AN INVESTIGATION OF THE METABOLIC RESPONSE TO CARDIOPULMONARY BYPASS AND THE EFFECTS OF TWO LEVELS OF INTRAOPERATIVE HYPOTHERMIA ON THE RESPONSE.

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Le seul bien qui me reste au monde

Est d'avoir quelquefois pleure

Alfred de Musset (1810-1857). Poemes

DEDICATED TO MY PARENTS, BROTHERS AND SISTERS.

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LIST OF ABBREVIATIONS

An attempt has been made to avoid the use of abbreviations. In certain circumstances, due to the size of a technical term or the frequency of its repetition, use of abbreviations was considered prudent. Consequently, the following abbreviations have been used.

APE	Atom percent excess
BMR	Basal metabolic rate
CPB	Cardiopulmonary bypass
CRP	C-reactive protein
DLW	Doubly labelled water
EPA	End product average
FFM	Fat free mass
IRMS	Isotope ratio mass spectrometry
LBM	Lean body mass
MAC	Mid-arm circumference
MAMC	Mid-arm muscle circumference
3MeH	3-methylhistidine
PCV	Packed cell volume
^{o}C	Degrees centigrade
SD	Standard deviation
SE	Standard error
REE	Resting energy expenditure
TEE	Total energy expenditure
TST	Triceps skinfold thickness
VO_2	Oxygen consumption (ml/min)
VCO_2	Carbon dioxide production (ml/min)
WBPB	Whole body protein breakdown
WBPS	Whole body protein synthesis
WBPT	Whole body protein turnover

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STATEMENT OF COLLABORATION

The idea for this thesis was conceived by myself. All studies were designed by myself.

The operations were performed by Professor DJ Wheatley, Mr IJ Reece, Mr KG Davidson, Mr WR Dimitri and Mr JCS Pollock.

All blood and urine sampling and administration of isotopes was performed by myself. I assisted in the initial processing of all specimens. Biochemical measurements were performed in the the Royal Infirmary under the supervision of Dr WD Fraser and Dr A Shenkin. Plasma catecholamine measurements were performed at the Materia Medica Unit, Stobhill Hospital, under the supervision of Dr H Elliot. Stable isotope measurements were supervised by Dr T Preston, Mr D McMillan and Dr T Fallick at the Scottish Universities Research and Reactor Centre, National Engineering Laboratories, East Kilbride.

Anthropometric measurements were performed by Miss R Richardson, Department of Biochemistry, Royal Infirmary, Glasgow and Miss C Lowis, Department of Physiology, Glasgow University. All indirect calorimetry measurements were performed by myself with the assistance of Miss Lowis and Miss Richardson.

All data in this thesis has been analysed and interpreted by myself. Statistical analysis was performed with the STATGRAPH statistical package and the advice of Dr. Gordon Murray. Dr T Preston and Mr D McMillan provided assistance in interpretation of the stable isotope data.

All references cited in the text, unless otherwise indicated, have been read by myself.

The entire contents of this thesis were typed by myself. The figures were prepared by myself with the help of the Medical Illustration Department, Royal Infirmary, Glasgow.

DECLARATION OF WORK PUBLISHED AND PRESENTED

Some studies contained in this thesis have been accepted for publication by scientific journals while other work has been presented at scientific meetings or is currently under consideration for publication.

Listed below are the publications, published abstracts and presentations to date to learned societies.

PUBLICATIONS

Taggart DP, Fraser WD, Fell GS, Wheatley DJ, Shenkin A. Plasma albumin and haemodilution: the problem of interpretation in sequential studies. **Ann Clin Biochem** (in press).

Taggart DP, Fraser WD, Borland WW, Shenkin A, Wheatley DJ. Hypothermia and the stress response to cardiopulmonary bypass. **Eur J Cardiovasc Surg** (in press).

McMillan DC, Preston T, Taggart DP. Analysis of O-18 enrichment in biological fluids by continuous flow-isotope ratio mass spectrometry. **Biomedical and environmental mass spectrometry (in Press)**

PUBLISHED ABSTRACTS

Taggart DP, Fraser WD, Gray CE, Wheatley DJ. Profound hypothermia modifies the endocrine response to cardiac surgery. **J Cardiovasc Surg 1988; 29 suppl 4: 83.**

Taggart DP, Preston T, McMillan D, Wheatley DJ, Shenkin A, Burns HJG. Profound intraoperative hypothermia modifies protein metabolism after cardiac surgery. **Clin Nutr 1988; 7 suppl: 36.**

Richardson RA, Taggart DP, McMillan DC, Hansell DT, Wheatley DJ, Burns HJG. Inability of conventional predictive formulae to estimate resting energy expenditure. **Clin Nutr 1988; 7 suppl: 21.**

PERSONAL PRESENTATIONS TO LEARNED SOCIETIES

NATIONAL

Hypothermia and the stress response to cardiopulmonary bypass.

TO: Society of Thoracic and Cardiovascular Surgeons of Great Britain and Ireland (University of Southampton, Southampton, Sept 88).

A comparison of operative temperature 28°C and 20°C on the metabolic response to cardiac surgery.

TO: Surgical Metabolic Group (University of Newcastle Upon Tyne, Nov 87).

Can hypothermia reduce the catabolic response to surgery?

TO: The James IVth Association of Surgeons (Royal College of Surgeons of Edinburgh, Sept 87).

INTERNATIONAL

Does hypothermia modify the stress response to cardiac surgery ?

TO: European Association of Cardiothoracic Surgeons, Bordeaux, September 1988.

Profound hypothermia modifies the endocrine response to cardiac surgery. (WINNER OF DOS SANTOS PRIZE)

TO: European Society of Cardiovascular Surgery, Helsinki, August 1988.

SUMMARY AND CONCLUSIONS

The metabolic response to trauma has been an area of intense clinical research since Studley's identification of an increase in postoperative mortality and morbidity in patients with preoperative weight loss (7). Consequently, considerable efforts have been made to modify the response, including the use of nutritional, pharmacologic and environmental manipulations, and have met with varying degrees of success. The concept of using intraoperative hypothermia to reduce the "stress response to surgery" was first postulated in the 1950s (8,9) but there has been little evidence to support this premise. Recently, however, a reduction in "post-traumatic proteolysis" following open-heart surgery, using a combination of moderate hypothermia (28^{o}C) and high dose anaesthetic agents, has been reported (10).

Cardiac surgery is a unique form of tissue injury. It combines conventional surgical trauma with the added insult of cardiopulmonary bypass (CPB) and perfusion cooling. CPB induces widespread activation of inflammatory mediators such as complement in the extracorporeal circuit resulting in the "post-perfusion syndrome", characterised by pulmonary, cardiac, renal, hepatic and haematological dysfunction (5,6). The systemic metabolic consequences of this "whole body inflammation" (5,6) are, however, poorly documented. Perfusion cooling is a standard part of the CPB technique, largely governed by the need to ensure adequate myocardial protection, but there is no consensus on the optimal level of intraoperative hypothermia and various temperatures from 30^{o}C to 20^{o}C have been proposed (11-14).

The aims of this thesis were to characterise the systemic metabolic sequelae of CPB and to determine the potential of profound intraoperative hypothermia (20^{o}C) to modify these responses. Systemic

hypothermia to 28°C was used in the "control" group as it is currently the most commonly used level of intraoperative hypothermia during CPB (11). The metabolic or "catabolic" response was characterised in terms of its four integrally related components, ie the acute phase response, the endocrine response, nitrogen balance and protein metabolism, and changes in energy expenditure. In examining the effects of profound intraoperative hypothermia (20°C) the primary objective therefore was to determine the feasibility of a therapeutic intervention at the time of tissue injury (in this case the use of systemic perfusion cooling) to modify fundamental pathophysiological responses to trauma. It was not the aim of these studies to prejudge whether modification of the response to trauma, nor the effects of two levels of hypothermia, might be beneficial or detrimental to clinical outcome.

Chapter 2. Historical Review. The first part of this chapter traces the development of the clinical application of hypothermia from its topical use in a variety of disease states, through the initial experience of surface cooling in patients undergoing heart surgery, to the modern era of perfusion cooling, and outlines key cardiovascular and physiological sequelae of the latter. The major components of the metabolic response to surgery are reviewed in Part Two, while deficiencies in the knowledge of these responses in relation to CPB, and in particular to the effects of hypothermia, are highlighted in Part Three. The role of pre-existing nutritional status in influencing postoperative morbidity and mortality is discussed in Part Four while Part Five summarises and classifies previous attempts to modify the metabolic response to surgery in environmental, nutritional and pharmacologic terms.

Chapter 3. Methods. This chapter describes the patients studied and details the surgical procedure in terms of anaesthetic and operative techniques, management of CPB and postoperative fluid administration. Anthropometric measurements are described and the theoretical and practical considerations involved in protein turnover studies, indirect calorimetry and measurement of energy expenditure, using Doubly Labelled Water, are discussed in detail.

Chapter 4. Nutritional assessment. Pre-existing nutritional status is a major determinant of the magnitude of the metabolic response to injury and a high incidence of malnutrition has been reported in various hospital populations, including those with valvular heart disease. In contrast patients with ischaemic heart disease are generally regarded as being "overnourished" although there is little objective evidence to support this. Sixty-one male patients admitted for coronary artery surgery underwent biochemical and anthropometric assessment of nutritional status. Most biochemical parameters were within the normal reference range although modest elevations in C-reactive protein and gamma glutamyl transferase and low concentrations of serum zinc were common. Most anthropometric parameters implied that the patients were at or above their predicted ideal value, while triceps skinfold thickness measurements suggested that almost 60% of patients were malnourished. It was concluded that triceps skinfold thickness is the least reliable anthropometric measurement in patients with a tendency to obesity and that routine biochemical and/or anthropometric assessments of nutritional status are not justified in this population.

Chapter 5. Measurement and prediction of basal metabolic rate. Measurement of the basal metabolic rate (BMR) is useful in certain clinical situations and important in studies of alteration in energy

expenditure. The current clinical "gold standard" measurement of BMR is by indirect calorimetry, although limited availability of appropriate equipment and expertise necessary for the technique in clinical practice have led to widespread use of predictive formulae. The BMR was measured in 20 patients with ischaemic heart disease by indirect calorimetry, and compared to that calculated from three predictive formulae. The measured BMR was 10-20% lower than that estimated by the predictive formulae and the discrepancy between measured and predicted values was greatest when BMR was related to lean body mass rather than weight. It is uncertain whether the lower than expected values for measured BMR in patients with ischaemic heart disease are due to the unsuitability of predictive formulae in this population, or a genuine phenomenon perhaps related to the effects of beta-blockade therapy on the autonomic nervous system.

Chapter 6. Correction for haemodilution. In view of the large volumes of fluid administered during CPB and the perioperative period, the efficacy of four formulae (three based on packed cell volume and one on alpha-2-macroglobulin) to correct for perioperative dilutional changes in serum albumin and caeruloplasmin was assessed in four patients undergoing coronary artery surgery and mimicked in an *in vitro* model. Predictive formulae could accurately correct for haemodilution *in vitro* but no formula consistently corrected for the effects of haemodilution *in vivo.* It was concluded that alterations in the concentrations of plasma proteins during CPB are not totally explained by haemodilution but also reflect different volumes of distribution. Correction for haemodilution *in vivo* is most easily achieved using serial changes in the PCV but limitations inherent in this method, particularly in the early post-CPB period, should be recognised.

