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INTRODUCTION

Studies by the Naval Medical Research Institute on humans after prolonged antarctic residence have shown possible intracellular thyroid hormone alterations. These studies demonstrated a rise in serum clearance of orally administered triiodothyronine (T_3), T_3 production and T_3 total volume distribution in naval personnel after a 42 week residence in Antarctica compared to a control period in California (21). The increased serum clearance and total pool of T_3 indicated a possible intracellular response which could not be further investigated using human subjects. An animal model permitted prolonged confinement of subjects in temperature controlled chambers and the use of radioisotopes to label T_3.

Pigs are a suitable substitute for humans being anatomically and physiologically similar. They have furless skin, little or no brown fat and depend, in part, on shivering thermogenesis for cold tolerance (11) as do humans. Also pigs have demonstrated physiological adjustments for survival in the cold (19). Rats, however, which have been used extensively for cold studies, have demonstrated an adaptive non-shivering thermogenesis associated with modulation of brown fat by the sympathetic nervous system, under permissive control of thyroid hormone (2), but with a different peripheral profile during prolonged cold exposure compared to humans (20).

The purpose of this study has been to develop a suitable porcine model in order to investigate the effects of prolonged exposure to cold or heat on T_3, its response, distribution and intracellular kinetics, and its physiological consequences.

MATERIALS AND METHODS

This cooperative study between the University of Alberta and the Naval Medical Research Institute (NMRI) has been approached in two studies over a two year period.

1990 STUDY

Twelve young-adult male pigs, (Camborough, Pig Improvement Canada Ltd.) 48-68 kg in weight were confined individually in stainless steel metabolism crates, six to each chamber, throughout the experimental period. The crates had a floor area of $1.05m^2$ constructed of metal rods 12.7cm apart, 65cm from the chamber floors, with plexiglass sides. The chambers had air volumes of $143m^3$ (warm) and $95m^3$ (cold). They received a standard pig grower ration (16% crude protein, 3241 Kcal/Kg gross energy, 92% dry matter) ad-lib and free access to water. Lights were maintained at 12 hours on, 12 hours off. The pigs were preconditioned to the experimental surroundings and human handling before any measurements were taken. During preconditioning, and for two further weeks when measurements commenced, the climate chambers were maintained at 22°C. A transition week followed, during which the cold chamber temperature was lowered steadily to reach 5°C. The chambers were then maintained at 5°C (cold) and 22°C (warm) for six weeks.

Food intake was recorded daily and the pigs were weighed weekly. Oxygen consumption, measured using an open circuit calorimetry system, was recorded for

selected animals in rotation throughout the experiment. The animal and its metabolic crate were enclosed in an air-tight plastic hood. From the flow of air from the hood, and the composition of the air entering and leaving the hood, the level of oxygen uptake and from this, the metabolic heat production and energy balance could be calculated.

During week four the pigs were fasted for 24 hours and blood samples were taken for hormone assay. The pigs then underwent surgery under general anaesthesia, using 1 ml/kg bodyweight of Stresnil I.M. to sedate followed by 1 ml/kg bodyweight Hypnodil I.V. and a 2% Halothane oxygen gas mixture, to insert two Silastic catheters into the external jugular vein. Two catheters were required one long, 28cm insertion for injection, the other short, 23cm insertion for sampling. Postoperative recovery was observed before experimental procedures continued. Catheters were flushed daily with 2 ml of heparinized saline, 20 i.u./ml, to keep them patent.

Blood samples were taken after recovery from surgery. These and the fasted pre-surgery samples were allowed to clot, centrifuged (1000 x g, 10 minutes) and the separated serum was assayed for total T_3 (tT_3), free T_3 (fT_3), total T_4 (tT_4), free T4 (fT_4), and thyroid stimulating hormone (TSH) using Coat-a-Count Radioimmunoassay kits (Diagnostic Products Corp. Los Angeles, CA.). Hematocrits were measured by micro-centrifugation.

Body composition and plasma volume were measured during the fifth week of treatment. A bolus of tritiated water, $3H_2O$ (New England Nuclear, Wilmington, DE; specific activity 70.27 micro Ci/g) 300 uCi/pig and a known concentration of Evans Blue dye expressed as a dose per litre, were injected into each animal. Timed serum samples were collected pre injection and at 2, 6, and 24 hours after. The samples were counted in a Packard Tri-carb counter to determine $3H_2O$ activity, and Evans Blue dye concentration was measured in a spectrophotometer. Total body water, from the $3H_2O$, and plasma volume from the Evans Blue dye, were calculated by extrapolation of these concentrations to time of injection. Body composition including water, protein, fat, and ash content were also calculated, using the equations of Ferrel and Cornelius (7).

T_3 kinetics studies to determine the serum clearance rate of T_3 were carried out on four pigs a day, two from the cold chamber, two from the warm, during week five. On the day prior to injection each pig received 250 mg of potassium iodide on apple slices twice daily, to block thyroid gland uptake of iodine. The isotope, L-3,5,3 '-[^{125}I] - triiodothyronine (New England Nuclear, Wilmington, DE; specific activity 2200 Ci/mmol) was sterilized by filtration through a 0.2 micrometer filter (Milipore corp, Bedford, MA). High pressure liquid chromatography showed there to be less than 2% free or organic iodide contaminants. Five minutes before injection the radiolabelled [^{125}I] T_3 was diluted to 5 ml with 1% autologous serum and administered to each animal as a bolus injection of 80uCi. This was flushed with an equal volume of heparinized saline. Blood samples of 6ml were taken at 0.08, 0.25, 0.50, 0.75, 1.0, 1.5, 2.0, 2.5, 3.0, 4.0, 8.0, 24 and 48 hours after injection. Samples were allowed to clot and serum was separated by centrifugation (1000 x g, 10 minutes) and stored at -70°C. The extraction and counting of labelled T_3 was carried out at the Naval Medical Research Institute.

T_3 distribution was measured in four pigs per day during week six, four or five days after receiving their first [^{125}I]T_3 injection. There is virtually complete body clearance of [^{125}I]T_3 in the pig by 72 hours. A blood sample was taken prior to injection of 80uCi into each animal as for the kinetics studies.

The animals received 250mg of potassium iodide twice daily as before. Each pig was sacrificed four hours following the $[^{125}I]T_3$ administration by injection of 0.3mg/kg bodyweight T-61 (Hoechst Regina, Sask.). The animal was then dissected and the wet weights of various organs were recorded. Samples were taken from a range of tissues and organs and were weighed, dried to determine % dry matter (DM) and counted in the Packard Tri-carb counter. The samples were later analyzed using kjeldahl digestion to determine crude protein and ether extraction to determine fat content (1). Samples of liver and thyroid gland were also taken and frozen at -70°C within 20 minutes of sacrifice. The measurements of I-5'D kinetic parameters were carried out at the Naval Medical Research Institute.

1991 STUDY

Sixteen young-adult male pigs 46-69kg in weight were confined individually in metabolism crates, six in two of the chambers and four in the third. The warm chamber and cold chamber were as previously described. The hot chamber had an air volume of $76m^3$. The animals received a lower energy diet than the first year, grower mix diluted with 50% alfalfa (12% crude protein, 2646 Kcal/Kg digestible energy, 88% dry matter), to slow their growth rates slightly.

The animals received two week pre-conditioning followed by two weeks when all chambers were kept at 22°C. During this period body composition was measured using $3H_2O$. A second measure of this was made during week six of the temperature treatment. Food intake was recorded daily, and for one full week all faeces were collected and weighed, and samples were taken for analysis. Dry matter % of feed and faeces were calculated, and the energy content of feed and faeces was measured using a bomb calorimeter. From this data, energy balance or feed digestibility, energy going in per gram DM, minus energy lost per gram DM, could be calculated. A second digestibility study was conducted during week four of temperature treatment. Oxygen consumption was measured on selected animals throughout the experiment as before. After a transition week when temperatures were steadily lowered or raised, the three chambers were maintained at 22°C (warm), 5°C (cold) and 40°C (hot) for seven weeks.

During weeks one to four of temperature treatment three animals at once underwent acute hot air tests (AHATs) or acute cold air tests (ACATs) in rotation. Control measurements of rectal temperature, side temperature, ear temperature, heart rate and respiration rate were made on one animal from each treatment in its own environment. For an AHAT, the pig from the warm and pig from the cold chamber were put into mobile metabolic crates and moved into the hot room. The same physiological measurements were made on all three animals in the hot, with the hot pig acting as control, after 30 minutes and 60 minutes. The animals were then returned to their own chambers. For an ACAT, the pig from the warm and the pig from the hot chamber were moved into the cold chamber for an hour. The change in the physiological parameters measured during an acute (one hour) exposure to an extreme in temperature could then be calculated.

The animals underwent surgery under general anaesthesia as before except that the injection and sampling catheters were inserted one into each of the external cephalic and internal cephalic veins. These lead into the jugular vein and in effect the catheters were in the same location as before. The catheters were separated into two veins and the cephalic cannulation, less tissue invasive than the jugular cannulation, was used to minimise risk of infection due to surgery.

In week six of treatment blood samples were taken from each pig immediately

before and after a 24 hour fast. The serum was separated and assayed for tT_3, fT_3, tT_4, fT_4, as for the first year samples, and also assayed for testosterone concentration. All assays were done using Coat-a-Count radioimmunoassay kits. Blood samples taken at this time were also sent to a commercial medical laboratory for blood characterisation and metabolite analysis including white and red blood cell count, hemoglobin level, calcium, phosphorous, glucose, protein, urea and cholesterol concentration.

The T_3 kinetics study was carried out during week six using the methods described for year one. This was followed by a T_3 distribution study as before for which the pigs were sacrificed. Samples of muscle (biceps) and liver were removed from each animal within minutes of sacrifice and stored for a minimum length of time in krebs bicarbonate buffer. Live tissue oxygen consumption was the measured on these samples using a Clark cell apparatus (Yellow Springs International).

Analysis of treatment effects was undertaken by analysis of variance using the SAS statistical package.

RESULTS

Group mean daily food intake was increased in the cold (+40% year 1), (+47% year 2) and decreased in the hot (-33%) (Figure 2, Figure 3). When total food intake was compared to total weight gain over the experimental period, it was seen that animals in the cold had the highest food intake for the lowest gain in bodyweight (Figure 4), a food conversion ratio of 0.13 compared to 0.24 and 0.25 in the warm and hot groups respectively.

There were no significant changes in body weight or composition over the time spent in the cold or in the hot (Figure 2, Figure 3, Figure 5). In the first year study, cold exposed animals had significantly heavier thyroid glands and kidneys, as a percentage of body weight (Table 2). No differences in organ weights were seen in the second year. No significant treatment differences were seen in the composition of liver, thyroid and muscle tissues measured in the first year study.

There were significant changes in the thyroid hormone levels. Serum concentrations were higher in the cold exposed animals (tT_3 +96% and +21%, fT_3 +119% and +36%, tT_4 +68% and +11%, fT_4 +49% and +7%), and lower in the hot group (tT_3 -44%, fT_3 -25%, tT_4 -11%, fT_4 -20%), compared to animals kept in the warm group (Figure 6). No change however was observed in TSH levels (Figure 7). Fasting was seen to reduce tT_3, fT_3, fT_4 and TSH in all animals over both years. In the hot treated animals tT_4 was also reduced. Within the fasted samples tT_3 tT_4 and fT_4 were significantly higher in the cold than in the warm (+110%, +52%, +100% respectively) during the first years study only (Figure 8). Animals in the hot room had lower fasted levels of tT_4 (-20%) and fT_4 (-31%) than those in the warm. Serum testosterone was lower in the cold exposed animals, (-83% fed, -20% fasted) (Figure 9). However fasting increased serum testosterone under all temperature treatments (+341% warm, +189% cold, +324% hot).

Mean group total oxygen consumption increased (+25%) in the cold exposed animals (Figure 7), and tissue oxygen consumption increased in muscle from the cold exposed animals and decreased in muscle from animals kept in the hot (Figure 10), however none of these observations were statistically significant. A slight reduction in food digestibility was also seen in the cold (Figure 10) but again this was not significant.

Total body water, blood volume and plasma volume were not altered by

temperature treatment (Figure 11) however hematocrit was seen to be significantly increased (+6%), by cold treatment. When blood characteristics and metabolite levels were measured no significant changes were seen in white blood cell count, calcium level, glucose level or urea level (Figure 12). However cold exposed animals were seen to have significantly higher red blood cell counts (+16%), hemoglobin levels (+13%), phosphorous levels (+14%) and albumen levels (+11%). Hot treatment was seen only to decrease albumen levels (-17%). Serum cholesterol levels were significantly higher (+21%) in the cold treated animals. Fasting was seen to increase cholesterol levels in all temperature treatments (+49% warm, +18% cold, +30% hot) (Figure 9).

After several weeks adaption to the different temperatures, little alteration in rectal temperature, as an indication of core temperature was seen, although a drop was measured in the cold during the first year study (Figure 13). Side temperature and ear temperature clearly fell (-13% and -23% side, -47% and -41% ear) in the cold exposed animals and rose (+21% side, +36% ear) in the hot exposed animals compared to those in the control, warm room. A small fall of heart rate (-14%) was seen in the hot animals. Respiration rate fell in the cold (-39%) and increased in the hot (+133%).

