of oxygen free radical injury were determined: catalase activity was comparable in MTy and CTy (means 14.82 and 6.3–10.8 mm/mg protein, respectively) but superoxide dismutase activity was low in CTy (means 0.01–0.29 and 4.75 U/mg protein, respectively). Malondialdehyde content after 48 h of normothermic HOC was higher in CTy than in MTy (means 2215 and 1275 nmol/g, respectively). The results show that CTy is injured by HOC under conditions tolerated by MTy, and that this difference is related to the greater sensitivity of CTy to oxygen free radical injury.

Key words: Hypertonic oxygen, thyroid gland graft – Culture, hyperbaric oxygen – Thyroid gland graft, culture – Free radicals, culture, thyroid gland graft

Hyperbaric oxygen culture (HOC) under certain conditions (95% O_2, 5% CO_2; 1.76 kg/cm^2; 37°C) can prevent murine thyroid allografts from being rejected after transplantation in non-immunosuppressed mice [6]. Duration of exposure to the high concentration of oxygen resulting from hyperbaria is an essential factor in this immunoalteration. The culture duration required to effect immunoalteration of murine thyroid tissue under normothermia (37°C) is 48 h. In previous studies, we have shown that murine thyroid gland tissue can also be immunoaltered under conditions that simulate hypothermic organ preservation [15, 16]. HOC of murine thyroid tissue was carried out at 5°C in University of Wisconsin (UW) cold storage solution in place of a tissue culture medium. Under hypothermia, it took 7 days of HOC to obtain the same result as after 48 h of HOC under normothermia. Furthermore, it could be demonstrated that the presence of oxygen free radical scavengers inhibited immunoalteration, suggesting an oxygen free radical mechanism in immunoalteration.

The next step in taking this method of immunoalteration closer to application to whole organs was a study in a higher animal species such as the dog [17]. Canine thyroid tissue was cultured with hyperbaric oxygen under conditions that had proved effective in mice, and transplanted under the kidney capsule as autografts and allografts. Unlike murine thyroid tissue, canine thyroid tissue was injured after 48 h of normothermic HOC. This species difference in the response of thyroid gland tissue to HOC was studied in relation to indicators of oxygen free radical processes.

Materials and methods

HOC of murine thyroid gland tissue

B10BR/SGSNJ and C57BL/10J mice were obtained from the Jackson Laboratory, Bar Harbor, Mass., USA. Methods of murine thyroid gland procurement and normothermic HOC have been described previously [6, 15, 16]. In this series, murine thyroid tissue was subjected to normothermic HOC for 24 h. Non-cultured (control) and cultured thyroid grafts from donor B10BR/SGSNJ mice were transplanted to recipient B10BR/SGSNJ mice (isogeneic transplants) or C57BL/10J mice (allogeneic transplants). Transplantation and graft assessment of murine thyroid lobes were performed as previously described [6, 15, 16].

Procurement of canine thyroid gland tissue

Adult mongrel dogs of either sex and weighing approximately 20 kg were used. The dogs were anesthetized with pentothal sodium (8 mg/kg) and maintained on 100% oxygen with halothane. In the dog both thyroid lobes are positioned lateral to the trachea [9]. The

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thyroid gland was exposed by a median incision in the neck and the left lobe was excised. (In the dog the right lobe is more closely associated with the recurrent laryngeal nerve than the left lobe.) The extirpated thyroid lobe was placed on ice and its fibrous capsule was dissected and removed. Thin slices (approximately 3 × 3 × 1 mm) of the thyroid gland tissue were cut.