Chapter 7. The acute phase response. Part 1: Plasma proteins. The plasma protein component of the acute phase response was measured in 20 male patients undergoing elective coronary artery surgery and randomised to an intraoperative blood temperature of 28°C or 20°C. The protein changes observed were typical of the acute phase response with increases in C-reactive protein, caeruloplasmin and ferritin and decreases in serum albumin, transferrin and prealbumin. The magnitude of changes in concentrations of these proteins was greater than that described for other operations. There was a greater fall in serum albumin in the 28°C group during CPB and a trend towards a smaller postoperative increase in C-reactive protein in the 20°C group.

Chapter 8. The acute phase response. Part 2: Trace metals and their protein binding ratios. Alterations in the serum concentrations of the trace elements iron, zinc and copper and in their binding ratios to their respective carrier proteins was examined in 20 male patients undergoing elective coronary artery surgery and randomised to an intraoperative blood temperature of 28°C or 20°C. Changes in serum iron have previously been described following cardiac surgery but not changes in serum zinc or copper. Serial examination of metal:protein binding ratios allows interpretation of changes in the affinity of trace metals for their carrier proteins irrespective of changes in the protein concentration and have not previously been described after operations. Typical decreases in serum iron and zinc, as part of the acute phase response, were preceded by early rises during CPB. Significant alterations in the molar binding ratios of metals to their carrier proteins preceded significant changes in the concentrations of the metals and occurred earliest with zinc:albumin binding. Changes in the latter constitute one of the earliest markers

of the stress response, being apparent by the time of skin incision, and may be useful in serial estimation of the acute phase response. A similar pattern of response was observed in both temperature groups although elevations in the serum concentrations of iron and zinc were significantly less in the 20°C temperature group during CPB.

Chapter 9. The endocrine response. Part 1: Plasma hormones. There has been no previous study of the effects of two levels of intraoperative hypothermia on the endocrine response to CPB. The plasma endocrine response was studied in 20 male patients undergoing elective coronary artery surgery and randomised to an intraoperative blood temperature of 28°C or 20°C. There was a progressive rise in adrenaline and noradrenaline during CPB in the 28°C group, similar to previous reports also using systemic hypothermia to 28°C. In contrast, in the 20°C group there was no further rise in catecholamines after the start of CPB. The cortisol response showed an exaggerated response in comparison to that observed after surgical stress without CPB and was similar in both groups. There was a marked fall in plasma T3 in both groups which may have implications for low cardiac output in the postoperative period. Thyroid stimulating hormone secretion increased in both groups during CPB implying preservation of pituitary function during pulsatile CPB. In summary the endocrine response observed in response to pulsatile CPB was typical of that following elective surgery. A more profound level of intraoperative hypothermia appeared to attenuate the plasma catecholamine response to CPB.

Chapter 10. The endocrine response. Part 2: Urinary excretion of hormones. Measurement of plasma hormone concentrations is necessary to detect rapid or subtle changes in the endocrine response to surgery while timed urinary collections for excretion of hormones

or their metabolites provide a more integrated assessment of the endocrine response. The effects of two levels of intraoperative hypothermia (28°C and 20°C) on the endocrine response to CPB were assessed by measuring the excretion of adrenaline, noradrenaline, dopamine and cortisol in 24 hour urine collections obtained preoperatively and on the first, second, third and seventh postoperative days. This demonstrated that a more profound level of intraoperative hypothermia attenuated the endocrine response to CPB with smaller increases in the postoperative excretion of adrenaline, noradrenaline, dopamine and cortisol which also returned to baseline excretion rates more quickly.

Chapter 11. Nitrogen balance and whole body protein turnover. Nitrogen balance and whole body protein metabolism were measured by standard techniques, pre and postoperatively, in 20 male patients undergoing elective coronary artery surgery and randomised to an intraoperative blood temperature of 28°C or 20°C. There was a significant postoperative increase in nitrogen excretion only in the 28°C group. The preoperative rate of whole body protein synthesis (WBPS) and breakdown (WBPB) was similar within each group when calculated from the urinary enrichment of urea, ammonium and the end product average (EPA). Isotopic enrichment of urinary urea implied that the postoperative development of a negative nitrogen balance was due predominantly to a fall in WBPS and that this decrease could be attenuated with a more profound level of intraoperative hypothermia. In contrast, isotopic enrichment of urinary ammonium suggested that a postoperative negative nitrogen balance was due to an increase in WBPB exceeding a concomitant rise in WBPS and that these changes were not attenuated with a more profound level of intraoperative hypothermia. It was concluded that the stochastic concept of a single protein

precursor pool is not tenable, at least in the postoperative situation. While the EPA may be a valid marker of protein turnover in the steady state, it obscures marked divergence in the visceral and peripheral protein precursor pools after surgery. In studies of WBPT, interpretation of protein metabolism based on the kinetics of a single end product is misleading and may account for some of the discrepancies regarding the aetiology of a negative nitrogen balance after surgery.

Chapter 12. Skeletal muscle metabolism. This study was performed simultaneously with the protein turnover studies reported in the previous chapter. The urinary excretion rates of three "markers" of skeletal muscle breakdown, 3-methylhistidine (3MeH), zinc and creatinine were measured in the pre and postoperative period as their urinary excretion is increased after surgery and appears to be related to the degree of trauma. There was a significant postoperative increase in 3MeH excretion in both groups and this was greater in the 20°C group. There was a postoperative increase in zinc excretion in both groups which was highly significant in the 20°C group but not significant in the 28°C group. Creatinine excretion increased significantly in both groups postoperatively and particularly so in the 20°C group. These findings were in accordance with the results of postoperative WBPB, as judged by isotopic enrichment of urinary ammonium, and implied that a more profound level of intraoperative hypothermia did not attenuate postoperative skeletal muscle breakdown.

Chapter 13. Alterations in postoperative energy expenditure. The effect of surgery on energy expenditure is controversial and there has been no report on the effect of cardiac surgery or hypothermia on energy expenditure. Total energy expenditure (TEE) was measured over 10 day periods, pre and postoperatively, in 16 patients undergoing

coronary artery surgery and randomised to an intraoperative temperature of 28°C or 20°C. TEE was measured with Doubly Labelled Water (DLW), a non-invasive stable isotope technique, which is theoretically simple, safe and of minimal inconvenience to the patient. DLW has only recently been introduced into clinical practice and this is the first study of the effect of uncomplicated major surgery on TEE. TEE gave a value for energy expenditure 30% higher than that calculated from the basal metabolic rate. There was a modest increase in TEE after surgery in both groups which was presumed to result from an increase in the basal metabolic rate as overall activity levels were reduced in the postoperative period.

Chapter 14. Clinical outcome. Clinical outcome was examined in sixty patients who were randomised to an intraoperative blood temperature of 28°C or 20°C. The temperature groups were well matched with respect to age and body weight but the 20°C temperature group received significantly more grafts than the 28°C group. Despite the longer mean cross-clamp (by 11 minutes) and bypass times (by 19 minutes) in the 20°C group, the mean postoperative ventilation periods were similar in the two groups. Fewer patients in the 20°C group required inotropic support and postoperative blood transfusion requirements were also less. There was no significant difference in the incidence of postoperative complications between the groups except for a reduction in the incidence of atrial fibrillation in the 20°C temperature group (1 v 6; $p < 0.05$) presumably due to more effective myocardial protection in this group.

In summary, these studies have demonstrated that a more profound level of intraoperative hypothermia during CPB can modify changes in some aspects of the acute phase response, the endocrine response,

nitrogen balance and protein metabolism. The postoperative increase in TEE was minimally reduced in the 20°C group. In contrast, postoperative changes in skeletal muscle breakdown were less susceptible to modification by hypothermia implying that the latter may be a more fundamental response to trauma.

The "control" group experienced hypothermia to 28°C and it is possible that blood cooling to this level attenuates the metabolic response which would be observed following normothermic CPB. Indeed there is already some evidence that moderate hypothermia may itself modify "post-traumatic proteolysis" (10). In the light of standard CPB techniques and current knowledge regarding myocardial preservation, however, normothermic CPB would be considered prejudicial to the safety of the myocardium (7,8). To elucidate the effects of moderate hypothermia on the metabolic response to trauma would require a control population undergoing major general surgical procedures at normothermia. Such a comparison would not be stictly valid, however, as both intraoperative temperature and the nature of the inflammatory stimulus - including or excluding CPB - would differ.

The mechanism by which hypothermia may modify some components of the metabolic response not only during, but also in the week following surgery, is uncertain. It is particularly surprising as the temperature difference between the two groups existed only for the duration of the cross-clamp period (when the heart and lungs are excluded from the systemic circulation) which was less than 60 minutes. It implies that the mediators which determine the magnitude and the duration of the metabolic response are present or are elaborated from the time of surgical insult. The complete control of this response is complex and not fully known but cytokine peptides, such as interleukin-1, 6 and tumour necrosis factor, are strongly

incriminated (21,22). The counter-regulatory hormones are also involved in the metabolic response to trauma since infusions of adrenaline, cortisol and glucagon can reproduce some aspects of the response in healthy volunteers (386-388). Furthermore, there is probably synergism between the cytokine mediators and the hypothalamic-pituitary-adrenal axis in stimulating the metabolic response (65).

Although the routine use of hypothermia to attenuate the metabolic response is impractical except in open heart surgery, these studies illustrate the potential of an intervention at the time of tissue injury to modify at least some components of the subsequent metabolic response. These studies were not designed to answer whether modification of the metabolic response to trauma is necessarily beneficial. The demonstration that postoperative morbidity and mortality correlate with loss of lean body mass (7) implies that prevention of the latter would be beneficial. Few would disagree that marked and persistent loss of large quantities of nitrogen, accompanied by overt loss of body mass, is detrimental to the severely injured or critically ill patient. On the other hand some aspects of the metabolic response presumably confer survival advantages, eg the acute phase response ensures synthesis of proteins which enhance bacterial killing and removal of toxic radicals while simultaneously altering plasma trace metal concentrations to deprive potential pathogens of essential elements for growth. As such, this component of the metabolic response is probably beneficial unless severe and protracted. Indeed almost two hundred years ago the Scottish surgeon John Hunter intimated in his "Treatise on the blood, inflammation and gun-shot wounds" (London, G.Nicol, 1794) that injury induced changes in the body that would provide "the means and disposition for cure of

the injuries".

Interest in modification of the metabolic response to trauma is likely to continue from both the clinical and research viewpoints. Since Studley's report (7), there has been a marked fall in morbidity and mortality from surgery attributable to a number of factors including improvements in the nutritional status of patients, anaesthetic and surgical practice and perioperative care. Success in these areas has, however, led to application of major surgical techniques to an increasingly elderly and often sicker population, while improvement in resuscitation techniques outside the hospital environment have resulted in severely traumatised patients surviving to require prolonged and complex hospital care. Consequently, the search for techniques with the potential of attenuating the metabolic response to trauma will continue. The studies described in this thesis have illustrated that hypothermia at the time of tissue injury can modify the subsequent metabolic response and it is likely that pharmacological manipulations would have at least similar potential.