During the hour long acute cold tests, warm animals did not undergo any changes significantly different to the cold animals already present, in any of the physiological criteria (Figure 14). Animals from the hot room however on average showed significant decreases in rectal (-0.5°C), side (-12.8°C) ear (-22.1°C) temperature, and respiration rate (-59 breaths/min). During the acute hot tests, both animals from the warm room and cold room showed rises in rectal (+1.6 and +1.2°C), side (+6.9 and +15°C) ear (+9.7 and +21.7°C) temperature and respiration rate (+92 and +73 breaths/min), significantly higher than the heat adapted animals. The increased levels measured in the cold animals were also significantly greater than those in the warm animals. No changes were seen in heart rate, probably due to the large variation in response.

DISCUSSION

Many studies have shown that exposing animals to a fall in ambient temperature results in an increased rate of production and utilization of thyroid hormones (6,8,15), and exposing animals to an increased ambient temperature decreases rate of thyroid hormone production (15,3,14). This rise in the cold of thyroid hormones was seen in the first year study, and fall in the heat in the second year. The greatest increases and decreases were in thyroxine, T_3 levels, as the biologically active form. T_4 can be converted to T_3 by deiodination at the 5' position, so T_4 levels act as a buffer for rapid T_3 changes. No significant differences in thyroid hormone levels between cold and control groups were seen in the second year, although the trend was there. In the second year animals were fed a diet high in alfalfa in an attempt to reduce growth rates. This did not happen because the animals compensated by eating more. Food intake was therefore higher in the second study. The increased fibre in the diet generates more heat as a by-product of digestion (23) and this could have been used to reduce heat production requirements in the cold.

To meet the increased or decreased energy demand for heat production in the cold or hot, food intake will be increased or decreased when available (13,3,14,6). This was seen in our pigs. There is some debate as to whether it is the increased energy demand that causes the adjustment of food intake and that this in turn has an effect on thyroid hormone levels, or whether it is the

thyroid hormones that stimulate the food intake adjustment. Evans and Ingram (6) have suggested that the initial rise in thyroid hormone is in response to an initial increase in TSH, due to temperature receptor stimulation, but that the ability to increase food intake is what maintains elevated hormones and TSH returns to control levels via feedback. Our animals showed no response in serum TSH after several weeks in the cold. Also fasting caused a decrease in thyroid hormones in all temperature treatments, and in the second year study temperature treatment differences seen in T_3 hot pigs were removed. Animals on the second year study high fibre diet may have experienced calorific restriction which limited the thyroid hormone response to cold. Macari et al (18) concluded from their work that the adjustment in food intake during acclimation could be dependant on thyroid hormone levels, from looking at the response in thyroidectomized swine. The relationship between nutrient intake and thyroid function is clearly complex.

Food digestibility has also been shown to decrease in the cold (9) however this is less predictable in swine than in ruminants and was not observed in this study.

Associated with a rise in thyroid hormone levels is a rise in metabolic rate. This results in increased heat production to meet body needs in the cold. When this increase in energy demand is coupled with ad-lib food intake, body composition can be maintained. No changes in water, fat or protein content due to temperature treatment were seen in our study. Some workers have seen changes (5,10) when young or restrict fed swine were used. Our mean group body weights did not differ between treatments, however overall gain was reduced in the cold despite elevated food intake. Food conversion efficiency in the cold was low since extra energy was channelled into heat production rather than body growth. Some workers have seen changes in certain organ weights (24). In the first year study thyroid weight and kidney weight as a percentage of bodyweight were increased in the cold, possibly indicative of increased activity of these organs, however no changes were seen in the adrenals or liver. The fat water and protein content of muscle, liver and thyroid tissues were also unchanged by temperature. Ad-lib feeding probably protected these body tissues.

Total oxygen consumption of the animals also increases in the cold as metabolic rate rises (15) and muscle oxygen consumption has also been seen to increase (12). These trends were evident in our study but were not statistically significant. Hematocrit, red blood cell count and hemoglobin levels in the cold exposed animals were all increased. These mechanisms would all increase the efficiency of oxygen transport as demand increased. Heart rate would have also been expected to increase but this was not clear due to large variation in the data. Animals startled by handling would have had a higher heart rate anyway. Cold temperature has been known to reduce plasma volume and total body water. Vasoconstriction results in less plasma volume required for circulation. This also contributes to the increase seen in hematocrit. Some problems with methodology were probably responsible for the lack of change in body fluids observed. A depression in circulating lymphocytes and an increase in heterophilic granulocytes has been shown as a stress response in chickens (22). The ratio of these were analyzed in this study but no changes in white blood cells were observed in cold or heat.

Circulating albumen can act as a binding protein for thyroid hormones. A clear increase in the cold and decrease in the hot was seen, suggesting that the change in albumen level could have been associated with changes in thyroid hormones.

Testicular size and level of steroidogenesis can be reduced by adrenal corticosteroids acting to lower plasma interstitial cell stimulating hormone (16). In swine testosterone has been shown to respond differently to an acute change in corticosteroids as opposed to a longer term change (17). In the cold, long term stress may have caused the depression in circulating levels observed. When the animals were fasted for 24 hours, a dramatic increase in testosterone was seen in all temperature treatments. Injection of ACTH in boars has been shown to rapidly increase testosterone (17), and this rise may have been caused by the acute stress of fasting.

Thyroid hormones are known to increase both production and clearance of cholesterol. Fed animals in the cold showed higher circulating levels, whilst fasting animals showed no temperature effect. Fasting cholesterol was raised possibly because the fall in thyroid hormones reduced the rate of clearance, so despite lower production, on balance, serum levels were higher. General mobilization of body fat reserves could also have contributed to the higher levels in the cold.

Rectal temperature has been shown to decrease in cold acclimated pigs in some cases (12) but not in others (4). If the animals are old enough and have access to food they are more likely to maintain their core body temperature. A response in the cold was seen in the first year study but not in the second. A rise in rectal temperature was seen in the hot treatment group. Clear changes were measured in skin temperature, both on the flank and ear in the hot and cold. Lowering the temperature of the outer body shell effectively increases thermal insulation of the core (11). In cases where a fall in rectal temperature has occurred, it has been suggested that this could be a possible adaptive mechanism in the cold, and that a slight decrease in body temperature decreases the metabolic demand on the animal (11). Vasoconstriction draws blood away from the extremities, particularly the ears, where a large surface area in swine would have a high potential for heat loss. In the heat, skin surface, both on ear and flank, rose almost to rectal temperature as blood circulated close to the skin surface to maximise heat loss. Respiration rate increased in the hot group as the pigs were panting. Behavioral changes were seen. Animals in the hot room spent little time standing or moving and most of the time lying stretched out. Conversely pigs in the cold spent a lot of time on their feet or lying with legs tucked under to minimise exposed skin area.

When animals were put through the ACATs and AHATs, the magnitude of change in the physiological parameters was considered. For hot animals in AHATs and cold animals in ACATs this change should have been and was very small over the hour. Any changes measured were probably due to the limit of accuracy of the thermometers. In the ACATs, pigs from the warm group did not undergo significant changes in temperature over the hour. Animals from the hot group however did. The hot group respiration rate also fell in the cold. In AHATs both the warm and cold pigs underwent significant changes in rectal and skin temperature over the hour, with cold acclimated pigs showing the greatest change. The bigger the gradient in environmental change that the pigs were exposed to, the bigger was the response seen. The animals had acclimated to their treatment temperatures and had to activate rapid mechanisms to cope with the change in environment.

Overall both years of the study were successfully completed, using swine as a model to investigate the effects of prolonged exposure to cold and heat on thyroid function and its physiological consequences. Material for the joint part of the study on intracellular kinetics of thyroid hormone response was also successfully collected and processed at the Naval Medical Research Institute.

There is now a clearer picture of the consequences of stationing naval personnel for long periods in cold or hot climates, what the changes in biological demands will be and what stresses will be involved in moving them rapidly from one extreme climate to another.

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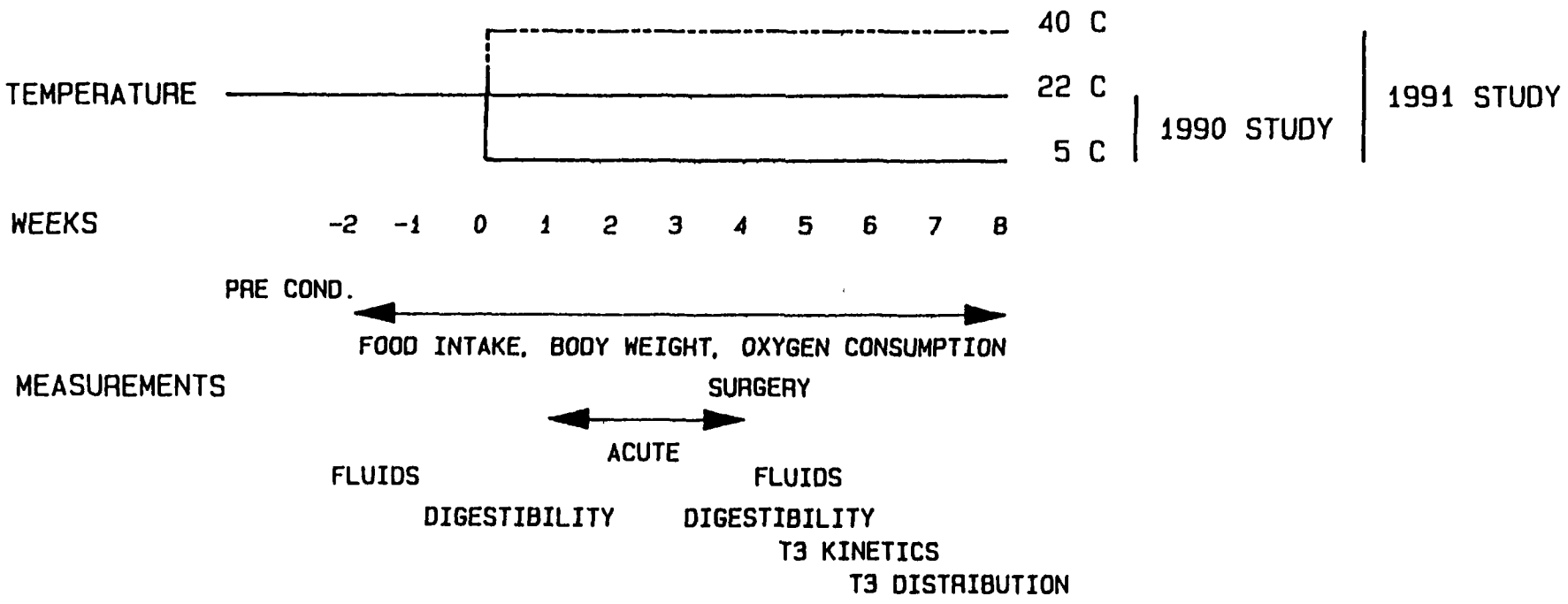
Figure 1

EXPERIMENTAL PLAN



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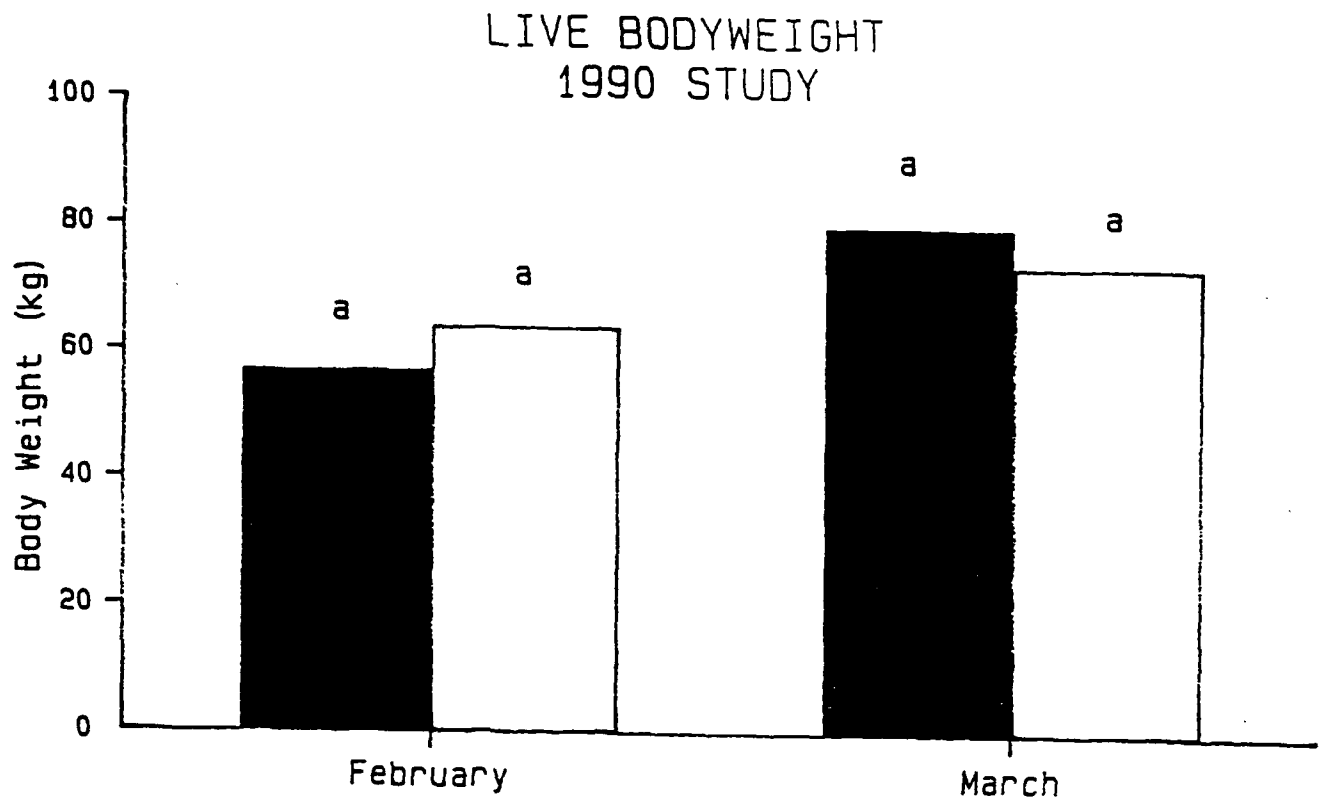