Normothermic and hypothermic HOC of canine thyroid tissue

For normothermic HOC, the canine thyroid tissue specimens were placed in minimal essential medium (Eagle's, Grand Island Biological, Grand Island, N.Y., USA) supplemented with 2% fetal calf serum, 1.1 g/l sodium bicarbonate, 10 mM sodium pyruvate, 1 mM glutamine, penicillin and streptomycin (pH 6.9). For hypothermic HOC, the thyroid tissue specimens were placed in UW preservation solution containing hydroxyethyl starch/lactobionate [11]. The agents allopurinol and glutathione in UW preservation solution were not included for these experiments following the results of a previous study [16]. Four thyroid tissue specimens from the same dog were incubated in 4 ml culture medium in a tissue culture dish (Falcon no. 1008, 60 mm). HOC was carried out in a pressure chamber maintained at either 37°C (normothermic HOC) or 5°C (hypothermic HOC). The pressure chamber was equilibrated with O_2/CO_2 (95%:5%) at a pressure of 1.76 kg/cm^2. The culture durations were 48 h and 24 h under normothermia, and were extended to 3, 4, 7 and 14 days, respectively, under hypothermia.

Transplantation of canine thyroid tissue and assessment of graft survival

Cultured and non-cultured (control) thyroid tissue specimens were transplanted as auto- and allografts under the kidney capsule. The kidneys were freed from the posterior abdominal wall and the graft was pushed under the kidney capsule, through a small incision in its posterior face. The site of implantation was marked with a 6-0 prolene suture. The incision in the kidney capsule was closed with a 5-0 silk suture. For each test, a cluster of three thyroid specimens was transplanted at the same time in the same animal.

Graft survival was determined 21 days after transplantation by functional assessment and histological examination. The dog was given 10 μCi ^{125}I (carrier free) by mouth 24 h before sacrifice on postoperative day 21 [10]. After removal of the kidneys, the implant sites were excised and placed in 10% formalin for determination of ^{125}I in a gamma counter (CliniGamma 1272, LKB Wallac). The implant sites were subsequently sectioned and stained for histological examination (H & E). Grafts were considered viable if radioactivity (cpm) in renal cortical tissue containing the thyroid graft exceeded ten times the count of renal cortical tissue away from the graft (background radioactivity). This was also correlated with histological evidence of intact and viable thyroid tissue.

^{125}I in vitro viability assay

This procedure was set up as a method for assessing graft viability in vitro [17]. The method was modified according to the procedure described by Bauer and Herzog [2]. A cultured thyroid specimen (3 × 3 × 1 mm) was transferred to a test-tube containing 0.5 ml Eagle's MEM to which 0.5 μCi ^{125}I (carrier free) was added. The test-tube was capped and incubated for 15–20 h at 5°C to maintain the viability of the thyroid tissue during incubation. The specimen was subsequently transferred to a test-tube containing 1 ml 10% formalin and kept at room temperature for 8 h. The specimen was then transferred to a test-tube containing 1 ml fresh 10% formalin and gently shaken. This procedure was repeated and both the test-tubes, containing the wash solution and the specimen, were counted for ^{125}I in a gamma counter (CliniGamma 1272, LKB Wallac). This procedure was repeated until the wash solution was essentially free of ^{125}I (less than 2% of the count of the specimen). The specimen was then dried and weighed, and its ^{125}I content expressed as cpm/mg tissue. In each assay, four equally treated thyroid tissue specimens obtained from the same animal were tested.

Canine thyroid xenograft studies in nude mice

To compare the results of the ^{125}I viability assay of canine thyroid tissue as described above with survival in vivo, tissue specimens of canine thyroid tissue were both tested for their in vitro ability to take up ^{125}I and implanted under the kidney capsule in nude mice. The methods of implantation and assessment of graft survival were essentially the same as in the murine thyroid transplantation experiments described above. Briefly, the nude mice were anesthetized with chloral hydrate 7% solution (0.10 ml/g body weight) and the left kidney was exposed. The canine thyroid graft (3 × 3 × 1 mm) was placed under the kidney capsule through a small incision made in the anterior renal capsule. Graft survival was determined 21 days after transplantation by functional and histological analysis. Functional analysis was by determination of the amount of ^{125}I in the kidney containing the transplanted thyroid and kidney receiving no thyroid tissue. Recipient mice were injected (i.p.) with 0.5 μCi ^{125}I (carrier free) in 0.5 ml normal saline 24 h before sacrifice. The next day both kidneys were removed and placed in saline containing 10% formalin, and ^{125}I determined in a gamma counter (CliniGamma 1272, LKB Wallac). The ratio of radioactivity (cpm) in the kidney containing the thyroid graft to that in the right kidney (no graft) was determined. Survival of the grafts was confirmed by histological examination (H & E).