CHAPTER 1

INTRODUCTION AND AIMS

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INTRODUCTION AND AIMS

"Surgery of the heart has probably reached the limits set by Nature to all surgery : no new method and no new discovery can overcome the natural difficulties....."

Sir Stephen Paget 1896 (1).

The last twenty five years have witnessed an explosion in the number of coronary artery bypass operations since Sabiston's description of the first aortocoronary bypass graft, using saphenous vein, in 1962 (2). It is now estimated that approximately 770 such operations per million of population are performed annually in the USA (3). In the United Kingdom's first consensus conference on the topic of ischaemic heart disease there were calls to double the annual number of coronary artery operations in the UK to 300 per million of population (4).

Cardiac surgery is a unique form of surgical insult combining standard operative injury with the additional trauma of cardiopulmonary bypass (CPB) and hypothermia. Coronary artery surgery requires the use of CPB to maintain systemic perfusion while the heart and lungs are excluded from the circulation. CPB is performed with an extracorporeal perfusion pump which causes "whole body inflammation" through systemic activation of inflammatory mediators, such as complement (5), resulting in the "post-perfusion syndrome" characterised by pulmonary, cardiac, renal, hepatic and haematological dysfunction (6). The effects of these additional insults on the systemic metabolic response to conventional surgical injury are poorly documented.

Modification of the metabolic response to surgery has been an area of intense clinical research over the last 50 years in view of the increased morbidity and mortality which accompanies a protracted catabolic response (7). Attempts to attenuate the response using a variety of anaesthetic, nutritional and hormonal manipulations have met with equivocal results. The concept of using intraoperative hypothermia to reduce the "stress response" to surgery was first mooted in the 1950's (8,9) but until recently there has been little objective evidence to support this premise. In 1986 Wilmore's group, at Harvard Medical School, reported that intraoperative hypothermia during open heart surgery reduces post-traumatic proteolysis (10). In addition to moderate hypothermia (28^{o}C), however, these workers used anaesthetic agents in doses themselves capable of modifying the metabolic response to surgery.

Standard surgical practice for coronary artery disease now involves some level of systemic hypothermia for all but the shortest procedures. A large number of factors influence the chosen level of intraoperative hypothermia but of particular relevance is the need for adequate myocardial protection. There is, however, no consensus on the optimal level of intraoperative hypothermia and although 28^{o}C is most commonly used (11) various temperatures have been advocated including 30^{o}C (12), 25^{o}C (13) and 20^{o}C (14). There is no objective evidence to demonstrate the overall superiority of any particular intraoperative temperature and the potential of hypothermia to modify the systemic metabolic response to CPB is unknown.

The aims of this thesis were:

(a) To examine the metabolic response to CPB by assessing its four distinct, but integrally related, components viz :

1] The acute phase response

2] The endocrine response

3] Whole body protein metabolism and nitrogen balance

4] Energy expenditure

(b) To determine if any component of the metabolic response could be modified at different levels of intraoperative hypothermia (28°C and 20°C).

The basic objective of these studies therefore was to examine the feasibility of manipulating systemic pathophysiological responses to trauma by an intervention (in this case the use of hypothermia) at the time of tissue injury. It was not a primary aim of these studies to determine the effects of modification of the response to trauma or the effects of two levels of hypothermia on clinical outcome.

CHAPTER 2

HISTORICAL REVIEW OF THE LITERATURE

CHAPTER 2

HISTORICAL REVIEW OF THE LITERATURE

" For this relief much thanks: 't is bitter cold
And I am sick at heart"

William Shakespeare (1564-1616). Hamlet I.1.8

PART 1: HYPOTHERMIA

EVOLUTION OF THE CLINICAL USE OF HYPOTHERMIA

Systemic hypothermia reduces the metabolic requirements of all organs. Consequently its use during cardiopulmonary bypass (CPB) allows a reduction in flow rates and pressures generated by the extracorporeal circuit thus diminishing non-coronary collateral blood flow, which tends both to rewarm the myocardium and obscure the surgeon's operating field (15). In a study of 54 patients during CPB the maximum rate of non-coronary collateral flow was 6% of the cardiac output, being highest in patients with severe coronary artery disease followed by those with aortic stenosis and least in patients with mitral valve disease (16). A reduction in the mean arterial pressure and flow rate during CPB minimises "washing out" of cold cardioplegic solution from the coronary circulation by non-coronary collateral flow thus maintaining a cold flaccid myocardium (15). Furthermore, systemic hypothermia provides a safety margin in the event that the extracorporeal circuit should fail for any reason.

The earliest record of the use of therapeutic hypothermia is in the Edwin Smith papyrus, dated at around 3,500 BC, for wounds of the head and for infected or ulcerated breasts (17). There are numerous examples of the use of topical hypothermia over subsequent centuries

as a panacea for many ailments.

A major incentive to the study of clinical applications of hypothermia was Smith and Fay's use of surface cooling to treat patients with advanced malignancy (18). Although topical hypothermia helped to control pain it did not increase longevity and subsequent post mortem examinations demonstrated no tumour regression (19). Other workers reported similarly negative findings and the clinical use of hypothermia fell into disrepute, particularly when Talbot reported that topical hypothermia had caused the death of two patients from heart failure (20). The use of topical hypothermia in various clinical problems had, however, stimulated Dill and Forbes to examine some of its metabolic and physiological consequences (21).

The first scientific evidence that hypothermia might have potential clinical application in the treatment of cardiovascular disease was the report by Bigelow and colleagues to the American Surgical Society in 1950 that surface cooling of dogs to 25°C allowed total arrest of circulatory flow for at least 15 minutes (22). The same authors showed a linear fall in oxygen consumption down to 20°C when oxygen utilisation was reduced by more than 80% in control animals (23). The implication that complete arrest of the circulation might allow intracardiac surgical procedures to be performed in a bloodless field, with temporary protection of the vital organs, was substantiated when Lewis and colleagues (24) reported the successful closure of atrial septal defects with the aid of topical hypothermia. Shortly after, Swan and colleagues reported the first series of patients undergoing open heart surgery for various congenital defects employing topical hypothermia and simultaneously documented a number of important physiological changes (25). Within a few years topical hypothermia was being used routinely during cardiac surgical

procedures in many centres in Europe, America and Japan.

In view of the difficulties in providing effective surface cooling and the time-consuming nature of the procedure, which involved transferring the patient from the operating table to ice baths and back, other workers began to consider alternative methods of achieving cooling through systemic perfusion. Boerema and colleagues (26) and Delorme (27) described arteriovenous bypass systems whereby an animal's own arterial pressure was used to drive heparinised blood through a heat exchanger back to a vein. Subsequently Brock and Ross described the use of a hand driven rotor pump to deliver blood through a heat exchanger (28). The first description of a true veno-arterial pump oxygenator was by Dogliotti and co-workers in 1953 (29). The aim of such systems was to provide a mechanism for achieving systemic hypothermia and only subsequently did exclusion of the heart and lungs, through the availability of pump-oxygenators, become the most important consideration. In America much pioneering work on the principles of pump-oxygenators was performed by Gibbon, a major force in the transition of cardiopulmonary bypass from an experimental to an invaluable clinical tool (30,31).

Such apparatus became rapidly and widely used in many centres because of the increased time it allowed to perform intracardiac procedures than when topical hypothermia was used alone. The employment of these systems allowed surgical intervention in many cardiac diseases which had previously been considered inoperable.

Hypothermia achieved by surface cooling differs considerably from hypothermia due to perfusion cooling. In the latter, cooling of the heart, liver, kidney and mid-oesophagus occurs quickly while the large muscular mass of the body remains warm (32). This differential cooling results in internal temperature gradients between organs and

cold blood perfusing warm tissue also results in an unfavourable oxyhaemoglobin dissociation curve, contributing to tissue hypoxia and progressive metabolic acidosis (32). Furthermore the difference between the core (cold) and peripheral (warm) tissues and the resulting acidosis may contribute to postoperative cardiac dysfunction. This problem was overcome by the introduction of more efficient heat exchangers, initially by Brown and colleagues, by which systemic hypothermia was more readily achieved (33). Effectively, systemic hypothermia and circulatory arrest, providing a clear operative field, had become the prime goal and the extracorporeal circuit merely a means of achieving this.

Standard cardiac surgical practice for coronary artery disease now involves some level of systemic hypothermia for all but the shortest procedures (11). In the USA more than 90% of surgeons use cold cardioplegic arrest of the myocardium rather than intermittent ischaemic arrest (34). A large number of factors influence the chosen level of systemic hypothermia but of particular importance is the need for adequate myocardial protection. Systemic hypothermia permits a reduction in arterial pressure and extracorporeal flow rates with a consequent fall in non-coronary collateral blood flow which otherwise "washes out" cardioplegia, diminishing myocardial protection (15,16). There is, however, no consensus on the optimal level of systemic intraoperative hypothermia and various temperatures have been advocated including 30°C (12), 28°C (11), 25°C (13) and 20°C (14).

PHYSIOLOGY OF HYPOTHERMIA

Hypothermia reduces the metabolic requirements of all organs and early studies suggested that the decline in metabolic rate bore a linear relationship to the fall in temperature (23). Subsequent

studies demonstrated, however, that the fall in oxygen requirement associated with a decline in systemic core temperature is approximately exponential (35). At 32°C the reduction is by 25-30%,at 30°C by 45-50%, at 28°C by 60%, at 25°C by 65-70%, at 20°C by 75% and at 10°C oxygen utilisation is approximately only 10% of normal (23,36). These figures are based on work derived mainly from animal experiments and in man it has been estimated that at 26°C oxygen requirements are reduced to 35-50% of normal (37). The variability in the reduction in oxygen requirements in response to hypothermia results from the shivering response to cold as shivering thermogenesis can triple oxygen consumption (21). The organs with the greatest metabolic rates such as liver, heart and muscle have the most significant reduction in metabolic rates with systemic hypothermia, particularly between 37°C and 30°C when the Van't Hoff-Arrhenius rule applies, viz. the rate of any chemical reaction doubles or halves for each respective 10°C rise or fall in temperature (38).

Cardiovascular system:

With modest levels of hypothermia, and in the absence of neuromuscular blockade, cardiac output is increased because of the rise in metabolic rate due to shivering thermogenesis. As systemic core temperature falls there is a parallel fall in heart rate whereby a sinus bradycardia will eventually progress to cardiac asystole (or ventricular fibrillation in the case of cardiac surgery) without further cooling. The progressive fall in cardiac output with increasing hypothermia is mainly related to a fall in heart rate as stroke volume remains relatively unchanged (39). Arterial blood pressure may remain within a normal range until systemic core temperature falls to less than 26°C (40). Peripheral resistance

increases with progressive hypothermia until 20°C when resistance decreases (41).