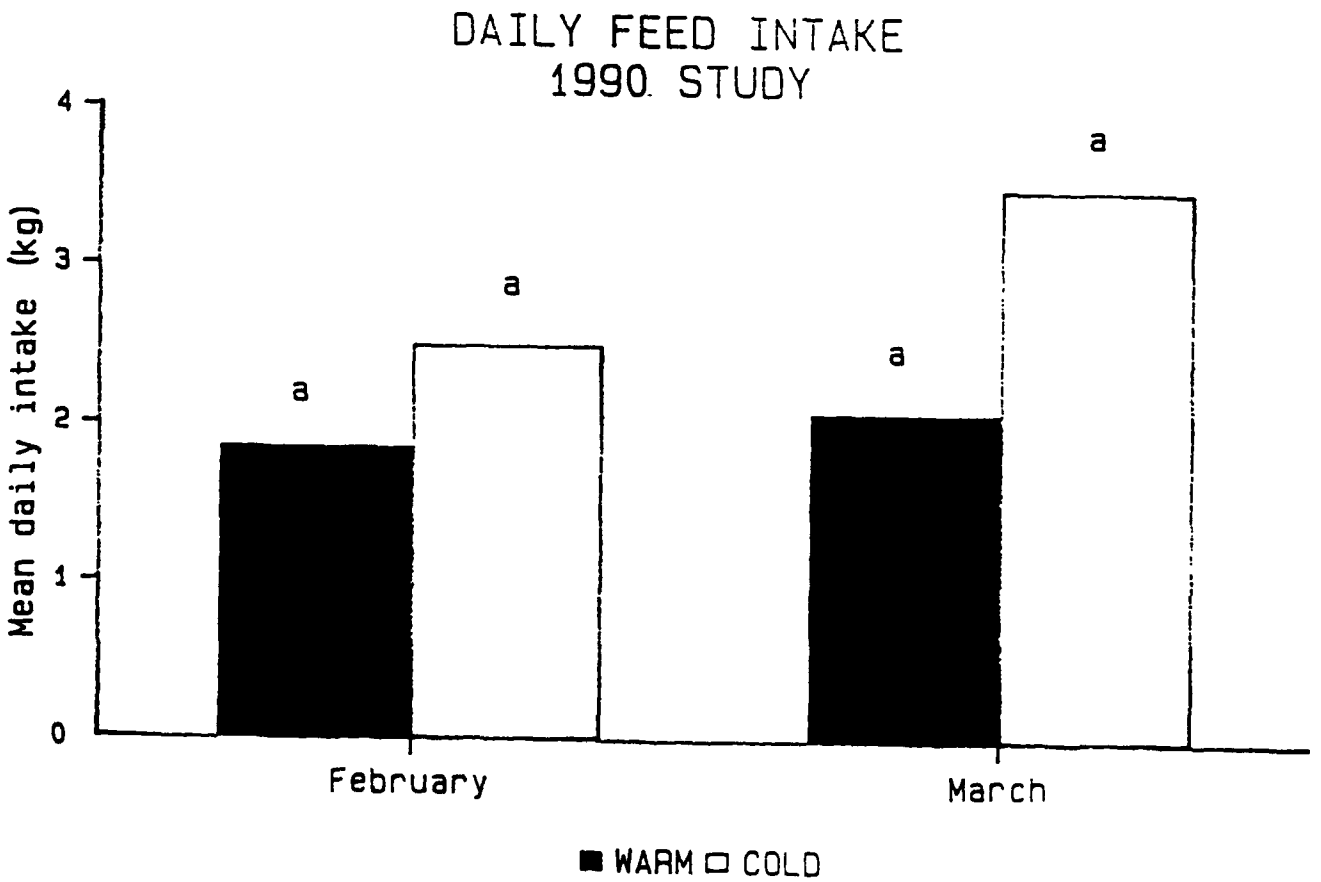

Different Superscripts Indicate Significance At P<0.05

Figure 3

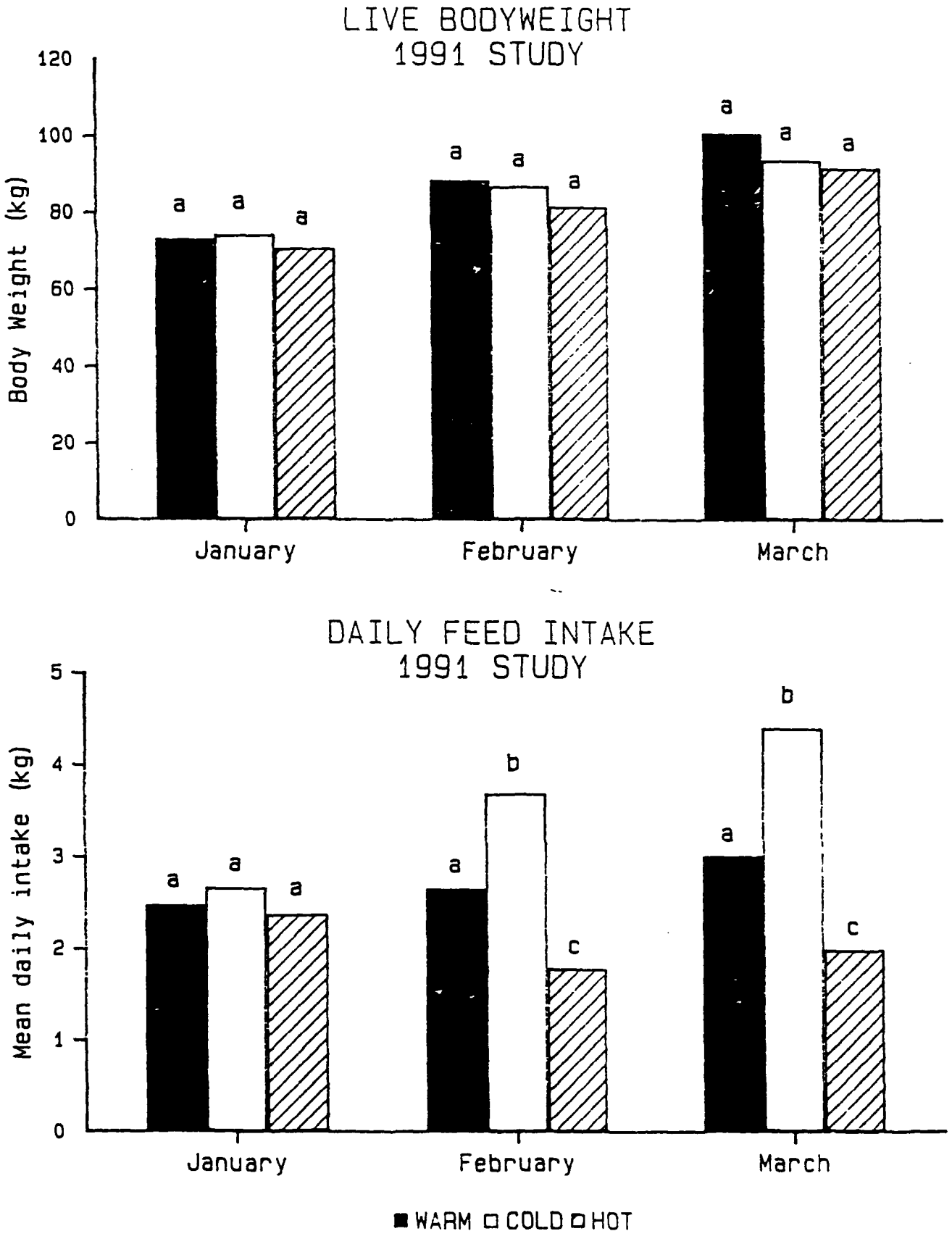

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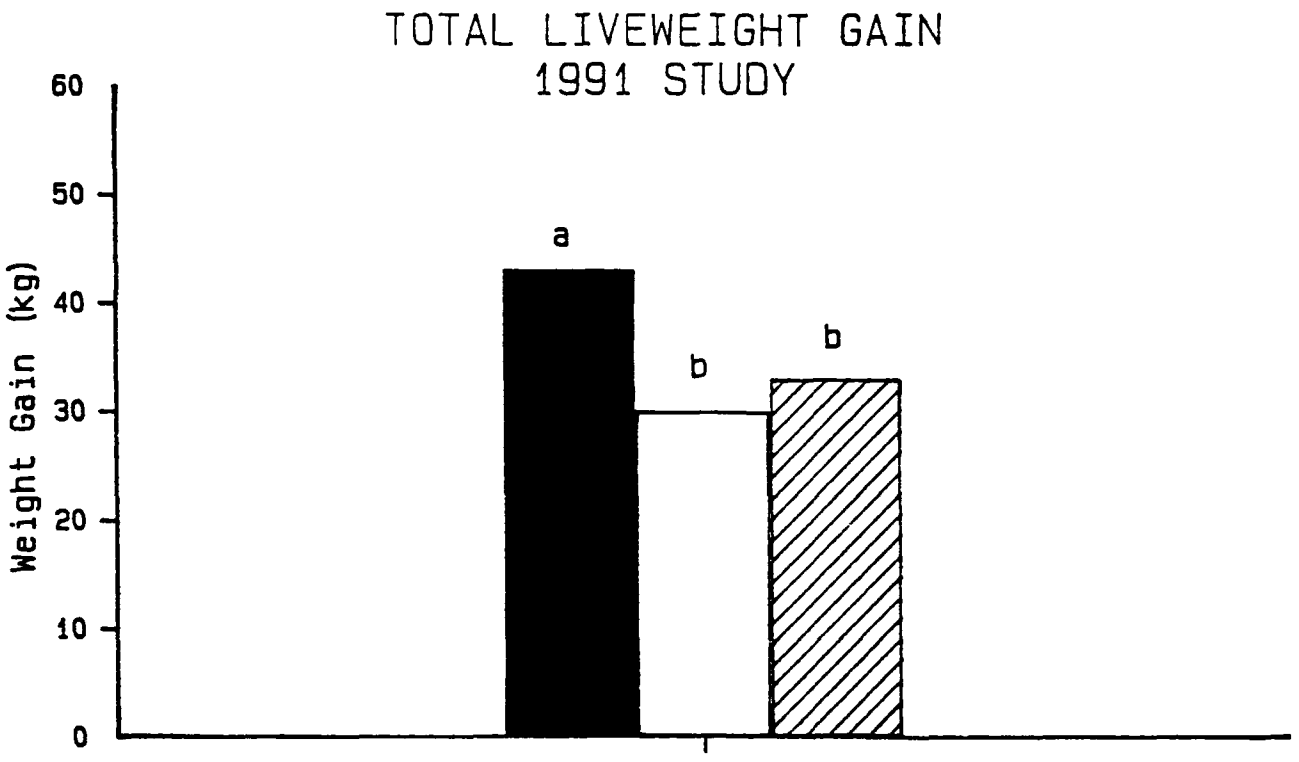
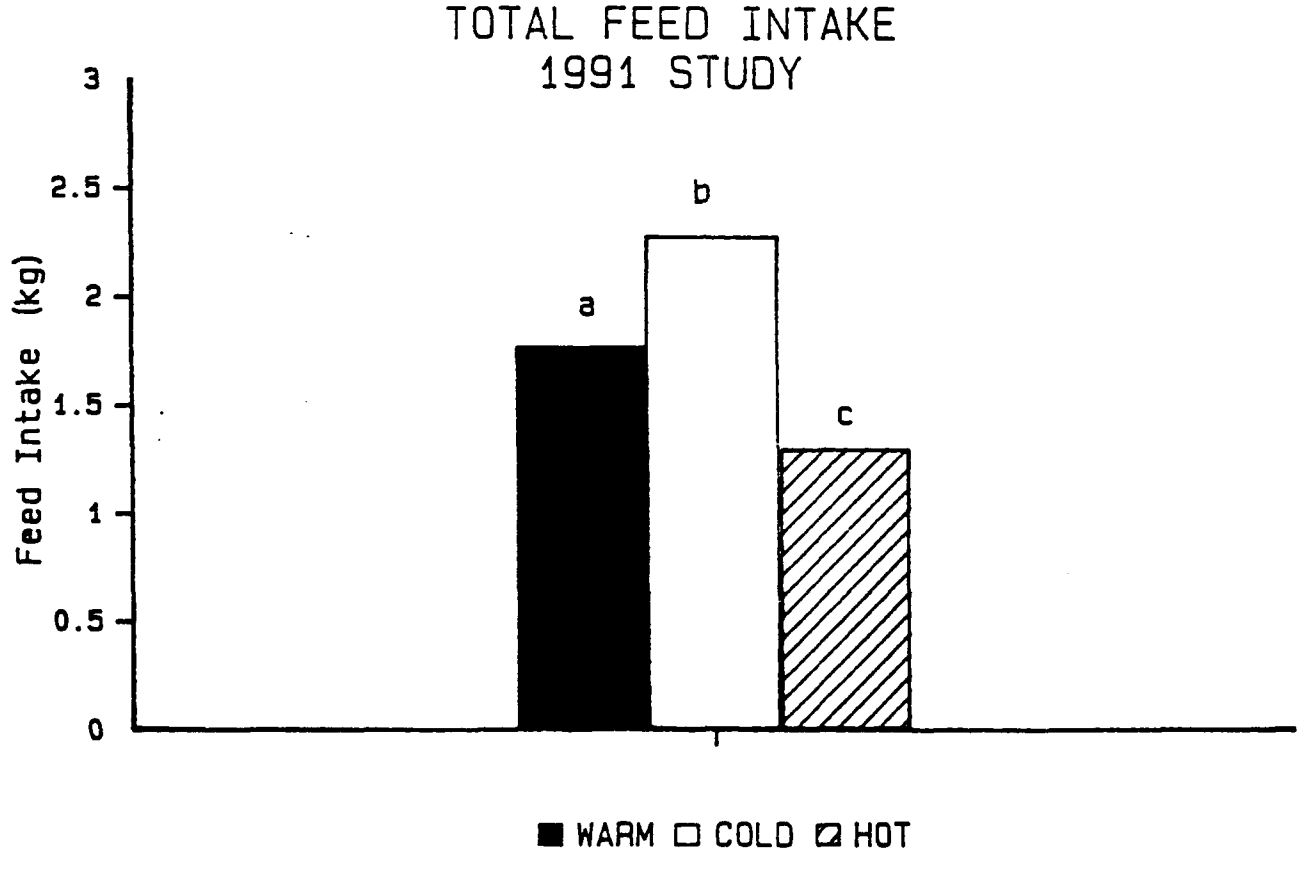
Figure 4