Canine thyroid specimens obtained from one dog were cultured for 48 h in hyperbaric oxygen (95% O_2, 5% CO_2; 1.76 kg/cm^2; 37°C) ($n = 8$) in room air at ambient pressure ($n = 8$) and in pressurized room air (25 psi) ($n = 8$). Four specimens of each group were subsequently assayed in vitro for ^{125}I uptake, while the other four were implanted in nude mice.

Superoxide dismutase, catalase and malondialdehyde assays

Superoxide dismutase (SOD) and catalase (CAT) activity of canine and murine thyroid tissue were determined using previously reported methods as described by Starling and Harms [13]. Thyroid tissue was obtained from three dogs and stored at − 80°C until assay. To have sufficient tissue for one assay, 24 thyroid lobes were obtained from mice. SOD and CAT activities were determined by combining a 5% homogenate of thyroid tissue with 0.05 M phosphate buffer, pH 7.8. Total tissue SOD content was assayed as described by Beauchamp and Fridovich [3] and reported as units per mg protein. Catalase activity was determined according to the method of Aebi using a phosphate buffer/H_2O_2 mixture [1]. Protein was determined using the method of Lowry et al. [7].

Malondialdehyde (MDA) was determined before and after 48 h normothermic HOC in thyroid tissue of six dogs. In addition, MDA

Table 1. Survival of murine thyroid isografts and allografts after normothermic (37°C) and hypothermic (5°C) HOC

	Controls (no culture)	37°C in Eagle's MEM[a]		5°C in UW solution[b]	
		24 h	48 h	4 days	7 days
Isografts	8/9 (88%)	3/3 (100%)	11/12 (92%)	2/3 (66%)	8/ 9 (89%)
Allografts	0/5 (0%)	1/5 (20%)	14/15 (93%)	2/5 (40%)	15/18 (83%)

[a] Eagle's minimal essential medium

[b] University of Wisconsin cold storage solution (without glutathione and allopurinol)

Table 2. Survival of canine thyroid autografts and allografts after normothermic (37°C) and hypothermic (5°C) HOC

	Controls (no culture)	37°C in Eagle's MEM[a]		5°C in UW-solution[b]		
		24 h	48 h	4 days	7 days	14 days
Autografts	20/24 (83%)	3/3 (100%)	0/ 8 (0%)	3/3 (100%)	3/3 (100%)	0/3 (0%)
Allografts	0/28 (0%)	0/3 (0%)	0/15 (0%)	0/3 (0%)	0/3 (0%)	0/3 (0%)

[a] Eagle's minimal essential medium
[b] University of Wisconsin cold storage solution (without glutathione and allopurinol)

was determined after 48 h normothermic culture in room air (ambient pressure). A total of 22 murine thyroid lobes were obtained for MDA determination: 11 lobes were assayed before, and 11 lobes after, 48 h HOC. MDA was measured using the thiobarbituric acid reaction as described by Sunderman et al. [14]. Briefly, thyroid tissue samples were homogenized and transferred to tubes containing cold H_3PO_4 solution. After addition of 0.6% thiobarbituric acid solution, the tubes were placed in a boiling water bath for 45 min. After cooling, butanol was added to each tube and the contents centrifuged (1000 rpm). Each butanol extract was determined in a spectrophotometer at 500–560 nm. The results were expressed as nmol MDA per g (wet weight) of tissue.

Results

Survival of murine thyroid isografts and allografts after normothermic and hypothermic HOC

The survival rate of isografts was 92% after 48 h of normothermic (37°C) HOC and 89% after 7 days hypothermic (5°C) HOC (Table 1), indicating that the viability of the thyroid grafts was not affected by the culture procedure. Allograft survival was 93% after 48 h of normothermic HOC, but was only 20% when the duration of normothermic HOC was reduced to 24 h (Table 1). Hypothermic HOC resulted in prolongation of murine allograft survival only after 7 days culture duration.