With progressive hypothermia the blood volume is reduced by up to 40% (42) and is accompanied by an increase in viscosity and red cell sequestration (43). The white blood cell count initially rises with moderate levels of hypothermia but then falls progressively with increasing hypothermia (44). Platelet concentration decreases with temperatures below 30°C and a marked thrombocytopaenia occurs when the temperature falls below 25° C (44) and these changes are greater with systemic than with surface cooling (45). There is a progressive rise in whole blood clotting times down to 25°C when it is significantly prolonged (44).

Endocrine system:

Adrenal gland: Moderate hypothermia is a stressful insult resulting in adrenal gland stimulation (46). With more profound hypothermia the adrenal gland becomes progressively unresponsive so that at temperatures below 28°C adrenal function is significantly inhibited and reported to be totally absent at temperatures below 25°C (47,48). Likewise there is a reduction in adrenaline and noradrenaline secretion with more severe levels of hypothermia (49-51). At lower temperatures noradrenaline becomes less effective as a pressor agent and the cold myocardium becomes unusually sensitive to adrenaline (51) and may explain, in part, the frequency with which ventricular fibrillation occurs in the cold heart .

Thyroid gland: Thyroid gland activity has been reported to become progressively reduced at temperatures less than 34°C (52) although the gland remains sensitive to exogenous thyroid stimulating hormone. At 15°C uptake of iodine 131 by the thyroid gland is

inhibited (53,54).

Pituitary gland: There is a decline in pituitary function with increasing hypothermia so that reduced secretion of antidiuretic hormone promotes a hypothermic diuresis (55). With progressive hypothermia there is a reduction in the production of thyroid stimulating hormone (52) and adrenocorticotrophin hormone (8,48,56).

Pancreas: Both the production (57) and the physiological action of insulin in reducing blood glucose concentration are inhibited (58,59) so that systemic hypothermia results in a relative hyperglycaemia.

PART 2: METABOLIC RESPONSE TO SURGERY

It is now more than half a century since Cuthbertson described the metabolic response to tissue injury, in patients with long bone fractures, as a progressive loss of body nitrogen, potassium and phosphorous (60). Cuthbertson subsequently described two components to this metabolic response: an "ebb phase", occurring for the first several hours, characterised by a reduced metabolic rate while the circulating volume and tissue perfusion were restored; the "flow phase" occurring after restoration of perfusion and typified by generalised catabolism and heat production due to increased sympathetic activity and increased secretion of the counter-regulatory hormones (61). In the current era of modern surgical practice with strict control of fluid requirements, analgesia, and careful surgical technique, the ebb phase may not exist.

The realisation that a myriad of stereotyped physiological and biochemical events occur after any critical stress, culminating in increased energy expenditure and the development of a negative nitrogen balance, has stimulated much basic research into regulatory

mechanisms. This "catabolic response" can be arbitrarily classified into four integrally related components:

i) the acute phase response

ii) the endocrine response

iii) whole body protein turnover and nitrogen balance

iv) alterations in energy expenditure

The most influential factors determining the magnitude of the catabolic response, and subsequent clinical outcome, are the severity of the injury and nutritional status of the patient.

ACUTE PHASE RESPONSE

The acute phase response is a stereotyped but non-specific response to tissue injury from any cause including trauma, surgery, sepsis, burns, malignancy or chronic illness. The exact role of the acute phase response remains to be fully determined but is believed to be an attempt to redress disturbed homeostasis after tissue injury through a variety of responses, including stimulation of the immune system and tissue repair. It is characterised by a dramatic increase in the synthesis of the acute phase reactant proteins and profound alterations in the concentrations of the trace elements iron, zinc and copper. Furthermore the acute phase response is a generalised host response whether the initiating factor is localised or systemic in nature.

The acute phase response plays an important and diverse role in protection of the host following injury. It involves production of proteins with antiprotease activity (eg alpha-1-antitrypsin) which bind and inactivate proteolytic enzymes such as elastase and collagenase, thereby limiting further cellular damage. Other actions include protection against bacterial invasion, elaboration of

coagulation factors such as fibrinogen and production of C-reactive protein (CRP) which promotes complement-induced opsonisation and lysis of bacteria.

The acute phase response is initiated and controlled primarily by a number of mediators generally referred to as cytokines. Knowledge about the cytokines is currently accumulating at a bewildering rate but amongst the most important are interleukin-1 (62), interleukin-6 (63) and tumour necrosis factor (64). In addition to their individual actions the cytokines may have synergistic potential and also interact with corticosteroids and catecholamines (65).

The most important factor in the initiation of the acute phase response is probably interleukin-1, previously known as Leukocyte Endogenous Mediator, Endogenous Pyrogen and Lympocyte Activating Factor. Interleukin-1 is a peptide, or group of closely related peptides, synthesised mainly by cells of the reticulo-endothelial system, in response to a wide variety of stimuli, which can reproduce both laboratory and clinical features of the acute phase response (62). The main sources of interleukin-1 are blood monocytes, phagocytic cells of the lung, liver and spleen, and tissue macrophages, although some specialised epithelial cells including keratinocytes have the capacity to produce smaller amounts (62). Although interleukin-1 is primarily produced from phagocytic cells and its main actions are on local tissues, it probably also enters the general circulation and acts on distant organs such as liver and hypothalamus. The biological activities of interleukin-1 are protean and include the production of fever, stimulation of the immune response and alterations in acute phase proteins and serum trace metals (62) although recent evidence suggests that interleukin-6 may

be the most important cytokine peptide (63). Interleukin-1, or a peptide fragment of the molecule, has also been incriminated in muscle proteolysis (66).

Plasma proteins and the acute phase response

In response to stress, the liver dramatically increases the synthesis of some proteins (the acute phase reactant proteins) while decreasing the production of others such as albumin, prealbumin and transferrin, thereby reversing the albumin:globulin ratio.

The acute phase reactant proteins increase in response to both transient and prolonged inflammatory stimuli and can be generally classified into two categories - i) proteins which are normally present in plasma but whose concentration increases several fold (eg. caeruloplasmin, ferritin, alpha- 1 antitrypsin, alpha- 1 acid glycoprotein, fibrinogen, haptoglobin) and ii) proteins which are normally absent or only present in minute concentrations in plasma but which increase several hundredfold in response to tissue injury (eg. CRP).

CRP (whose name derives from its ability to precipitate with pneumococcal C-polysaccharide) is the classical acute phase protein (67). In response to most forms of tissue injury its plasma concentration increases several hundredfold, from barely detectable levels, within 24 to 48 hours. With resolution of the initiating stimulus the serum concentration rapidly declines (67). CRP is synthesised by hepatocytes under the influence of cytokines including at least interleukin-1 (68) and, although its exact role is uncertain, it appears to have a number of functions in host defence mechanisms including effects on agglutination reactions, phagocytosis, complement formation and cellular immune responses (67).

Trace elements and the acute phase response

A decreased concentration of serum iron and zinc and increased concentration of serum copper are invariable consequences of the acute phase response whatever the initiating stimulus (69). A significant fall in serum iron is one of the earliest markers of the acute phase response and may even precede the development of fever (70). In response to any insult, including surgery (71), the serum concentration of iron may fall to barely detectable levels within 12 hours and remain so for the duration of the stimulus (72). If an infectious process becomes chronic, the sequestration of iron can lead to the so called "anaemia of infection" (73). The rapid drop in iron is due to sequestration of the cation in storage sites within the liver, the reticulo-endothelial system and bone marrow in the form of ferritin or haemosiderin, making it unavailable for red blood cell production. This rapid shift of iron from plasma to liver is probably mediated by interleukin-1 as is the production of ferritin - a protein with a very strong affinity for free iron (62). The profound decrease in serum iron in association with tissue injury, particularly when mediated by infection, may be explained on a teleological basis as a non-specific host defence mechanism aimed at depriving micro-organisms of a trace element essential for growth, replication and toxin production (74).

In response to tissue injury there is a large flux of zinc from plasma to liver, mediated by cytokines such as interleukin-1 (62), where it is an essential co-enzyme for many of the metabolic pathways involved in synthesis of the acute phase proteins. Whereas serum iron falls to barely detectable levels, as part of the acute phase response, there is a less precipitous fall in serum zinc, to approximately 50% of normal concentrations (69). Unlike iron there is

normally no intracellular protein which can bind zinc in a storage form and therefore the liver is stimulated into rapid synthesis of zinc binding metallothioneins (75). Like iron, zinc is essential for the growth and reproduction of invading micro-organisms and its rapid decline in plasma, as part of the acute phase response, may be another part of non-specific host defence mechanisms (76).

A slow rise in the concentration of serum copper accompanies the rapid falls in iron and zinc during the acute phase response. The increase in serum copper is due to an increased rate of synthesis of its binding protein caeruloplasmin, an acute phase protein whose synthesis is stimulated by interleukin-1 (77). Copper has anti-inflammatory properties (78), is an essential requirement for immune competence (79), and may be important in the scavenging of oxygen free radicals (80). Because caeruloplasmin has a long half-life the return of elevated plasma copper concentration towards normal is prolonged during convalescence (69).

ENDOCRINE RESPONSE

Alterations in circulating levels of hormones (from the Greek word "hormaein" meaning to arouse or set in motion) following a wide variety of insults - including trauma, elective surgery, burns and myocardial infarction - are well documented (81). Numerous hormonal changes occur in response to stress and can be classified into those predominantly concerned with fluid and electrolyte balance, eg aldosterone, antidiuretic hormone, renin and angiotensin, and those with an important influence on body composition, eg insulin, catecholamines, cortisol, glucagon, thyroid and growth hormones. The latter hormones may be broadly classified, depending on function, as anabolic or catabolic. Insulin and growth hormone are anabolic

hormones while cortisol, adrenaline, noradrenaline and glucagon are the major catabolic or counter-regulatory hormones.

Insulin: Insulin is released from the Beta cells of the pancreas and promotes amino acid and nitrogen retention and the storage of lipids and carbohydrates. Plasma levels of insulin usually fall in the early stages of illness despite elevated blood glucose concentrations (82), although insulin concentrations eventually rise to an appropriate level for the degree of hyperglycaemia. In severe illness there exists an "insulin resistance" characterised by the failure of normally insulin-sensitive tissue, such as skeletal muscle, to utilise plasma glucose (83) probably because of a failure of carbohydrate transport. Insulin decreases the rate of protein catabolism in response to stress, both by providing glucose for cell oxidation, thereby sparing amino-acids, and by independently stimulating protein synthesis (84). Provision of glucose, insulin and potassium to critically ill patients has been shown, at least in the short term, to improve the function of various tissues, such as cardiac muscle, to correct the "sick-cell" syndrome, and to decrease nitrogen excretion (85).

Cortisol: Cortisol is a glucocorticoid, released from the adrenal gland, and is essential for the survival of the stressed patient. Increased levels of plasma cortisol occur in response to a wide variety of stresses including surgery (86). The release of cortisol in response to stress is probably mediated via the hypothalamic-pituitary-adrenal axis. The predominant metabolic effects of cortisol are to increase hepatic glycogenesis, gluconeogenesis, and protein catabolism, i.e. an anti-insulin effect which serves to raise blood glucose concentrations (although it may stimulate protein synthesis in liver in rats) (87).