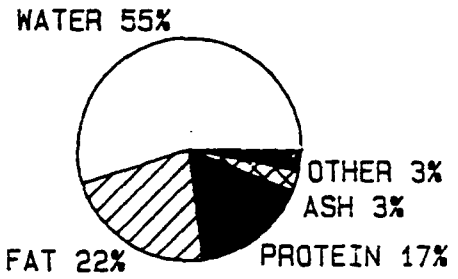




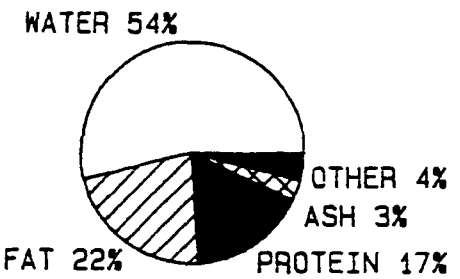
Different Superscripts Indicate Significance At P<0.05

. Figure 5

BODY COMPOSITION
1990 STUDY

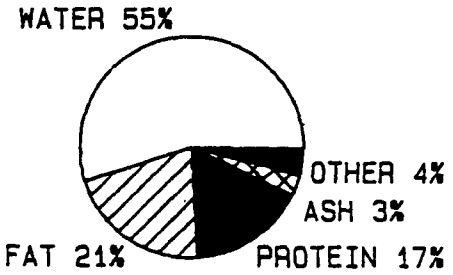


WARM

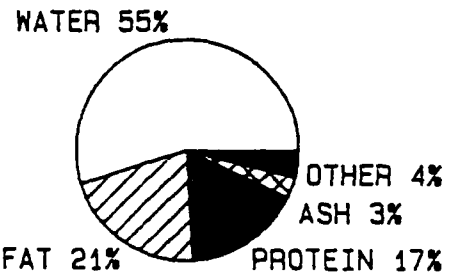


COLD

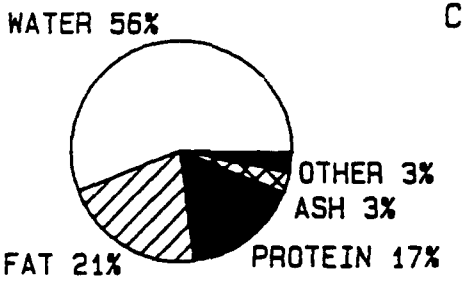
BODY COMPOSITION
1991 STUDY