Survival of canine thyroid autografts and allografts after normothermic and hypothermic HOC

Of 24 fresh (no culture) thyroid autografts implanted under the kidney capsule, 20 were retrieved at sacrifice (Table 2). These demonstrated high ^{125}I counts and histologically showed viable thyroid tissue with neovascularization and intact follicles. None of 28 fresh allografts survived. Counts of ^{125}I did not exceed background radioactivity and histologically scar tissue, with occasionally some residual cellular infiltrate, was found at the site of implantation.

Eight canine thyroid autografts, which were subjected to 48 h normothermic HOC in Eagle's MEM, did not survive (Table 2). Histological examination showed scar tissue only at the implant site. Reduction of the time of normothermic HOC to 24 h resulted in the survival of autografts, indicating that the viability of the grafts was maintained. The allografts, however, were rejected.

Canine thyroid grafts subjected to hypothermic HOC in UW solution remained viable for up to 7 days (Table 2). The allografts, however, were all rejected. When the duration of hypothermic HOC was extended to 14 days, neither autografts nor allografts survived.

Histology of cultured murine and canine thyroid tissue specimens

Histological examination of murine thyroid tissue following 48 h HOC showed viable thyroid tissue with intact follicles. The canine thyroid tissue after 48 h HOC, however,

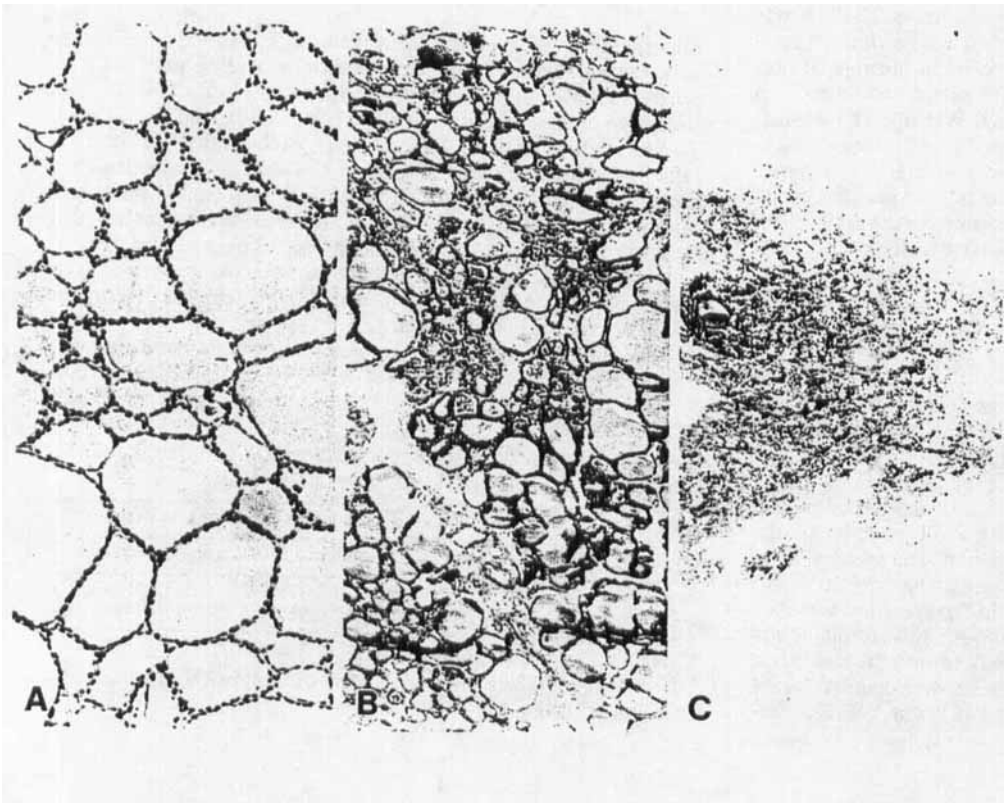

Fig. 1 A–C. Micrographs showing the histological appearance of normal and cultured canine thyroid gland tissue. **A** Normal configuration of canine thyroid tissue consisting of packed follicles. **B** Canine thyroid tissue after 24 h normothermic HOC showing focal areas of necrosis. **C** Canine thyroid tissue after 48 h normothermic HOC showing massive necrosis and disintegration of follicles (H & E; original magnification ×40)