Catecholamines: Like cortisol, plasma catecholamines are elevated in response to a wide variety of stresses and both plasma and urinary catecholamine levels can be correlated with an increased metabolic rate proportional to the severity of the stress (88). In addition to their effects on the cardiovascular system, plasma catecholamines exert metabolic effects including glycogenolysis in liver and skeletal muscle, mobilisation of fats (to free fatty acids and glycerol), stimulation of the metabolic rate (89) and synthesis of some acute phase proteins in rats. Catecholamines also have an anti-insulin effect, both by inhibiting release of insulin from beta cells of the pancreas and by stimulating the release of glucagon from Alpha cells (87).

Glucagon: Glucagon is released from Alpha cells of the pancreas when stimulated by B adrenergic activity and the elevation in its plasma concentration is usually proportional to the severity of the insult (90). Its metabolic actions include glycogenolysis, gluconeogenesis and lipolysis (91) and it occurs in higher concentrations in the portal than in the systemic circulation (91).

Growth Hormone: Plasma levels of growth hormone increase in response to stress, even in the presence of elevated concentrations of glucose which would usually suppress plasma growth hormone secretion (87). Current evidence suggests that growth hormone may play an important role in nitrogen retention and promote positive nitrogen balance (92).

NITROGEN BALANCE AND WHOLE BODY PROTEIN TURNOVER

The development of a negative nitrogen balance is the predominant feature of the catabolic response to a wide variety of stresses including surgery, sepsis, burns and trauma. The loss of

nitrogen following trauma is proportional to the magnitude of the insult, the age and sex of the patient (primarily reflecting lean body mass), and the pre-existing nutritional status of the patient (60).

Nitrogen balance studies reflect net gain or loss of body protein between a subject and the environment and give no insight into the dynamics of protein synthesis or breakdown which ultimately determine nitrogen balance. Thus a negative nitrogen balance may result if protein breakdown rates increase while protein synthesis rates remain constant, or if protein breakdown rates remain constant while synthesis rates decrease. Alternatively, both the rates of synthesis and breakdown may increase but a negative nitrogen balance can still result from an increase in breakdown rates exceeding a concomitant increase in synthesis rates.

Whole body protein turnover is the sum of the metabolism (breakdown or synthesis) of many individual proteins in the same way that the basal metabolic rate reflects the oxygen consumption of many individual tissues. The original description of the use of amino acids, labelled with stable (and therefore non-radioactive) isotopes, to allow quantification of alterations in protein synthesis and breakdown rates was by Sprinson and Rittenberg in 1949 (93) and further developed by San Pietro and Rittenberg in 1953 (94). Only over the last 15 years, however, have isotopically labelled amino acids achieved widespread utilisation in the investigation of protein metabolism in malignancy, trauma, sepsis and elective surgery using the stochastic method of Picou and Taylor-Roberts (95).

Reduction of food intake in the normal individual causes a marked fall in protein synthesis within two days (96). Conversely, increasing nitrogen intake alone, above the usual requirements for a healthy adult, has no effect on body protein synthesis rates although

these can be increased if excess energy substrate is added to a normal nitrogen intake (97). Nutritional therapy will increase protein synthesis rates in malnourished subjects, particularly children (95).

Exercise counteracts the fall in protein synthesis seen in immobilised individuals, particularly those in plaster casts (98). Strenuous exercise may cause an initial increase in rates of protein breakdown followed by an increase in the rates of synthesis (99).

The negative nitrogen balance observed in response to injury has classically been attributed to an accelerated breakdown of skeletal muscle (60) in accordance with the frequent clinical observation of wasting of the skeletal muscle mass. However protein turnover studies, using isotopically labelled amino acids, have demonstrated that the development of a negative nitrogen balance in patients undergoing general surgical procedures (100) and elective orthopaedic operations (101) results predominantly from a fall in protein synthesis with little or no alteration in protein breakdown, similar to the response known to occur in starved (97) and immobilised (98) patients. On the basis of their observations in patients undergoing orthopaedic operations, Crane and colleagues suggested that injury may produce a "block" in protein synthesis activity (101). Conversely following major elective surgical trauma (102), major skeletal trauma (103), burns (104) and sepsis (105), a negative nitrogen balance may result from an increase in protein breakdown exceeding a simultaneous increase in protein synthesis.

It has therefore been postulated that in "severe stress" a different mechanism for protein loss exists from that observed in response to surgery of moderate traumatic severity (106). Severe injury accelerates nitrogen turnover as both protein synthesis and breakdown are increased. In the fasted patient protein breakdown

rates exceed those of synthesis and a negative nitrogen balance results. The rationale of nutritional support in severely stressed patients is to increase the rate of protein synthesis towards the accelerated rates of protein breakdown.

3-METHYLHISTIDINE (3MeH)

Evidence that skeletal muscle is involved in the catabolic response to surgery, and in particular the development of a negative nitrogen balance, was demonstrated in the studies of Cuthbertson where an increased urinary excretion of potassium, zinc and creatine occurred during the flow phase of injury (60,107). Although evidence for the release of amino acids from muscle protein can be demonstrated by measuring arteriovenous differences in amino acid concentrations across a limb, the technique is difficult and interpretation of the results complicated by re-use of some amino acids (108).

Studies of the effects of trauma on skeletal muscle metabolism were hampered by the lack of a specific marker of muscle protein breakdown until the amino acid 3-Methylhistidine (3MeH) was identified as a constituent of the actin and heavy chain of myosin, in the myofibrillar component of white muscle, by Asatoor and Armstrong (109). Since then 3MeH has achieved widespread clinical use as an indicator of muscle protein breakdown. Actin and myosin account for 65% of muscle protein and approximately 35% of the total body protein pool (110). Skeletal muscle in man is estimated to account for 90% of the total body content of 3MeH with the remainder being equally derived from the gastrointestinal tract and skin (111). Radioactive tracer studies have confirmed that 3MeH is neither metabolised nor re-utilised for protein synthesis and is rapidly excreted in urine (112). Since the total body pool of skeletal muscle can be estimated

from the rate of creatinine excretion, 98% of which is derived from skeletal muscle (113), then the ratio of excretion of 3MeH to creatinine can give an estimate of the fractional turnover of muscle protein. The fractional rate of muscle protein breakdown, as calculated by this ratio, has been investigated in a variety of clinical conditions including hyperthyroidism (114), trauma (115,116), burns (117), sepsis (118), malnutrition (119) and surgery (120).

Since 3MeH can be derived from ingested dietary muscle protein should be avoided in the diet for three days prior to any urinary collection (121).

From a theoretical point of view, Rennie has disputed the validity and clinical usefulness of 3MeH as a marker of skeletal muscle breakdown (122), suggesting that the smaller gastrointestinal pool of 3MeH may have a much more rapid turnover than skeletal muscle and may consequently provide a relatively greater contribution to the urinary excretion of 3MeH than would be anticipated from the size of the gastrointestinal pool. Nevertheless 3MeH is a valid marker of whole body protein breakdown (122,123) although it is not exclusively derived from skeletal muscle breakdown.

ENERGY EXPENDITURE AND SURGICAL TRAUMA

Total energy expenditure (TEE) consists of two components - resting energy expenditure (REE) and activity energy expenditure (AEE). REE is that energy expended in maintaining body temperature and is relatively constant, while AEE depends on exercise and consequently is much more variable. REE can be further sub-divided into basal metabolic rate (BMR) and the energy expended in the digestion of food, known as the specific dynamic action of food, and is altered in disease states.

The BMR results from essential metabolic processes necessary to maintain the cellular integrity and function of specific organs, and accounts for up to 90% of REE (124). Grande has shown that liver, brain, heart and kidney account for only 5% of body weight but 70% of the total BMR, while muscle accounts for 40% of body weight but only 20-30% of the BMR (124).

The BMR correlates closely with body size and can be predicted from variables such as height, weight and body surface area corrected for age and sex. Although the BMR is reproducible in the same individual, the variability amongst individuals in large groups is approximately 5-10% (125). As the BMR is essentially a predictor of the "active cell mass", differences attributed to age, sex and body composition largely disappear when differences in adipose tissue are taken into consideration (126).

The BMR is primarily controlled by thyroid hormones and, indeed, between 1920 and 1950 estimation of BMR was an important clinical investigation in patients with suspected thyroid disease. In the steady state there may be circadian changes in the BMR reflecting circadian alterations in hormonal status (127). The BMR is affected by food intake because of the energy expended in digestion and absorption of food (specific dynamic action of food) which accounts for approximately 10% of the former (128) and may be due to alterations in thyroid hormone metabolism (129). It appears that the the specific dynamic action of food is largely due to its protein content (130). In contrast, glucose must be given in considerable excess of normal requirements before increasing energy expenditure (129) and, conversely, REE falls in the fasting state by 10-20% (131,132).

Alterations in systemic temperature are amongst the most

important determinants of the metabolic rate, so that an increase in body temperature of 1°C from any cause increases the metabolic rate by approximately 12% (133).

Physical trauma, burns and sepsis, separately or in combination, can increase core temperature and energy expenditure. These increases are secondary to endocrine and metabolic changes resulting in an increased turnover of glucose, fatty acids and protein, and increased production of the counter-regulatory hormones. The magnitude of change in energy expenditure after trauma is dependent on the severity of the insult (132). Elective surgery may increase energy expenditure by up to 10%, major trauma by 20-30%, severe sepsis by 40-60% and third degree burns involving more than 20% of the body surface area by 80-100% (132).

PART 3: METABOLIC RESPONSE TO CARDIOPULMONARY BYPASS

Cardiac surgery produces a unique form of tissue injury as it combines the additional insults of CPB and hypothermia with more conventional operative trauma. CPB promotes "whole body inflammation" through the activation of inflammatory mediators such as complement (5) resulting in the "post-perfusion syndrome" characterised by pulmonary, cardiac, renal, hepatic and haematological dysfunction (6). The effects of these cumulative insults on the catabolic response to surgical injury are poorly documented. Furthermore there are no prospective studies examining the effects of varying levels of intraoperative hypothermia on the systemic metabolic response to CPB.

THE ACUTE PHASE RESPONSE TO CARDIOPULMONARY BYPASS

There have been no detailed studies of the acute phase response following CPB although serial estimations of CRP following cardiac

surgery have been reported to be useful predictors of sepsis (134) and outcome (135).

Fitzsimons and Ballantyne documented an increase in serum iron in the early stage following CPB (136) but did not investigate the effect of different levels of intraoperative hypothermia on these changes. There are no data on changes in the acute phase reactant trace metals zinc and copper in response to CPB.

THE ENDOCRINE RESPONSE TO CARDIOPULMONARY BYPASS

In contrast to the acute phase response, the endocrine response to CPB has been exhaustively investigated. Most studies have either been observational or have examined the effects of pulsatile and nonpulsatile extracorporeal perfusion on the response. No study has specifically addressed the effects of different intraoperative temperatures on the endocrine response during CPB or in the week following surgery. It is not known whether modification of the endocrine response by a more profound level of intraoperative hypothermia would result in a reduction in the catabolic response.