WARM

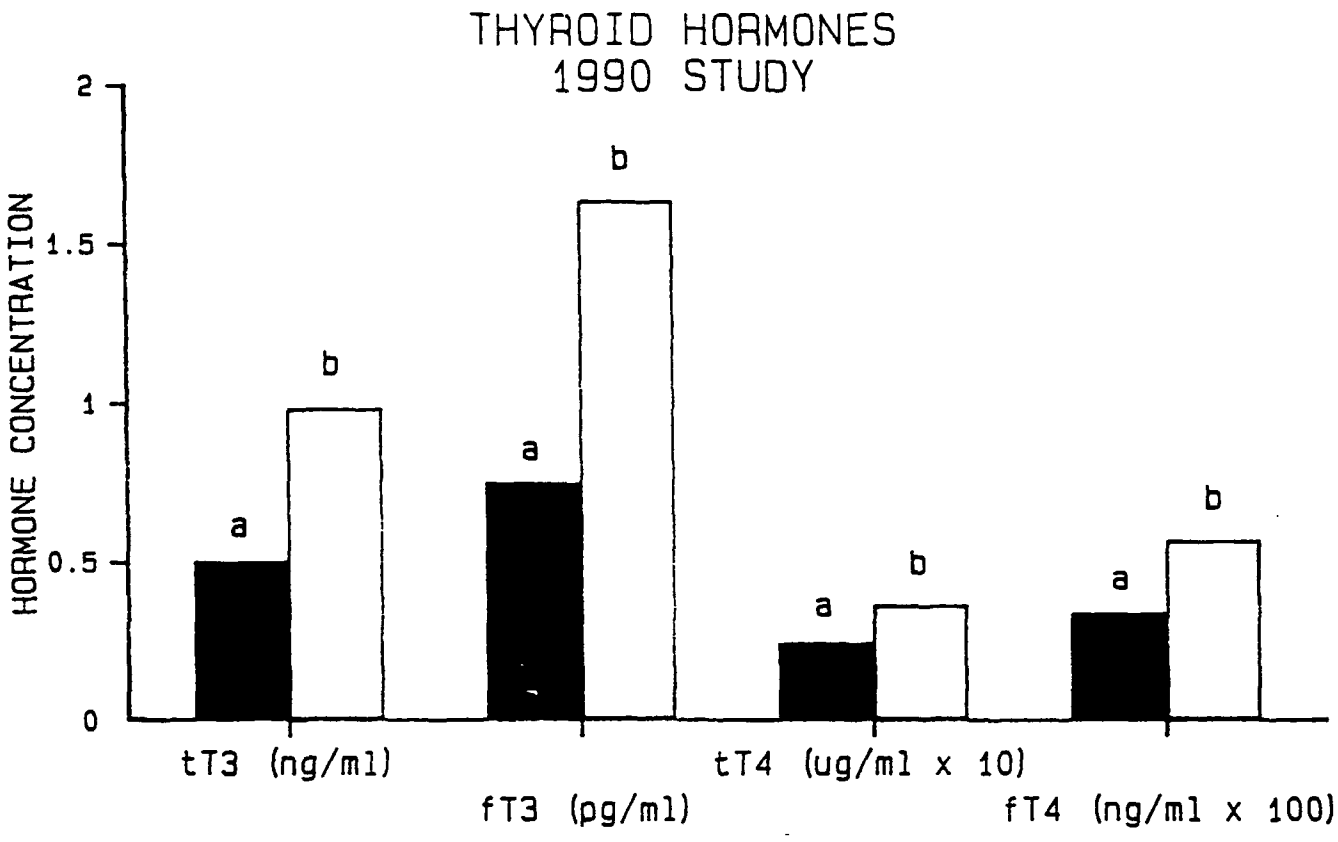


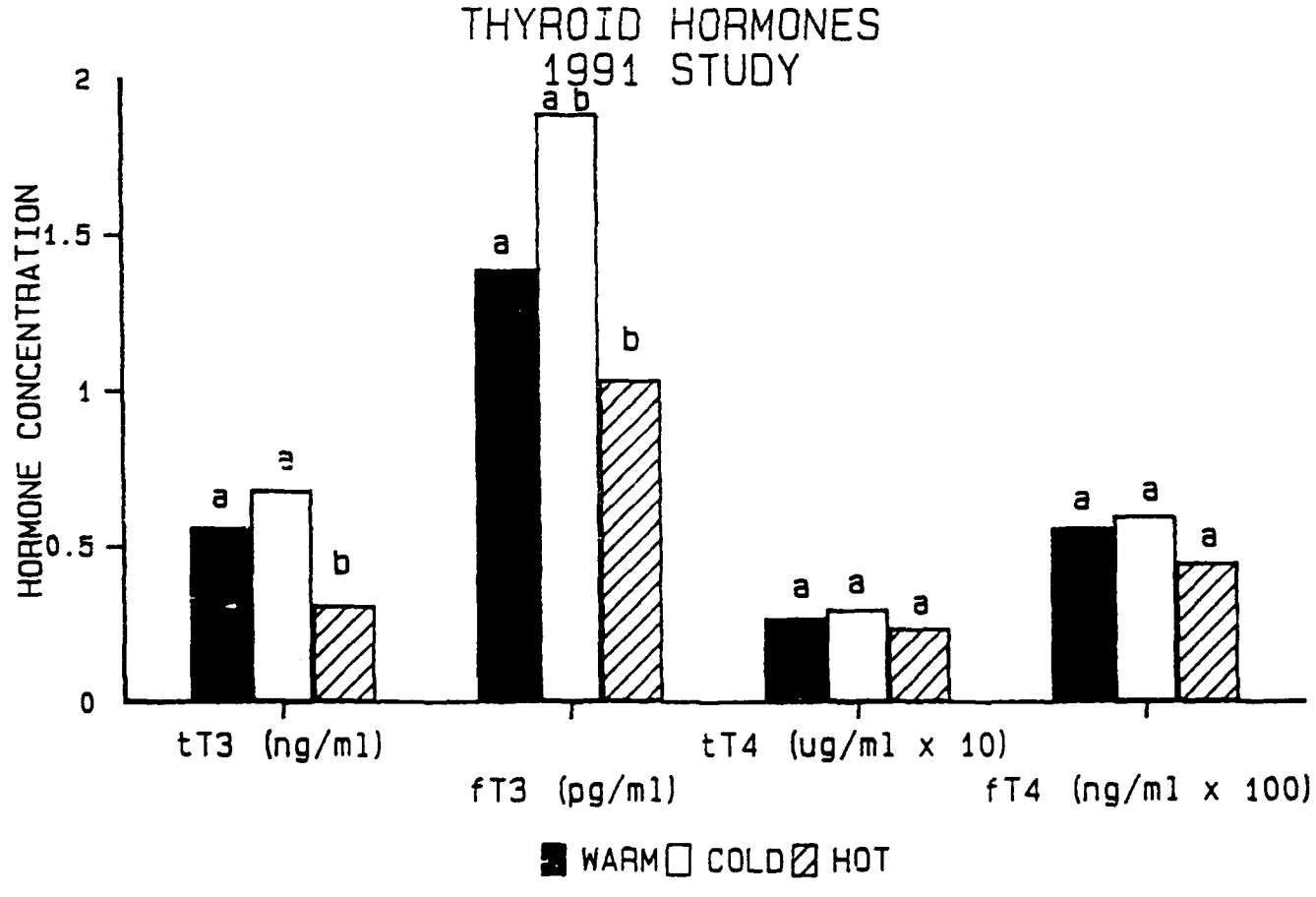
COLD



HOT

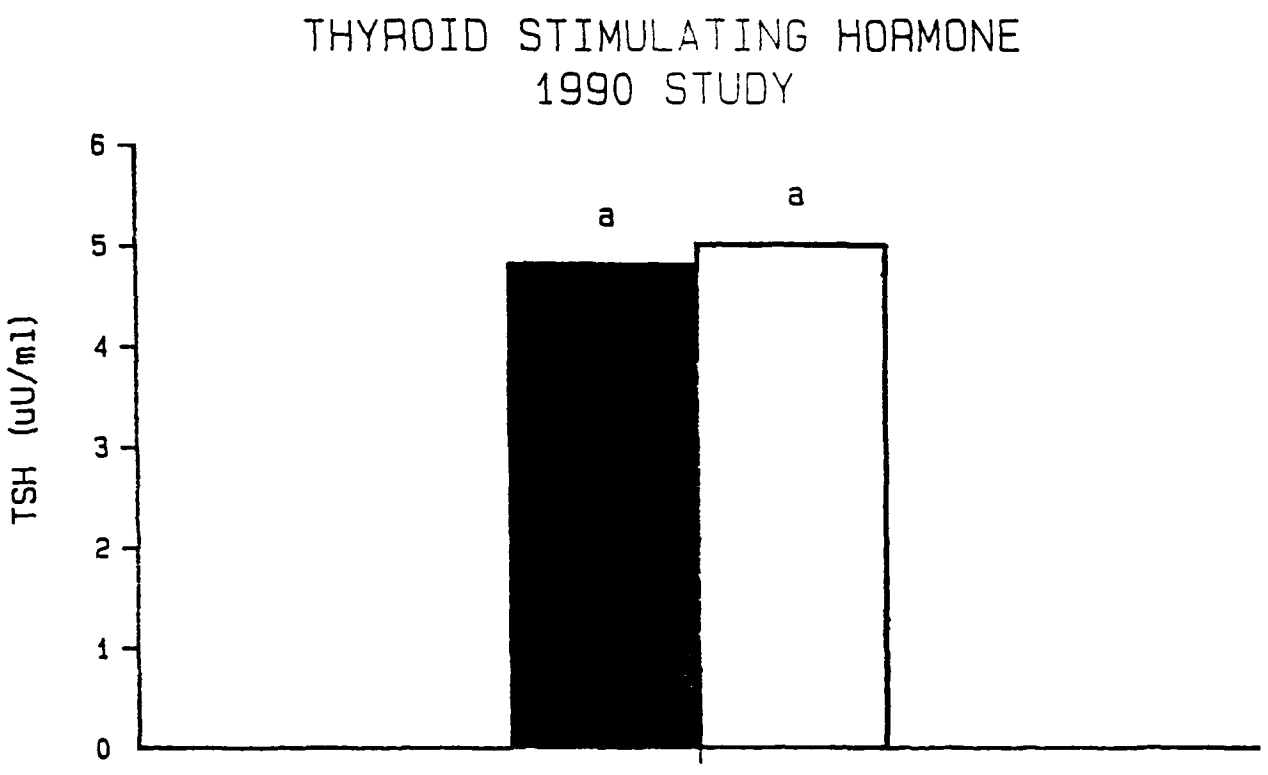
Figure 6

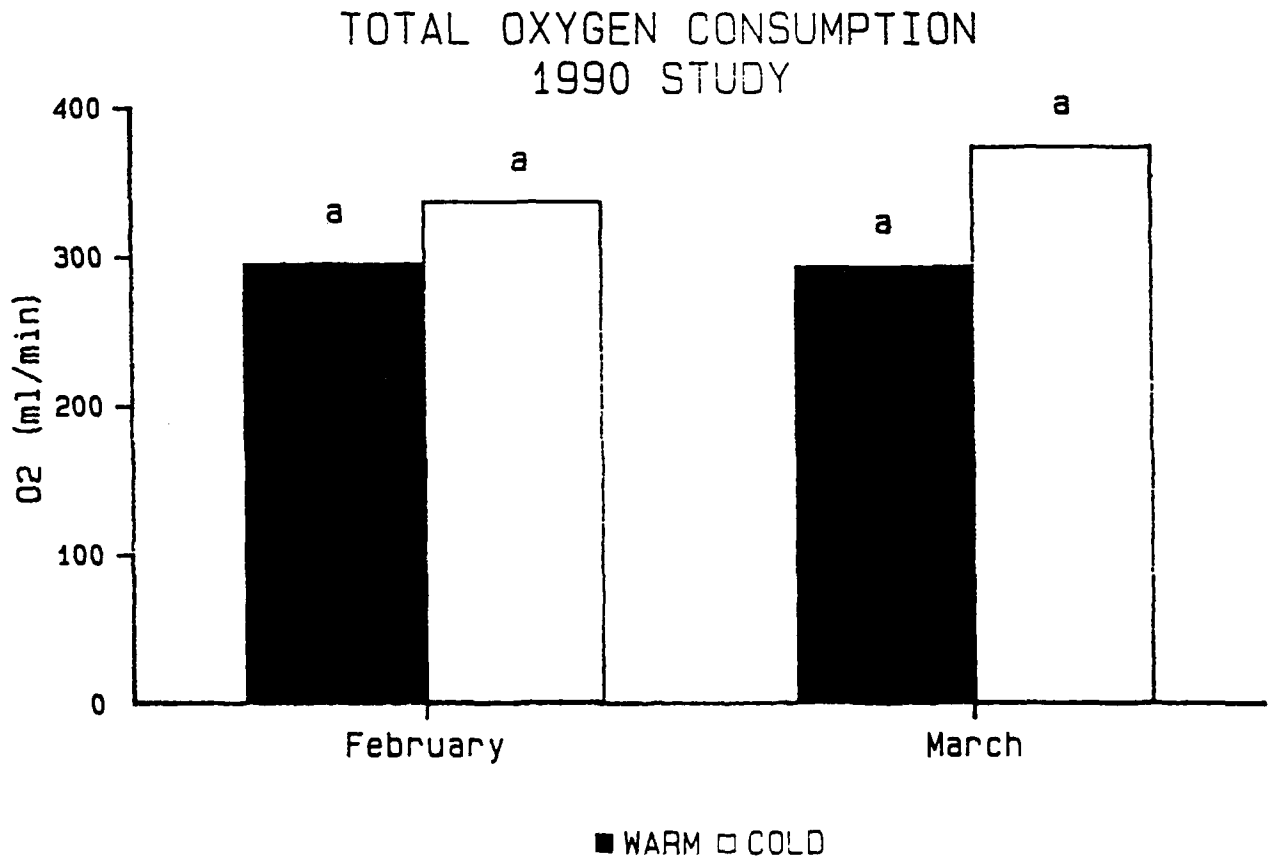




Different Superscripts Indicate Significance At P<0.05

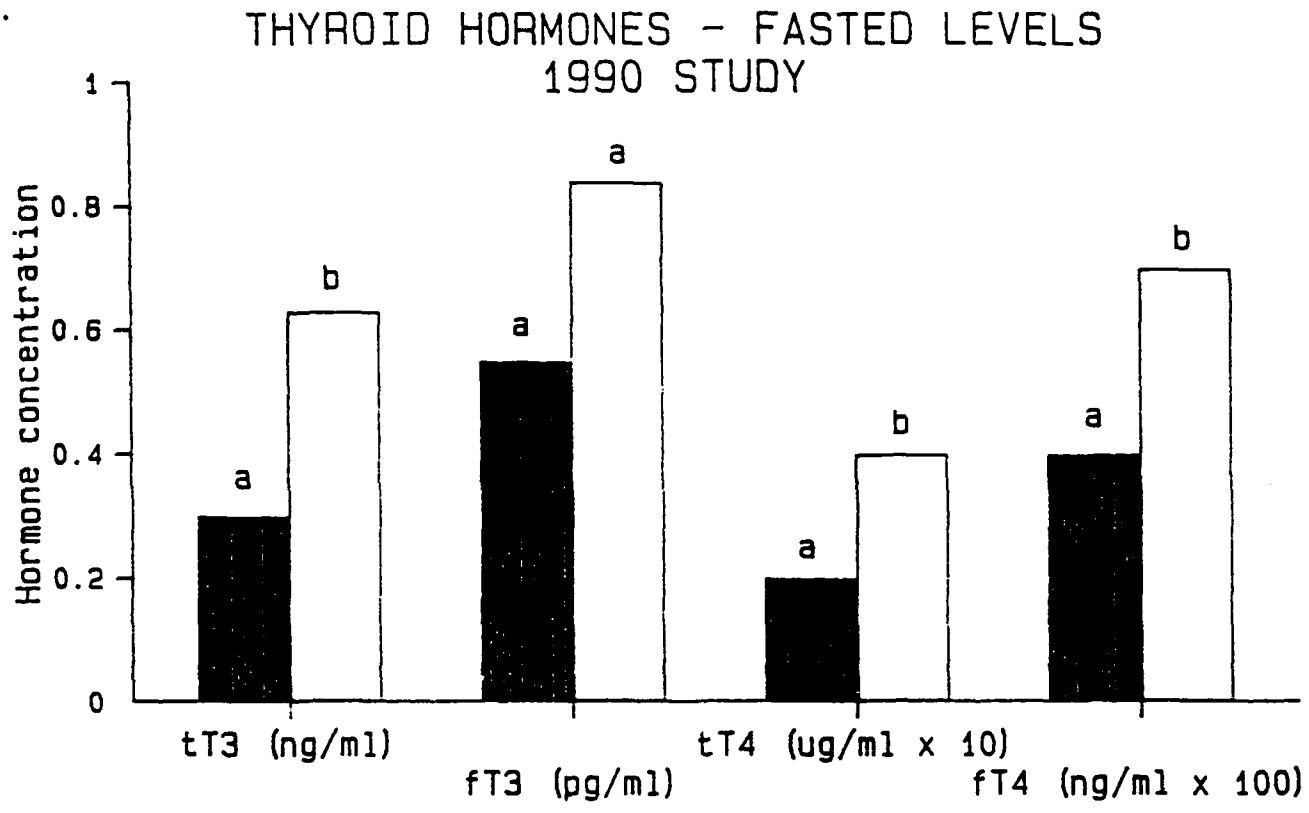
Figure 7

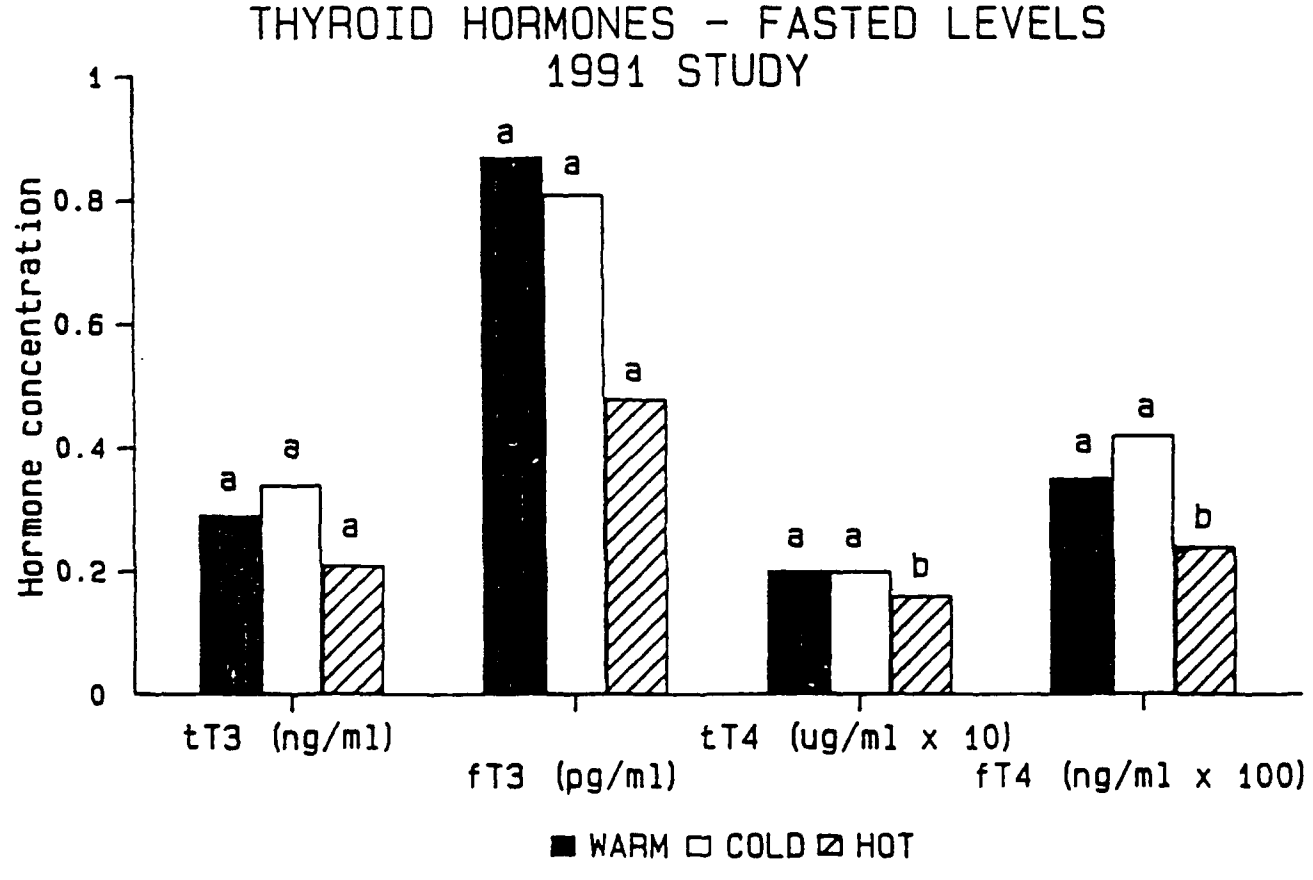