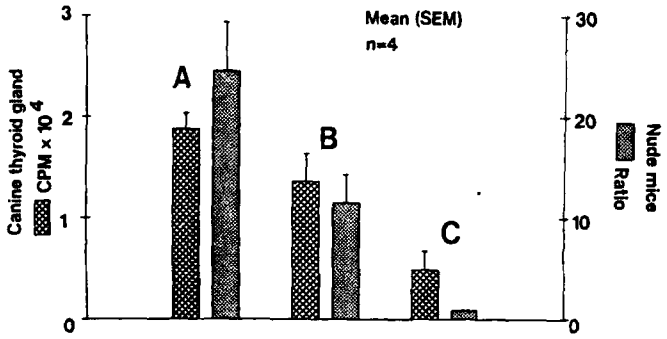

Fig. 2. Graph showing the relation between the capability of canine thyroid tissue to take up ^{125}I in vitro *(left axis)* and the uptake of ^{125}I by grafted thyroid tissue in nude mice after 21 days of implantation *(right axis)*. Canine thyroid tissue was cultured (37°C) for 48 h in room air at ambient pressure *(A)*, in room air pressurized to 1.76 kg/cm^2 *(B)* and with hyperbaric oxygen (95% O_2, 5% CO_2; 1.76 kg/cm^2) *(C)*. Four specimens of each group were assayed in vitro for ^{125}I uptake, and four specimens were implanted under the kidney capsule in nude mice. Increased oxygen concentration during culture was associated with decreased uptake of ^{125}I in vitro, and decreased viability as tested in nude mice

invariably showed massive necrosis with only a few isolated follicles remaining. Reduction of the duration of HOC to 24 h resulted in the thyroid structure being largely unaffected. Necrosis was limited to focal areas within normal thyroid tissue (Fig. 1). The same histological appearance was found after 7 days of hypothermic (5°C) HOC. Exposure of thyroid tissue to 95% O_2 under ambient pressure for 48 h resulted in necrosis restricted to the periphery of the specimens, leaving the central part intact. Conversely, subjecting thyroid tissue to room air under normal pressure gave necrosis centrally, with intact tissue at the periphery of the specimens.

^{125}I in vitro viability assay of canine thyroid tissue

To define the injury caused by culturing under various oxygen conditions, an in vitro viability assay of canine thyroid tissue was devised. Viability of the canine thyroid tissue was assessed using two criteria: (1) the ability of the thyroid tissue to take up ^{125}I in vitro and (2) evidence of graft survival after concomitant transplantation in nude mice.

Considerable variation has been found in the ability of fresh, native canine thyroid tissue to take up ^{125}I [17]. Therefore, a decrease in the uptake of ^{125}I after HOC was expressed as a percentage of the value determined for the thyroid tissue sample after culturing in room air under normal pressure. In a preliminary experiment, canine thyroid tissue specimens ($n = 4$) were incubated for 48 h with the poison NaCN (1.25%) in room air under ambient pressure. ^{125}I uptake was suppressed to 11% of that in controls cultured under similar conditions without NaCN, demonstrating that loss of viability could be adequately detected by decreased ^{125}I uptake.

The results of the in vitro ^{125}I uptake assay and the in vivo study in nude mice are shown in Fig. 2. HOC of canine thyroid tissue for 48 h resulted in a significant decrease in ^{125}I uptake (up to 74%) compared with tissue cultured in room air under ambient pressure. The same thyroid tissue transplanted into nude mice did not survive, as shown from their inability to concentrate ^{125}I (mean ratio 0.93) and from histological evidence indicating only some residual scar tissue at the implant site. The thyroid tissue cultured in room air under ambient pressure survived in the nude mice (mean ratio 24.5 ± 4.7) with histological evidence indicating viable and intact thyroid graft tissue. Thyroid tissue cultured in pressurized room air (1.76 kg/cm^2) for 48 h resulted in a decrease in ^{125}I uptake (27%) and, after transplantation, showed a lower ratio (mean ratio 11.6 ± 2.7) as compared with tissue cultured in room air under ambient pressure. These grafts, however, had all survived at histological examination. These results show that the ability of the thyroid tissue to take up ^{125}I decreases with enhanced exposure of the thyroid tissue to oxygen, and this decrease is associated with reduced function of the thyroid tissue in vivo.