In contrast to the elevated plasma levels of cortisol seen in most forms of stress, cortisol concentration has been reported to fall in patients undergoing nonpulsatile cardiopulmonary bypass (137-139). Such adrenal hypofunction resulted in the empirical use of exogenous corticosteroids (140) despite the demonstration of adequate levels of circulating unbound, physiologically active, free cortisol (141). The reduction in plasma cortisol at the start of bypass has been attributed to haemodilution due to the priming fluid for the pump (138) and inhibition of the pituitary gland during bypass (139). Taylor and colleagues demonstrated that the use of pulsatile, as opposed to nonpulsatile, bypass preserved the plasma cortisol response

to surgical injury and attributed this to an improvement in organ metabolism with pulsatile perfusion (142). The same workers reported that the absence of a pituitary response to thyroid stimulating hormone during nonpulsatile perfusion, could be restored by pulsatile perfusion (143). Taylor's work was performed at normothermia and in a historically controlled study of normothermic and hypothermic CPB, Kuntschen and colleagues reported that cortisol levels were significantly lower at the end of hypothermic CPB (144).

Several investigators have demonstrated increased adrenal catecholamine release during CPB (145-147) but there has been no prospective study to examine the effects of different levels of intraoperative hypothermia on this response.

NITROGEN BALANCE AND PROTEIN TURNOVER STUDIES

A number of studies have investigated nitrogen balance following cardiac surgery but are difficult to interpret in view of the differing clinical and nutritional status of the patients and the nature of the cardiac operation performed. Manners reported a cumulative negative nitrogen balance of 48 grams, over a six day period in patients undergoing coronary artery surgery and emphasised the difficulties in obtaining accurate measurements (148). There has been no study of the effects of different levels of intraoperative hypothermia on postoperative nitrogen excretion and there are no data on the effects of CPB or hypothermia on the kinetics of whole body protein synthesis and breakdown.

ENERGY EXPENDITURE FOLLOWING CARDIOPULMONARY BYPASS

There has been no formal study of energy expenditure following cardiac surgical procedures although Savino and colleagues, as part of

a larger study, reported the basal energy expenditure in three patients following coronary artery surgery as being between 1500 and 2000 Kcal/day during mechanical ventilation (149).

PART 4: MALNUTRITION PREVALENCE AND ASSESSMENT

In 1936 Studley reported a mortality of 33% in 46 patients undergoing peptic ulcer surgery who had lost more than 20% of their pre-illness body weight, compared with a mortality of 3.5% in patients with no weight loss (7). Subsequently Rhoads and Alexander demonstrated an association between poor nutritional status and susceptibility to infection (150).

Numerous studies over the last decade have confirmed a relationship between malnutrition and increased morbidity and mortality in hospital patients (151-154). Furthermore, a number of reports have documented a high incidence of protein calorie malnutrition in overtly nutritionally replete patients in general hospital wards (155-157) and those with valvular heart disease (158-160).

The pathogenesis of cardiac cachexia has been well-described by Pittman and Cohen (161,162) where cardiac failure causes relative tissue hypoxia and anorexia but an increase in the metabolic rate due to increased catecholamine production. As a consequence, there is wasting of muscle protein and adipose tissue which may culminate in cardiac atrophy promoting further heart failure (163). There is ample evidence that malnourished patients with valvular heart disease have an increase in morbidity and mortality following surgery compared to nutritionally replete patients (158-160). In patients with ischaemic heart disease Abel and colleagues (164) found little evidence of malnutrition although there was a greater incidence of postoperative

complications in male patients who were below their ideal body weight.

ASSESSMENT OF NUTRITIONAL STATUS

Numerous anthropometric, biochemical and immunological variables have been used to identify patients with evidence of protein calorie malnutrition. Baker and colleagues suggested that clinical examination could detect and assess malnutrition as accurately as laboratory tests (165) but their studies and interpretations have been criticised (166). Pettigrew and colleagues, in a prospective study of nutritional assessment, reported that even experienced clinicians on clinical examination alone could miss up to 50% of malnourished patients (167).

Anthropometry refers to the scientific measurement of body size, weight and composition, using measurements such as weight:height ratio, triceps skin fold thickness, mid-arm circumference and calculated mid-arm muscle circumference. These parameters provide useful information in overt protein calorie malnutrition but are of limited value in more subtle forms (168). Anthropometric indices have also been successfully used to predict energy expenditure (169).

A large number of biochemical parameters have been used in an attempt to identify patients with less overt forms of protein calorie malnutrition. The most commonly used indices have been plasma concentrations of albumin, transferrin, prealbumin and retinol binding protein. The same proteins can be used to assess the response to nutritional therapy depending on their half-lives of respectively 30 days, 8 days, 2 days and 12 hours (170). More recently fibronectin has been proposed as a sensitive indicator of nutritional status (171). The use of plasma proteins as markers of nutritional status is complicated by the fact that their plasma concentrations are dependent

on numerous factors including protein synthesis, catabolism and redistribution with proteins in the extravascular space.

Tests of delayed hypersensitivity have been recommended by some workers as useful parameters of nutritional status (172). These tests essentially examine cell-mediated immunity by the ability of the patient to mount an immune response to intra-dermally administered antigens such as purified protein derivatives, trichophytin, candida and streptokinase streptodormase (172). The usefulness of such tests in predicting postoperative morbidity and mortality and the relationship of these to nutritional status is, however, disputed (173).

Despite the well-established relationship between malnutrition and an increase in postoperative morbidity and mortality, no single parameter or estimate of nutritional assessment has been shown to have a predictive value by itself. Consequently some workers have combined several different variables of nutritional status to try and improve prognostic accuracy. Buzby and Mullen (153) have recommended the use of four factors (serum albumin and transferrin, triceps skin fold thickness and delayed hypersensitivity) as having prognostic significance, while demonstrating that absolute changes in weight, *per se*, do not correlate with clinical outcome. Indeed, recently Windsor and Hill have demonstrated that weight loss is a basic indicator of surgical risk only when associated with clinically obvious impairment of organ function (174).

PART 5: MANIPULATION OF THE METABOLIC RESPONSE

The recognition that the catabolic response to surgery results in the development of a negative nitrogen balance, through the loss of body protein (60,61,175), has stimulated intense research into

techniques to attenuate the response. These can be conveniently classified as environmental, nutritional and pharmacological.

ENVIRONMENTAL

Cuthbertson and colleagues demonstrated that nursing patients with long bone fractures at an ambient temperature of 28°C-30°C resulted in a significant reduction in nitrogen excretion compared to patients nursed at 20°C-22°C (107). It was subsequently reported that nursing burn patients in a warm, dry atmosphere could reduce mortality (176) and was presumed to result from energy required to evaporate water from burned tissues being met from the warmed environment rather than from the patient's own body fuels (177). Other workers demonstrated no significant metabolic benefit in nursing patients undergoing elective abdominal surgery of moderate severity in an increased environmental temperature (178).

The concept of using intraoperative hypothermia to modify the "stress response" to surgery was first proposed in the 1950's (8,9) but was abandoned when moderate topical hypothermia was found to produce a postoperative rebound increase in cortisol secretion. More recently Wilmore's group reported that "hypothermic anesthesia" during cardiac surgery reduces post traumatic proteolysis (10). This group, however, examined only moderate hypothermia (28°C) and used fentanyl anaesthesia in doses (100-200 ug/Kg) which can themselves modify the catabolic response to surgery (*vide infra*).

NUTRITIONAL

Since Studley's report (7) of increased mortality in malnourished patients undergoing peptic ulcer surgery, a large body of clinical evidence has confirmed the adverse effects of protein-energy

malnutrition on clinical outcome (151-154).

As a consequence, the use of nutritional support to reduce postoperative complications in malnourished patients has been widely recommended (179). The rationale lies in the demonstration that nutritional support accelerates the rate of protein synthesis towards the accelerated rate of protein breakdown which accompanies the catabolic response, thereby sparing body protein (180). Nutritional support can be provided enterally or parenterally (Total Parenteral Nutrition) in the case of gastrointestinal tract failure.

Despite numerous studies there is no consistent evidence that perioperative nutritional support reduces the mortality of surgery (181). Some groups have reported a reduction in mortality and morbidity with postoperative nutritional support (182,183) but this is disputed by others (184,185). Similarly there is no evidence that preoperative nutritional support reduces postoperative complications (186) although this may reflect too short a preoperative feeding regimen.

Apart from doubts over the efficacy of Total Parenteral Nutrition in reducing postoperative morbidity and mortality, such feeding is expensive and has potentially serious side effects. In view of these complications some workers have promoted the concept of peripheral intravenous nutrition (187).

PHARMACOLOGICAL

Anaesthesia employing large doses of opiate-based preparations such as morphine (2-4 mg/Kg body weight) or fentanyl (50-100 ug/Kg body weight) can attenuate the early endocrine response to general surgical trauma but not that during CPB nor postoperative nitrogen excretion (188,189). The use of the sedative etomidate has been

abandoned after demonstration of suppression of cortisol secretion in severely ill patients (190) with a consequent rise in mortality (191).

Regional neurogenic blockade, using epidural or spinal local anaesthetics, has been reported to modify the catabolic response to surgery with varying success. Brandt and colleagues (192) reported that epidural analgesia significantly improved postoperative nitrogen balance following hysterectomy while others found no such effect after upper abdominal surgery (193). It is possible that while epidural anaesthesia can block both afferent and efferent autonomic pathways in the lower abdomen, the autonomic system is less effectively blocked in the upper abdomen. Some workers have reported that splanchnic nerve blockade can modify the stress response to surgery (194) although the cortisol response is not affected (195).

Naftidrofuryl has been reported to significantly reduce cumulative postoperative nitrogen excretion and its effect was presumed to be due to more efficient metabolism of endogenous carbohydrate and fat (196). These results have been disputed by others (197) who found no beneficial effect of naftidrofuryl on nitrogen balance after elective surgery.

Anabolic steroids have been reported to improve postoperative nitrogen balance (198) in both the catabolic (199) and anabolic components of the response to trauma (200). Their clinical usefulness has so far been limited by their tendency to cause virilising effects as well as water retention (201). Anabolic steroids have been reported to promote positive nitrogen balance in patients receiving parenteral nutritional support (202) but this has been disputed by others (201). There is, however, no evidence that any beneficial effect of steroids on nitrogen balance has led to a reduction in postoperative morbidity or mortality.

Although plasma levels of insulin are elevated in response to trauma it appears to be less metabolically active than usual, giving rise to the concept of "insulin resistance" (83), and explaining the hyperglycaemia, losses of body nitrogen and potassium, and defects of cell membrane function (203) observed after severe injury. Some workers have reported that administration of exogenous insulin with glucose and potassium can reduce nitrogen and potassium losses in seriously burned patients (85,204).

Corticosteroids have been used to treat septic shock (205) and the adult respiratory distress syndrome (206) and although there has been no convincing evidence of a reduction in mortality, it has been postulated that this reflects their late administration to critically ill patients. The question of their efficacy remains unproven and trials are currently underway to determine if their administration earlier in the course of septic illness will favourably influence outcome. A large prospective randomised trial of steroids administered early in the course of the adult respiratory distress syndrome showed no beneficial effect on clinical outcome (207).