Different Superscripts Indicate Significance At P<0.05

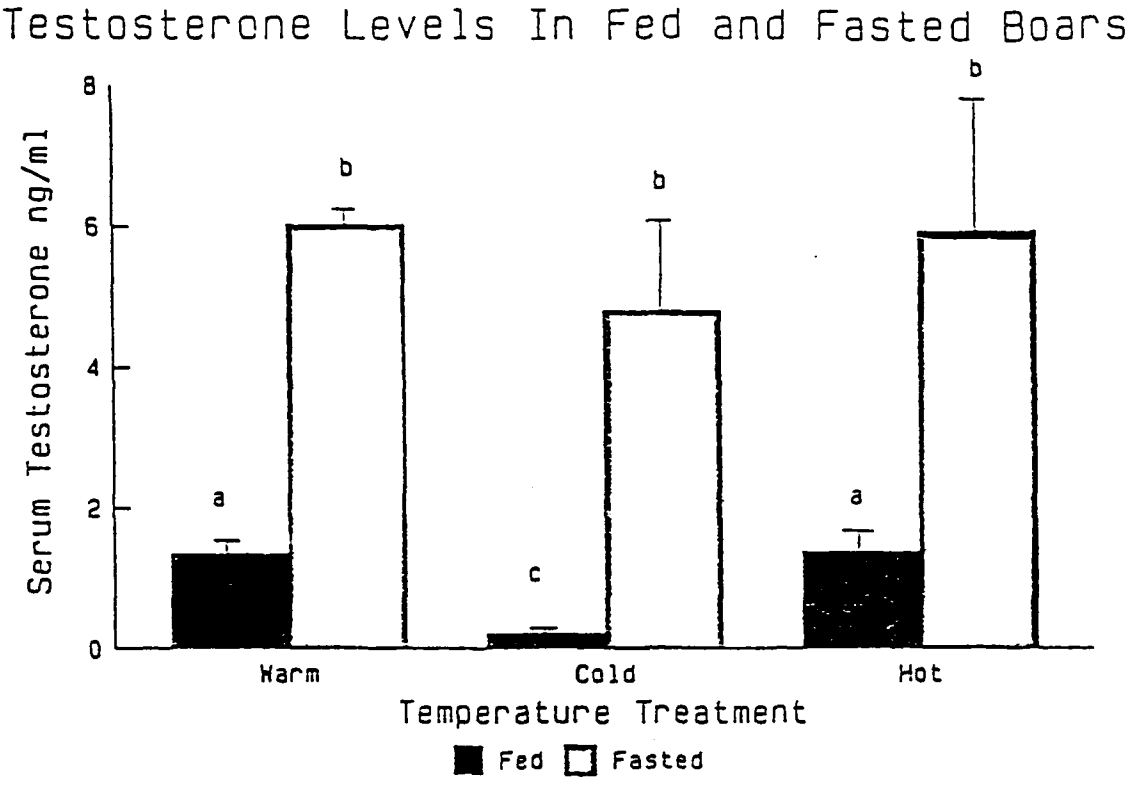
Figure 8



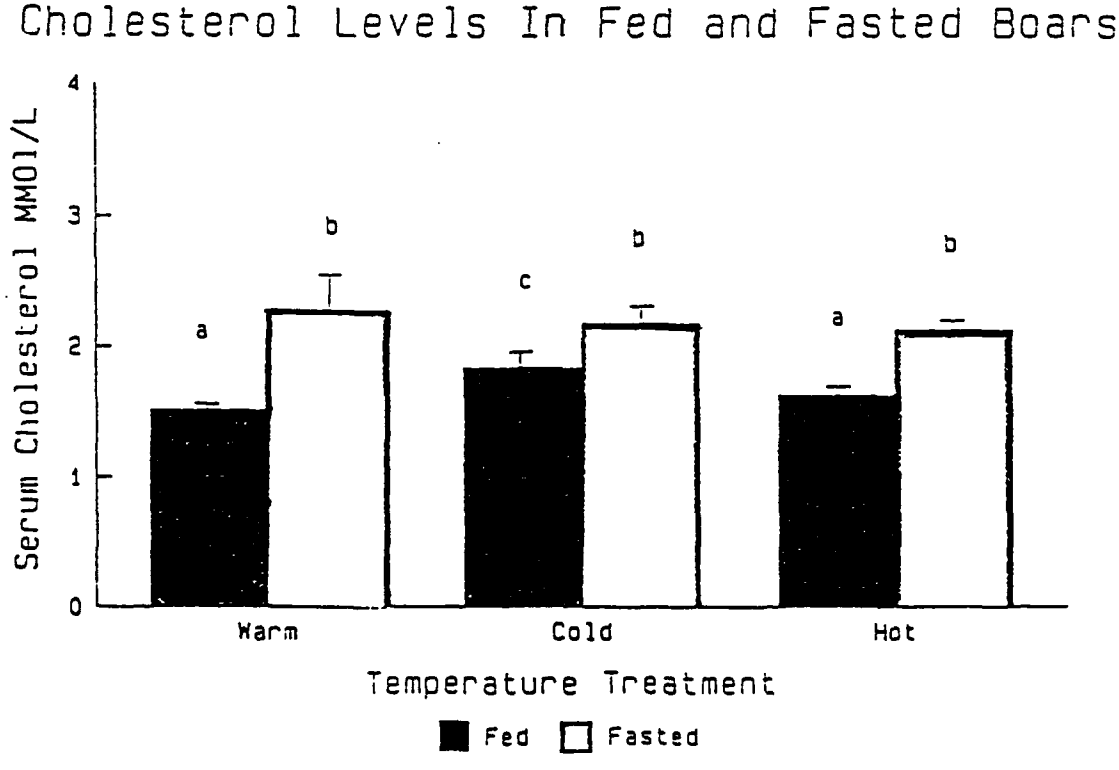


Different Superscripts Indicate Significance At P<0.05

Figure 9

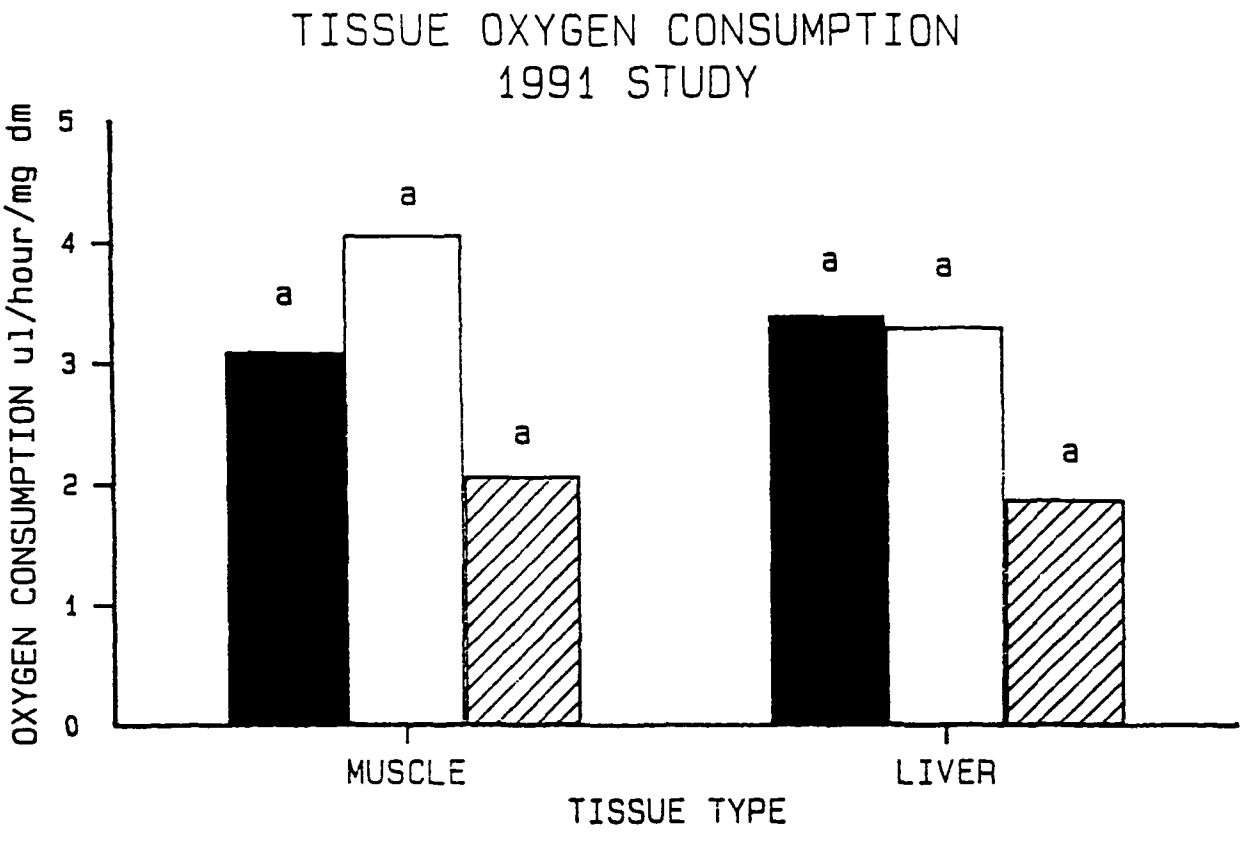


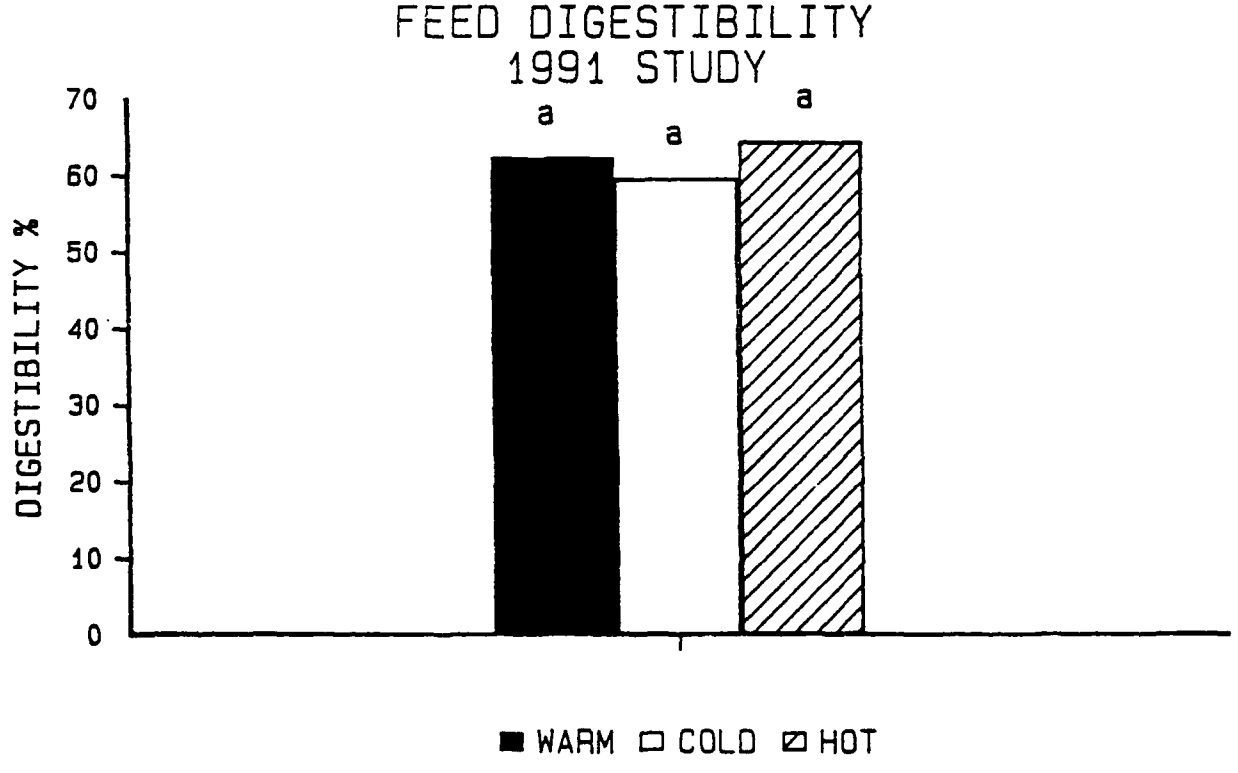
Different superscripts indicate significance at $p<0.001$