Superoxide dismutase, catalase and malondialdehyde determinations

CAT activity in murine thyroid tissue was comparable to the amount of activity found in dog thyroid (means 14.82 and 6.3–10.8 mm/mg protein respectively; Table 3). An additional assay of CAT activity in murine thyroid tissue exposed to 48 h normothermic HOC showed no significant increase in CAT activity (means 17.83 vs 14.82 mm/mg protein, respectively). In canine thyroid tissue, CAT activity was increased after 48 h HOC (20.4 vs 6.3–10.8 mm/mg protein).

SOD activity was low or hardly detectable in canine thyroid tissue (means 0.01–0.29 U/mg protein; Table 3). In murine thyroid tissue, SOD activity was considerably greater (4.75 U/mg protein). After 48 h of normothermic HOC, SOD activity was increased in canine thyroid tissue whereas in murine thyroid tissue SOD activity was not altered (1.37 and 5.03 U/mg protein, respectively).

MDA content of canine thyroid tissue was markedly elevated after 48 h normothermic HOC, with an average of an eight-fold increase compared with non-cultured thy-

Table 3. Results of catalase (CAT), superoxide dismutase (SOD) and malondialdehyde (MDA) assays in canine and murine thyroid tissue (± SEM)

	No. of samples	Dog thyroid				Mouse thyroid	
		Fresh			After HOC[a]	Fresh	After HOC[a]
		Dog 1	Dog 2	Dog 3	Dog 3		
CAT (mm/mg protein)	4	6.3 ±2.3	6.8 ± 1.7	10.8±2.6	20.4±1.9	14.82	17.83
SOD (U/mg protein)	4	0.29±0.17	0.49± 0.30	0.01	1.37	4.75	5.03
MDA (nmol/g)	2		269.5 ±40.9		2215 ± 198.5	301 ±28	1275 ± 65

[a] Hyperbaric oxygen culture (95% O_2: 5% CO_2, 25 psi, 37°C, 48 h)

roid tissue (means 2215 nmol/g after HOC vs 269 nmol/g in non-cultured thyroid tissue; Table 3). Normothermic culture of canine thyroid tissue in room air at ambient pressure showed only a two-fold increase in MDA content (results not shown). Murine thyroid tissue showed a four-fold increase in MDA after 48 h of normothermic HOC (means 1275 nmol/g after HOC vs 301 nmol/g in non-cultured thyroid tissue; Table 3).

Discussion

HOC at normothermic temperature is an effective method for immunoaltering murine thyroid gland tissue [6, 15, 16]. The altered immunoreactivity of the thyroid tissue is supposedly the result of inactivation of tissue passenger leukocytes and alteration of class I HLA antigens [6]. Evidence that this method of allograft modification is effective has been derived from studies performed exclusively in rodents. Before testing HOC with solid organs, it is necessary to determine if the same method of immunoalteration can be successfully applied using thyroid tissue of a higher species such as the dog.

In this study, HOC was injurious to canine thyroid tissue under the same conditions required to accomplish immunoalteration in murine thyroid tissue under normothermia. The loss of viability of canine thyroid tissue appeared to be related to the duration of exposure to normothermic HOC. Hypothermia, on the other hand, attenuated the injurious effects of HOC on canine thyroid tissue. Apparently, the processes that are activated by HOC, and cause the adverse effects in canine thyroid tissue, are suppressed under hypothermia.

Canine thyroid tissue that remained viable after normothermic HOC (24 h) and hypothermic HOC (7 days) was rejected within 21 days, indicating that prolongation of allograft survival did not occur as in mice. In this connection, it should be noted that in the mouse studies, two genetically defined, inbred strains were used with a full MHC difference, whereas the dog study used outbred dogs and so was uncontrolled in this respect.