Growth hormone has been demonstrated to improve nitrogen and potassium retention in burn patients, presumably by increasing insulin levels (208). It is likely that the availability of growth hormone from genetically programmed bacteria will lead to further studies on its therapeutic potential. It has recently been reported that biosynthetic growth hormone maintained a positive nitrogen balance in ill patients undergoing major gastrointestinal surgery who received a hypocaloric and low nitrogen-containing (7 grams) intravenous feeding regimen (209).

CHAPTER 3

METHODS

CHAPTER 3

METHODS

Little flower- but if I could understand
What you are, root and all, and all in all,
I should know what God and man is.

Alfred, Lord Tennyson (1809-1982). Flower in the crannied wall.

PART 1: POPULATION STUDIED

The patients studied in this thesis presented to the Cardiothoracic Unit of the Royal Infirmary in Glasgow, between August 1986 and September 1988.

All were male patients in the age range 40-64 years, undergoing elective coronary artery surgery. They were expected to receive at least two coronary artery grafts using saphenous vein and/or internal mammary artery.

All patients studied had isolated ischaemic heart disease. Patients who had undergone major surgery in the preceding six months were excluded as were those with diabetes mellitus, hyperlipidaemia, endocrine disorders and renal or hepatic impairment. No patient had unstable angina or was in cardiac failure.

In addition to normal hospital procedures a careful note was taken of anti-anginal medical therapy such as nitrates, beta-blockers and calcium antagonists.

Informed consent was obtained from all patients and the studies approved by the Hospital Ethical Committee.

Patients were randomised to one of two levels of intra-operative hypothermia ($28^{o}C$ or $20^{o}C$) on the morning of surgery. The surgical

operations were performed by one of five consultant surgeons using standardised procedures.

PART 2: OPERATIVE PROCEDURE

ANAESTHETIC TECHNIQUE

All patients were prescribed a benzodiazepine (20 mg temazepam) on the night before surgery.

On admission to the anaesthetic room the patient was given oxygen to breathe while ECG monitoring was commenced and the blood pressure checked. Anaesthesia was induced with a combination of an opiate (morphine), narcotic (fentanyl) and benzodiazepine (midazolam). Intubation was performed after administration of a non-depolarising neuromuscular blocking agent (pancuronium) and subsequently a radial artery cannula, central venous line (double lumen), nasopharyngeal temperature probe and urinary catheter were inserted.

Anaesthesia was maintained during surgery by a combination of morphine, fentanyl, midazolam (benzodiazepine), pancuronium and isoflurane (inhalational volatile anaesthetic). No patient received fentanyl in excess of 20 ug/Kg body weight or morphine in excess of 2 mg/Kg of body weight.

During the surgical procedure all patients were continuously monitored for heart rate and rhythm, blood pressure, central venous pressure, temperature, and urinary output. Blood temperature was continuously recorded in the extracorporeal circuit.

At the end of the operation the patient was returned, ventilated, to the Cardiac Surgical Intensive Care Unit. The patient remained ventilated until fully rewarmed (as core temperature normally falls during closure of the sternotomy wound) and haemodynamically stable. Prior to extubation, usually eight to twelve hours after

surgery, boluses of midazolam (2 mg) and morphine (2 mg) were used to provide adequate sedation and analgesia. Vercuronium (2-4 mg) was used to paralyse the patient if mechanical ventilation was not optimal. Dopamine was infused in inotropic doses (5-10 ug/ Kg/min) to maintain arterial blood pressure >100 mmHg when necessary. More commonly vasodilators (sodium nitroprusside and/or glyceroltrinitrate) were administered to prevent systolic arterial pressure rising above 140 mmHg.

OPERATIVE TECHNIQUE

The surgical operation commenced with the opening of the chest via a median sternotomy, using a Stryker saw, while long saphenous vein was simultaneously harvested from one or both legs. When appropriate the internal mammary artery was dissected from the sternum with a diathermy blade.

After opening of the pericardium, purse string sutures were placed in the aorta and right atrium at the proposed sites of cannulation. Heparin was administered via the right atrium in a dose of 300 units/Kg of body weight and the ascending aorta was cannulated with a Sarn 8 mm cannula. The right atrium was cannulated next, either with two cannulae respectively placed into the inferior and superior venae cavae or a single, double lumen, cannula. If deemed necessary a pulmonary artery or aortic arch vent was then inserted to ensure optimal drainage.

CPB was commenced when the activated blood clotting time exceeded 400 seconds as measured by the Haemochron 400 (International Technidyne Corporation). Systemic cooling was commenced and pulmonary ventilation stopped when a satisfactory flow rate was obtained. After identification of the proposed sites for coronary artery grafting the

ascending aorta was cross-clamped and 1 litre of cold cardioplegic solution (4° C) infused, in divided doses and under pressure, proximal to the cross-clamp to produce a rapid, flaccid paralysis of the myocardium. Topical cooling of the heart was achieved with physiological saline at 4°C.

The distal anastomoses were performed first by a continuous suturing technique for saphenous vein and an interrupted or continuous suturing technique for the internal mammary artery depending on the preference of the surgeon (210). The anastomoses were performed with 7/0 Prolene. Shortly prior to completion of the last distal anastomosis, systemic rewarming was commenced and on completion of the last anastomosis the cross-clamp was released to allow myocardial reperfusion. During systemic re-warming a segment of the ascending aorta was isolated with a clamp and a disc of aorta removed using a Goosen punch. The proximal anastomoses were performed with a continuous suturing technique using 6/0 Prolene. On completion of the proximal anastomoses the patient was weaned from bypass and the atrial cannula(e) removed. When haemostasis was considered to be satisfactory, protamine (3 mg/Kg body weight) was administered to reverse the effects of heparin, as judged by the restoration of a normal activated clotting time, and the aortic cannula removed.

The sternum was approximated with steel wires over two drains placed in the pericardial cavity. The subcutaneous and subcuticular layers were closed with Dexon and the legs with subcutaneous Dexon and clips for skin.

CARDIOPULMONARY BYPASS (Figure 3.1,3.2).

CPB was performed using pulsatile perfusion (Stockert pulsatile roller pump, Stockert Instruments, Munich, West Germany), a Harvey

H1700 bubble oxygenator (Bard Ltd., Pennywell Industrial Estate, Sunderland, UK) and a 40 micron pore size arterial filter (AF-1040C heparin-coated arterial filter, Edwards CVS Division, Bentley Laboratories Ltd., Wallingford Rd., Compton, Berkshire, UK).

The priming fluid for the extra-corporeal circuit consisted of 2 litres of Ringer's lactate, 8,000 units of heparin, 50 mmol of sodium bicarbonate, 10 g of mannitol, 15 mmol of potassium chloride and 750 mg of sodium cefuroxime.

Alteration in blood temperature in the extracorporeal circuit was achieved with a Normo-/Hypothermic heat exchanger (Stockert Instruments, Munich, West Germany) capable of decreasing or increasing blood temperature at approximately $1^{o}C$ per minute. To obtain the selected blood temperature, circulating water in the heat exchanger was cooled to $20^{o}C$ prior to CPB when the desired blood temperature was $28^{o}C$ and to $14^{o}C$ when the desired blood temperature was $20^{o}C$. Shortly prior to completion of the last distal anastamosis, systemic rewarming was commenced and continued during completion of the proximal anastamoses until a core temperature of $37^{o}C$ was attained.

The intraoperative blood temperature was continuously recorded in the extracorporeal circuit throughout the operation (Therm-A, Edwards CVS Division, Bentley Laboratories Ltd., Wallingford Rd., Compton, Berkshire, UK). Simultaneously, core temperature was measured in the nasopharynx (Yellow Springs series 401 temperature probe, Siemens Ltd., Napier Court, Wardpark North, Cumbernauld, Scotland, UK) and continuously displayed, along with blood temperature, on a Siemens Sirecust 404 patient monitoring system (Siemens, Munich, West Germany).

The perfusion flow rate was calculated from the formula that at normothermia full flow was equivalent to 2.4 L m^{-2} min^{-1} (11). During

intraoperative hypothermia the flow rate was reduced but did not fall below 1.5 L m^{-2} min^{-1}.

One litre of 4°C cardioplegic solution (St. Thomas's Hospital formula) consisting of 3.253 g of magnesium chloride, 1.193 g of potassium chloride and 273 mg of procaine hydrochloride in 1 litre of Ringer's lactate was administered in divided doses during the cross-clamp period. The heart was topically cooled with physiologic saline at 4°C.

It is current practice in this centre to control acid-base status by "pH-STAT" management, which entails alterations in CO_2 during CPB depending on pH. This technique is based on the fact that as body temperature falls during the hypothermic period of CPB, CO_2 solubility increases while the pH tends to rise because the dissociation constant of water (pK_W), the primary source of H^+ ions, falls. To maintain acid-base status during hypothermia, by pH-stat, requires increasing the total CO_2 content of blood either by addition of CO_2 or by hypoventilation, so that the pH remains at 7.40 even during hypothermia (211). There is, however, growing evidence that pH-stat management of acid-base balance during hypothermia leads to an uncoupling of cerebral blood flow and metabolism, so that below a mean arterial blood pressure of 55 mmHg there is loss of cerebral autoregulation and cerebral blood flow becomes pressure dependent. This subject has been extensively reviewed by Pearson who indicates the physiological superiority of "alpha-stat" management of acid-base balance, which maintains autoregulation of cerebral blood flow and therefore cellular metabolism, at a lower mean arterial pressure (212).

On return to the Cardiac Intensive Care Unit boluses of morphine (1-2 mg), midazolam (1-2 mg) and vercuronium (2 mg) were used to

maintain analgesia and permit mechanical ventilation until the patient was fully rewarmed, haemodynamically stable and ready for extubation.

POSTOPERATIVE FLUID REGIMEN

Fluid management was standardised in the perioperative period (213). Fluid balance charts were used routinely for all patients, initially on an hourly basis (for the first 48 hours) and then on a daily basis, the aim being to keep each patient in a slightly negative fluid balance (200 - 500 ml) over each 24 hour period. Patients received a standard fluid regimen consisting of 40 mls of 5% dextrose/ hour and 40 mls of 5% dextrose with 5 mmol potassium/ hour. Additional volume was administered in accordance with haemodynamic criteria (arterial blood pressure, central venous pressure, skin/core temperature gradient and urine output) and was predominantly with crystalloids unless required rapidly when colloid was administered. Blood loss was replaced with autologous blood from the "pump" or banked "packed cells" for excessive loss accompanied by a packed cell volume consistently less than 30%. Likewise blood was only administered in the remainder of the postoperative period for a persistently low haemoglobin (< 10 g/dl).

A positive fluid balance, in excess of 500 ml over a 24 hour period, was an indication for diuretic therapy (intravenous boluses of 10 mg of frusemide).

PART 3: BIOCHEMICAL METHODS.

Biochemical methods are described in the relevant chapters.

PART 4: ANTHROPOMETRY.