Different superscripts indicate significance at $p<0.05$

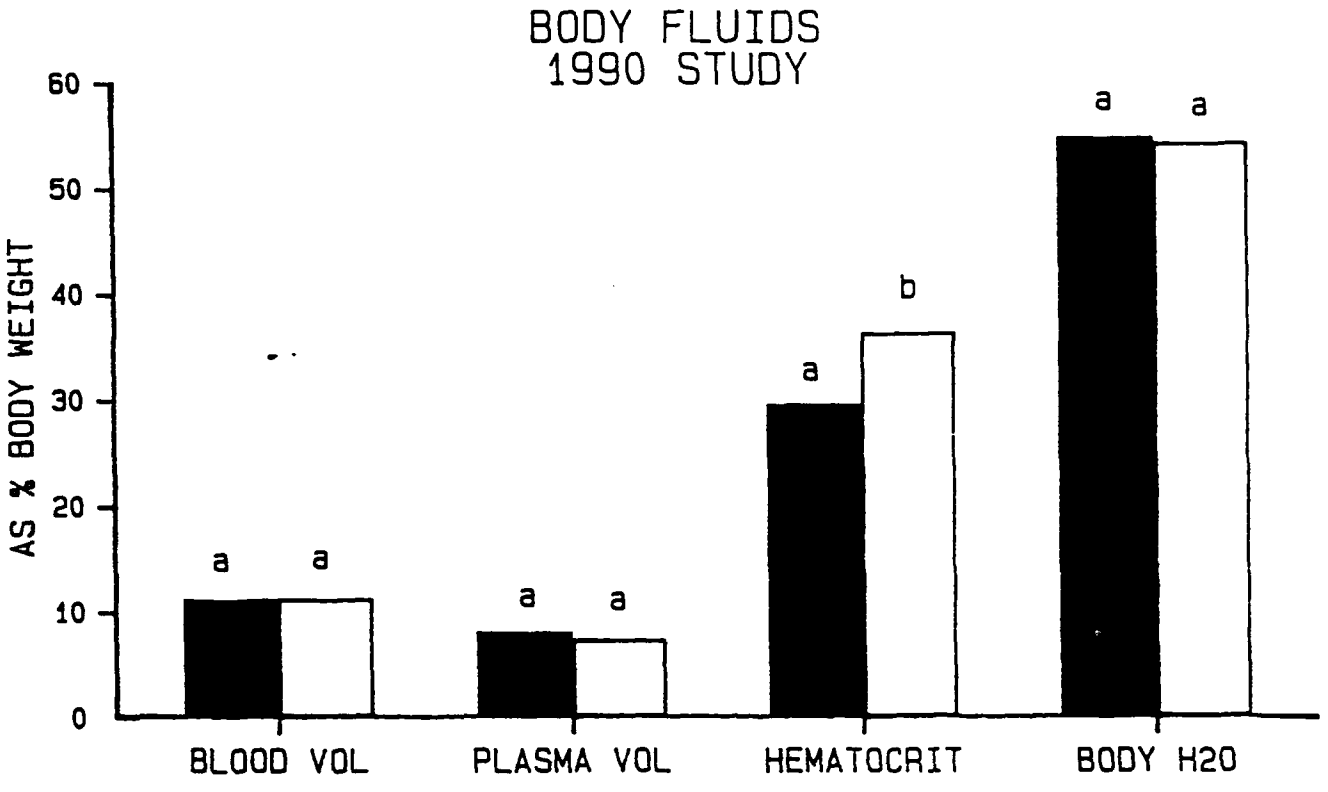
Figure 10

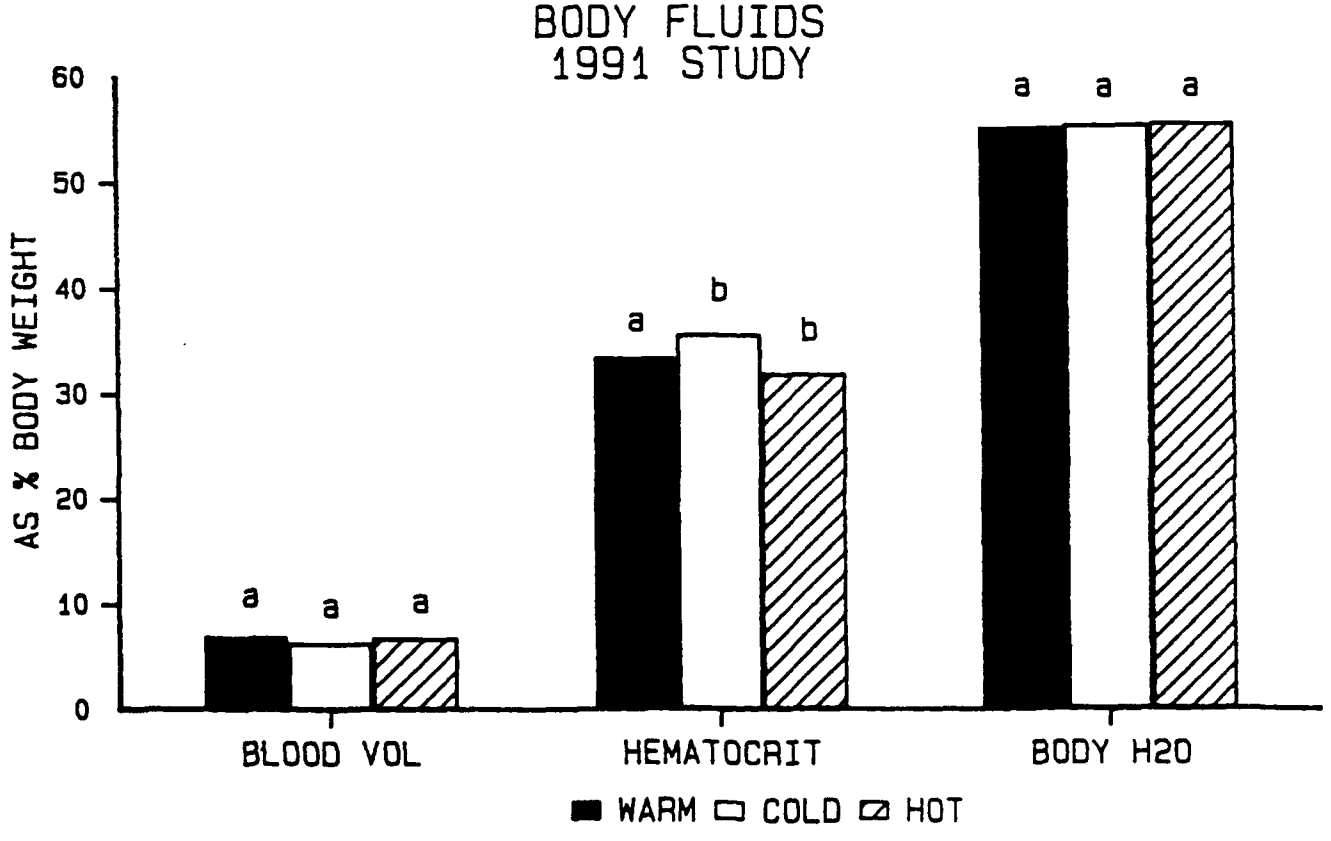




Different Superscripts Indicate Significance At P<0.05

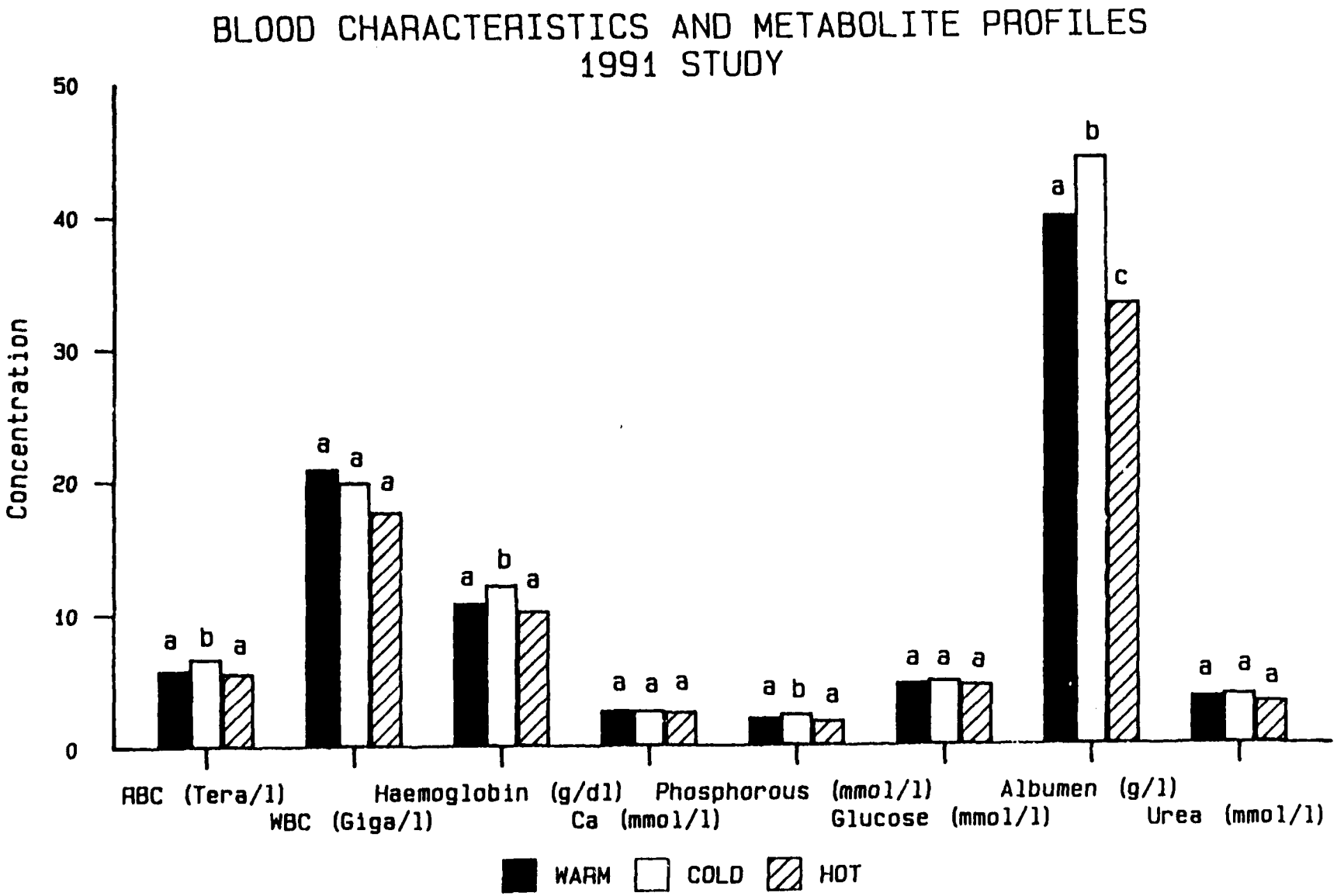
Figur 11





Different Superscripts Indicate Significance At P<0.05

Figure 12

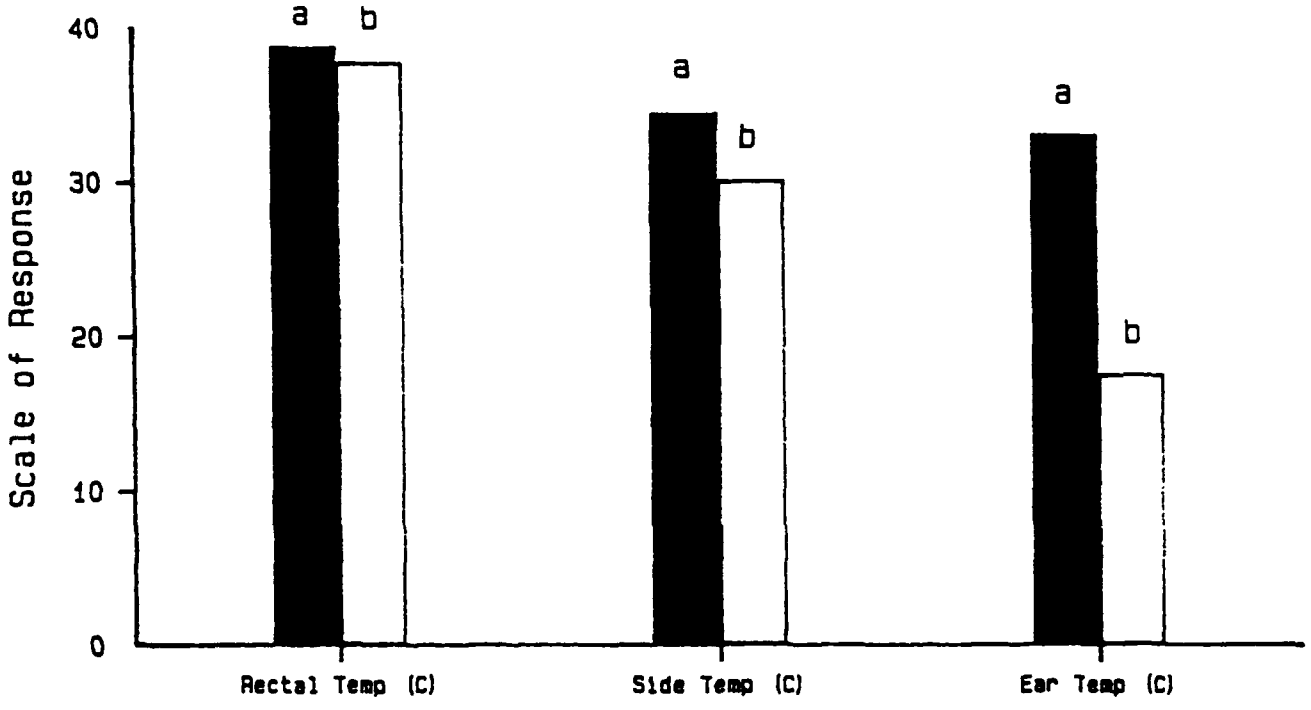


RBC - red blood cell count
WBC - white blood cell count

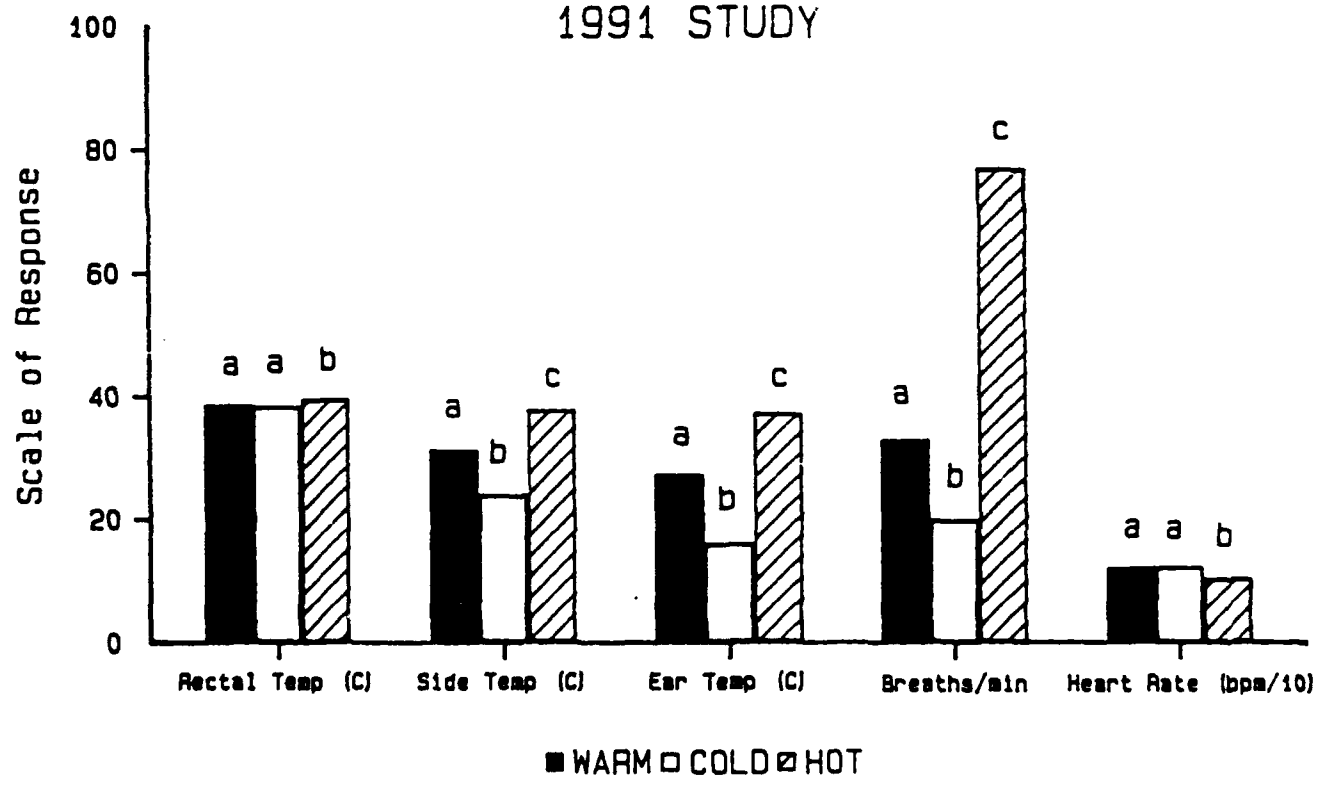
Different Superscripts Indicate Significance At P<0.05

Figure 13

PHYSIOLOGICAL ADAPTATION TO ENVIRONMENT
1990 STUDY

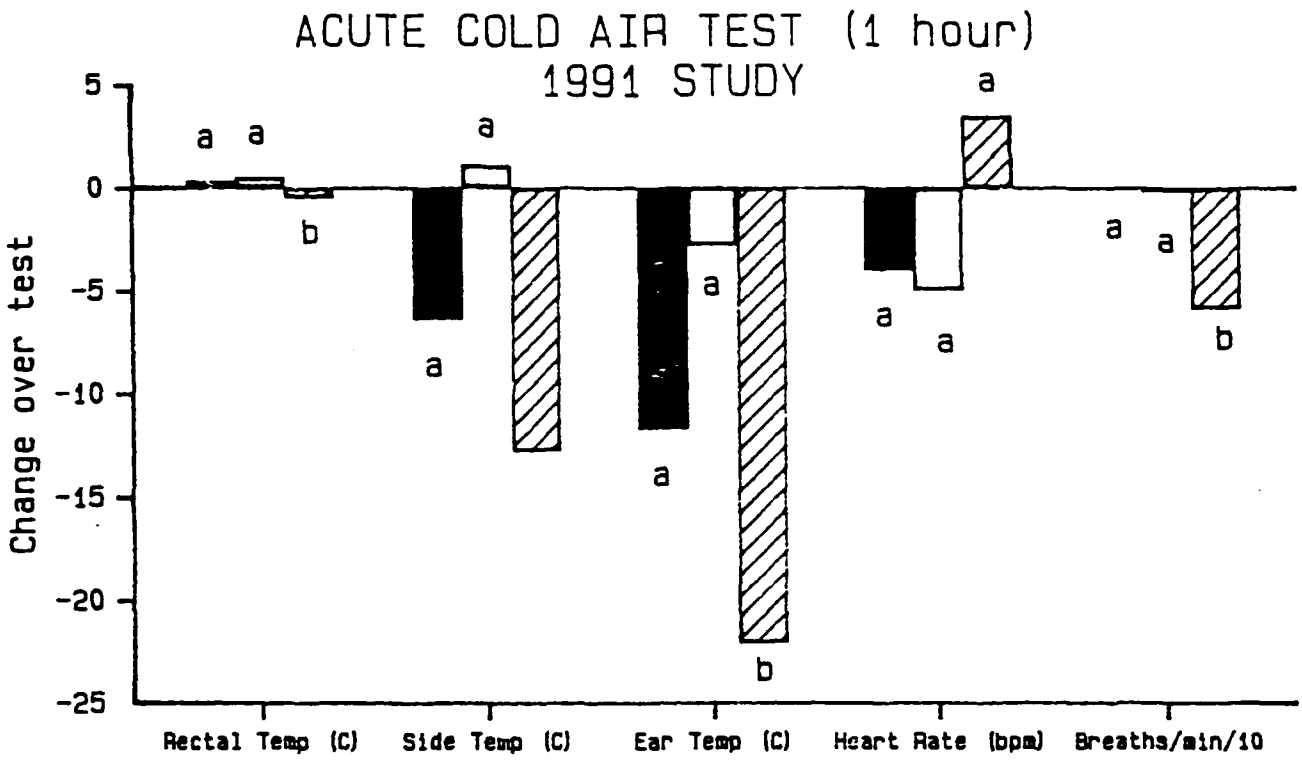


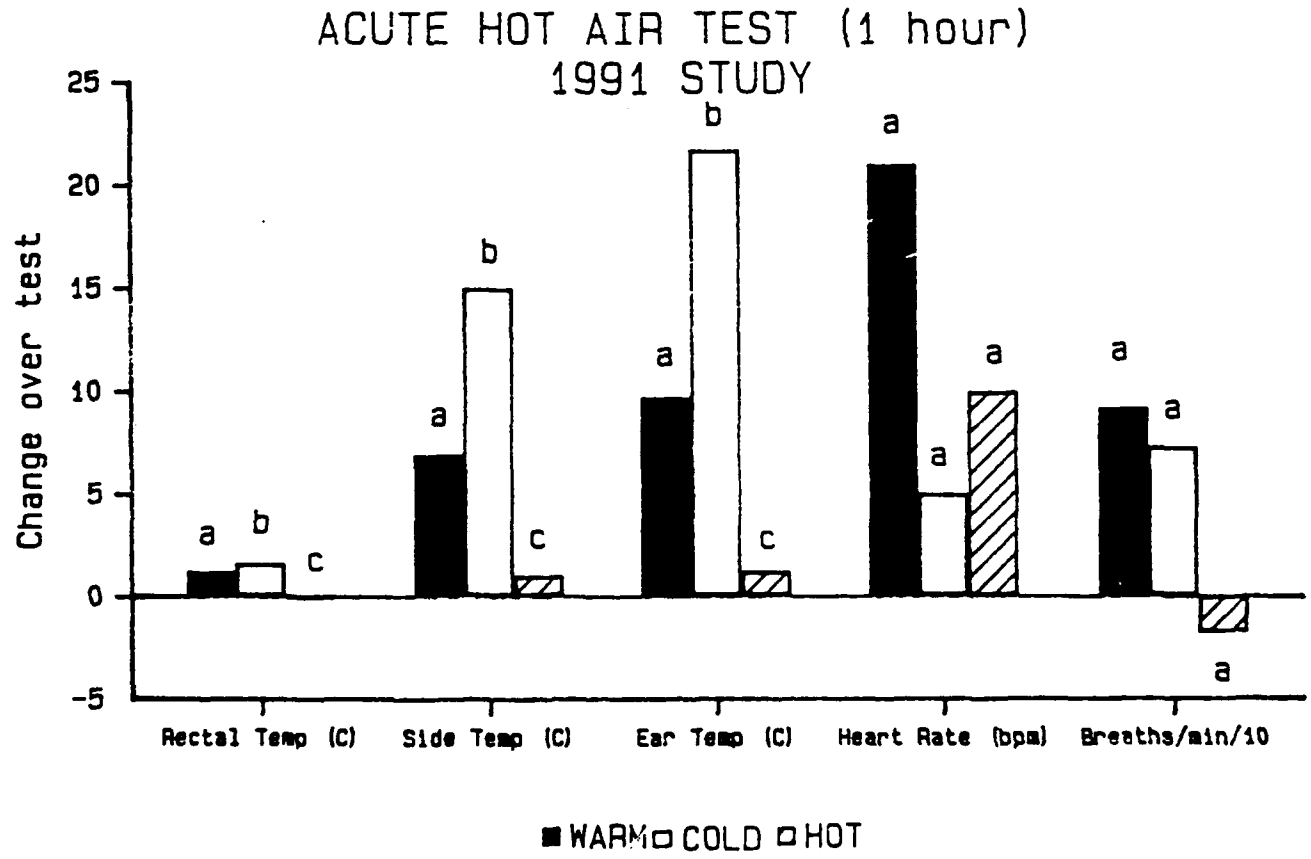
PHYSIOLOGICAL ADAPTATION TO ENVIRONMENT
1991 STUDY



Different Superscripts Indicate Significance At $P<0.05$

Figure 14





Different Superscripts Indicate Significance At P<0.05

Table 1

ROOM TEMPERATURE
1990 STUDY

		WARM ROOM	COLD ROOM
JANUARY	MAX C	22	23.7
	MIN C	20.2	19.1
	RH%		
FEBUARY	MAX C	20.7	13.8
	MIN C	17.9	6.7
	RH%	42	51