Exposure of canine thyroid tissue to 95% O_2 under ambient pressure for 48 h (37°C) resulted in the peripheral thyroid follicles being damaged, whereas the central follicles remained viable. When 95% O_2 was combined with high pressure (1.76 kg/cm^2), the centrally located thyroid follicles were also injured. Reduction in the oxygen concentration, such as with room air alone, also resulted in necrosis of only the central thyroid follicles, presumably because of the lack of sufficient oxygen concentration centrally in the tissue. A similar sequence of events has been described for thyroid mini-organs in culture [2]. These observations suggest that there is only a thin line between the beneficial effects and the adverse effects of oxygen as far as canine thyroid gland tissue is concerned.

The results of the viability assay showed a correlation between the ability of the thyroid tissue to take up ^{125}I in vitro, and the uptake of ^{125}I by the grafted thyroid tissue in vivo in nude mice after 21 days of implantation. The in vitro assay of ^{125}I uptake may, therefore, provide a useful tool for screening thyroid graft viability before testing the tissue in the transplant model. It is emphasized, however, that for comparative studies the same source of thyroid tissue should be used, since the ability of native thyroid tissue to take up ^{125}I may vary considerably between dogs [17].

An increase in oxygen concentration during culture was associated with a decrease in uptake of ^{125}I in vitro, and decreased viability in vivo. A decrease in ^{125}I uptake by canine thyroid tissue in vitro could likewise be obtained by adding the poison NaCN to the culture in room air and ambient pressure, suggesting that a toxic effect of the high-oxygen environment of HOC was responsible for cell death. These findings strongly suggest the involvement of oxygen free radicals in HOC. Increased oxygen concentrations are associated with the generation of more free radical species and an increase in H_2O_2 formation [4]. The injurious effects of oxygen free radicals, ultimately resulting in cell death, and the protective antioxidant mechanisms on which cell systems rely, have been extensively reviewed [5, 12]. During HOC, the antioxidant defenses of thyroid tissue are confronted with an increased rate of oxygen free radical production. The resistance of the thyroid tissue to HOC may, therefore, largely depend on the availability of naturally occurring antioxidant systems, of which the combination of SOD and CAT is a major representative. Marklund [8] pointed out that the content of extracellular SOD differs among mammalian species. The overall extracellular SOD content was distinctly less in dog tissues than in mouse tissues. This notion seems to offer at least one explanation for the divergent interspecies sensitivity to HOC observed in these studies.

The SOD activity in murine thyroid tissue was clearly higher than in dog thyroid tissue, suggesting that the murine tissue was more protected against the injurious effects of HOC. The activity of CAT and SOD in murine thyroid tissue did not increase in response to 48 h normothermic HOC, indicating that the quantity of endogenous CAT and SOD available in the murine tissue was apparently sufficient to counteract the oxidative stress of HOC. In canine thyroid tissue, however, both CAT and SOD activities were increased following 48 h of normothermic HOC.

Lipid peroxidation is an oxygen free radical-mediated process implicated in cell injury through oxidative degradation of unsaturated lipids occurring in the plasma membrane. One of the by-products of this peroxidation process is MDA [5]. The increase in MDA found in canine thyroid tissue following 48 h HOC largely surpassed the increase in MDA measured in murine thyroid tissue under similar HOC conditions. This difference may reflect the smaller amount of lipid peroxidation induced by HOC in murine thyroid tissue due to the greater activity of oxygen free radical scavengers. In a previous study focussing on hypothermic HOC of murine thyroid gland tissue, we showed that the presence of oxygen free radical scavengers in the culture medium (UW solution) inhibited immunoalteration [16]. The evidence presented in this study further supports the notion that an oxygen free radical-mediated mechanism is involved in immunoalteration of murine thyroid gland tissue.

Acknowledgement. The authors wish to thank Mrs. Barbara Imm for her technical assistance during the determinations of superoxide dismutase and catalase.

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