MARCH	MAX C	22.9	7.7
	MIN C	20.5	2.0
	RH%	48	56

ROOM TEMPERATURE
1991 STUDY

		WARM ROOM	COLD ROOM	HOT ROOM
JANUARY	MAX C	19.9	16.5	22.8
	MIN C		9	26.6
	RH%	34	54	33.9
FEBUARY	MAX C	21.5	6.6	38
	MIN C	16.6	4.5	35
	RH%	56.5	80.6	32.9
MARCH	MAX C	21.4	7.6	39.2
	MIN C	17.2	4.1	35.8
	RH%	61.6	81.1	37

Table 2

ORGAN WEIGHTS
1990 STUDY

		WARM ROOM	COLD ROOM	
WET WEIGHT	THYROID /g	8.7 ± 1.9	16.2 ± 4.6	*
	KIDNEY /g	314.6 ± 39.9	456.9 ± 120.5	
	ADRENALS /g	4.3 ± 1.2	5.2 ± 1.1	
% BODYWEIGHT	THYROID /g	0.012 ± 0.003	0.020 ± 0.005	*
	KIDNEY /g	0.431 ± 0.069	0.565 ± 0.097	*
	ADRENALS /g	0.006 ± 0.002	0.007 ± 0.001	

ORGAN WEIGHTS
1991 STUDY

		WARM ROOM	COLD ROOM	HOT ROOM
WET WEIGHT	THYROID /g	10.8 ± 0.8	11.6 ± 0.9	9.6 ± 0.7
	KIDNEY /g	331.8 ± 55	421.5 ± 39.7	306.5 ± 29.8
	ADRENALS /g	4.3 ± 0.5	5.3 ± 0.9	4.82 ± 0.4
% BODYWEIGHT	THYROID /g	0.011 ± 0.002	0.012 ± 0.002	0.010 ± 0.002
	KIDNEY /g	0.318 ± 0.04	0.445 ± 0.039	0.330 ± 0.017
	ADRENALS /g	0.004 ± 0.009	0.005 ± 0.002	0.005 ± 0.002

* Indicate Significance At $P<0.05$