The anthropometric assessments were performed by two experienced

observers. Ms Rosemary Richardson (Research Dietitian, Department of Biochemistry, Royal Infirmary, Glasgow) performed the anthropometric assessments reported in Chapters 4 and 13. Ms Carol Lowis (PhD student, Department of Physiology, Glasgow University) performed the anthropometric assessments reported in Chapter 5.

The patients were weighed in light attire (pyjamas only) and heights were recorded without footwear. Mid-arm circumference (MAC) was measured in the dependent and non-dominant arm, with the elbow flexed to 90^{o}, at a point midway between the acromion and olecranon process using Harpenden Skinfold Calipers (British Indicators, Holtain Ltd, Bryberian, Crymmych, Pembrokeshire, UK). Triceps skinfold thickness (TST) was based on the mean of three readings of a fold of skin, pinched between thumb and forefinger overlying the triceps, at the point where MAC was measured.

Arm muscle circumference (AMC) was calculated from the formula AMC = MAC -(0.314 TST) where AMC and MAC are in cm and TST in mm (214). AMC and TST were expressed as percentages of expected standard where in males AMC=25.3 mm and TST =12.5 mm (215).

PART 5: PROTEIN TURNOVER STUDIES (Figure 3.3, 3.4).

THEORETICAL ASSUMPTIONS (Figure 3.3).

Whole body protein turnover rates were calculated by the stochastic method of Picou and Taylor-Roberts (95). This method assumes the existence of a single metabolic pool of low molecular weight nitrogen compounds such as urea, amino acids and ammonia. The pool derives protein from two sources: from food intake (I) and from the breakdown of body proteins (B); protein is lost from this metabolic pool either through the synthesis of new body proteins (S) or, after degradation, by loss as nitrogenous waste products (E)

mainly as urinary urea but also through faecal and skin losses. In the steady state the amount of protein entering the pool is equal to the amount being lost, ie. I+B=S+E. Total protein turnover (Q) is equal to the amount of protein entering or leaving the pool per unit of time, ie. Q = I+B =S+E.

In the fasting state, since protein intake equals zero, the rate of protein turnover (Q) is equal to the rate of protein breakdown (Q=B) and the rate of protein synthesis equal to protein turnover less the nitrogen lost through excretion (S=Q-E).

The method of Picou and Taylor-Roberts (95) assumes that if an isotope is administered as a continuous infusion then a point is reached when the total amount of isotope entering the body's metabolic protein pool is equal to the amount leaving (ie. isotopic steady state). The specific activity of the pool can be estimated by measuring the specific activity of one of the products of the pool (in this case the chosen end products of protein metabolism were urinary urea and ammonium). Protein turnover was calculated using the formula:

$Q=D/E$ where Q= protein turnover

D= quantity of isotope infused per unit time

E= isotopic enrichment of chosen urinary end product

The method assumes that:

1) the size of the protein pool remains constant during the plateau period.
2) there is no significant re-entry of isotope into the protein pool during the plateau period (because relatively high levels of enrichment are achieved during the plateau, any isotope re-entering the pool is unlikely to significantly alter the final enrichment).
3) amino-acids from dietary sources are used in a similar way to

those derived from breakdown of body proteins.

4) there is no discrimination against the isotope.

5) the isotope labelled amino acid (in this case ^{15}N glycine) is a valid tracer for total amino-nitrogen and is utilised in the same way as 14 nitrogen.

PRACTICAL CONSIDERATIONS (Figure 3.4).

Protein turnover studies were performed by the method described by Sim and colleagues (216,217) using a primed, continuous 24 hour infusion of the amino acid glycine labelled with the stable isotope ^{15}N. The use of a primed infusion has been shown to shorten the time to plateau (216).

On the day prior to the study the patient ate a standardised hospital diet. After an overnight fast the patient voided at approximately 07.00 hours and this urine was discarded. At 07.50 the patient voided a second time and this urine specimen was retained for determination of baseline enrichment of ^{15}N in urinary ammonia and urea. At 08.00 a primed infusion of ^{15}N glycine was commenced and simultaneously a 24 hour urine collection, in two 12 hour aliquots, was begun (216). The ^{15}N glycine was obtained from Amersham International and sterilised by microfiltration. The priming dose of ^{15}N glycine was 50 mg and the 24 hour infusion contained a further 50 mg in 1 litre of normal saline. The ^{15}N glycine infusion was administered into a peripheral vein via a peristaltic infusion pump (Ivac, Harrow, UK).

The pre-operative study was performed in the 48-24 hour period before surgery. During this period the patient was allowed free access to non-sugar and non-milk containing fluids but not to solid food. The patient was permitted to perform minimum ward activities,

eg. lying in bed or sitting watching television. The study was repeated in the 24-48 hour post-operative period, after obtaining a base-line specimen of urine to determine ^{15}N enrichment of urinary urea and ammonium, while the patient was confined to bed.

Sample preparation

Total urinary nitrogen was measured on a Technicon autoanalyser following kjeldahl digestion (218).

The isotopic enrichment of urinary urea and ammonium was measured in urine collected over the 12-24 hour infusion period, as Sim and colleagues have demonstrated that during this period plateau isotopic enrichment occurs (216).

Ammonia was extracted from the urine specimen using a cation exchange resin in the sodium/potassium form (Bio-rad Laboratories Ltd., Hertfordshire, UK), in a modification of the technique initially described by Read and co-workers (219), leaving an aliquot of ammonium-free urine. The urea in this latter specimen was converted to ammonium by hydrolysis with urease and then extracted using the same type of cation exchange resin. Ammonium was liberated from the dry resin by treatment with 2.5M $KHSO_4$ and 50-100 uL subsamples (approximately 1-4 umol N_2) were pipetted into pre-frozen aluminium combustion chambers and freeze-dried for subsequent analysis.

^{15}N enrichment in urinary urea and ammonium was measured by continuous flow isotope ratio mass spectrometry (220). This consisted of a Roboprep biological sample convertor (Europa Scientific Ltd., Crewe, UK) interfaced directly to a MM 602 dual collector isotope ratio mass spectrometer (VG Isogas, Winsford, UK), by a simple variable leak valve. The sample preparation system and autosampler were computer controlled, making the isotopic analysis fully

automatic. The coefficient of variation was 0.10% for these analyses.

The atom per cent excess of ^{15}N in each sample was calculated from the mass 28 and 29 peak heights. By integrating and summing isotope peaks, the total elemental content of the analyte was quantified. The ^{15}N atom per cent excess for ammonium and urea in each patient was derived by subtracting the measured baseline enrichment of each end-product prior to the study. This pre-infusion isotopic enrichment was subtracted not only from the preoperative measurement of whole body protein turnover (WBPT) and whole body protein breakdown (WBPB), but also from the postoperative measurement. This was done as the postoperative pre-infusion isotopic measurement in urea and ammonium end-products reflected recycling of the ^{15}N label.

Measurements were performed by Dr. T. Preston (Scottish Universities Research and Reactor Centre, National Engineering Laboratory, East Kilbride) and Mr. D. McMillan (University Department of Surgery, Royal Infirmary, Glasgow).

PART 6: MEASUREMENT OF ENERGY EXPENDITURE

Total energy expenditure consists of two components: a relatively constant resting energy expenditure and a more variable activity energy expenditure. Resting energy expenditure is that energy used to maintain body temperature and can be further subdivided into basal metabolic rate (BMR) and energy expenditure due to food intake (specific dynamic action of food).

Energy expenditure can be calculated by measurement of heat loss (direct calorimetry) or estimation of heat production (indirect calorimetry).

During direct calorimetry the subject is enclosed within a

specially constructed chamber surrounded by water or ventilated by controlled air. Energy expenditure is calculated from the rise in temperature of the water or air, but the method depends on the subject being undisturbed for long periods and is therefore unsuitable in the clinical setting.

Indirect calorimetry provides an estimate of BMR. The maintenance of cellular integrity and the function of specific organs involves the combustion of body fuels which utilises oxygen (O_2) and produces carbon dioxide (CO_2). Measurement of gaseous exchange of O_2 and CO_2 through the lungs, by collecting all gases inspired from and expired into a canopy placed around the subject's head, provides an indirect estimation of energy expenditure. Furthermore the ratio of CO_2 production to O_2 consumption (ie. the respiratory quotient: RQ) is a useful indicator of the type of fuel being utilised. Useful measurements depend on the subject being undisturbed for at least 40 minutes and this technique is therefore unsuitable in ill patients where easy and frequent access must be available to medical and nursing staff.

Indirect calorimetry thus provides a factorial computation of energy expenditure over 24 hour periods which is difficult to validate in the postoperative patient and whose relationship to true total energy expenditure remains uncertain.

INDIRECT CALORIMETRY (Figure 3.5)

The indirect calorimeter used in this study was a ventilated hood system, used over the last ten years in the Physiology Department at Glasgow University. It consists of a flexible plastic hood placed around the subject's head and shoulders. Room air of a known O_2 and CO_2 concentration is drawn through the hood at a controlled rate (1

litre per Kg of body weight per minute). A sample line from the hood outflow allows the mixture of inspired air and patient's expired gases to be continuously analysed for O_2 and CO_2 content.

Technique

Calorimetry was performed at 08.00 after an overnight fast. The patient remained in bed after wakening and was not permitted to bathe or undergo any physical activity prior to a calorimetry run. A thirty minute acclimatisation period enabled the patient to become used to the apparatus and to reach a steady state before a thirty minute calorimetry run. Every 30 seconds the oxygen concentration of the air leaving the hood was recorded and the mean concentration in each ten minute period calculated. A sample of room air collected simultaneously and analysed at the end of each measurement period provided a mean estimate of inspired O_2 content.

The gases were dehumidified using silica gel prior to measurement. Oxygen concentration was measured with a paramagnetic oxygen analyser (Servomex 570A, Servomex Ltd., Crowborough, Sussex, UK) and the CO_2 concentration by a single channel infra-red carbon dioxide analyser (Seiger Ltd., Poole, Dorset, UK).

After correction of O_2 and CO_2 concentration for temperature and barometric pressure the oxygen consumption (VO_2) and carbon dioxide production (VCO_2) was calculated by comparing differences between room air and mixed expired gases.

The oxygen analyser was calibrated each morning using oxygen free nitrogen and inspired air and the CO_2 analyser using 0.8% CO_2. The integrity of the ventilated hood was established both before and after the study by means of a gas recovery test and was found to be accurate to +/- 2%.

Calculation of oxygen consumption

oxygen consumption= vol O_2 inspired - vol O_2 expired

$$= Vi \times \frac{20.9}{100} - Ve \times \frac{\%O_{2e}}{100} \quad (1)$$

where Vi =vol air inspired

Ve =vol air expired

20.9% =% oxygen in inspired air (measured in the current study)

$\%O_{2e}$ =% oxygen in expired air

therefore oxygen consumption $= \frac{(20.9 - \%O_{2e})}{100} \times$ flow (2)

Calculation of metabolic rate

The calorific equivalent of oxygen is equal to the amount of heat released for every litre of O_2 used and averages 5 Kcal/L.

The metabolic rate = O_2 consumption x calorific equivalent of O_2 and taking equation 2:

$$\text{metabolic rate} = \frac{(20.9 - \%O_{2e})}{100} \times \text{flow} \times 5$$

$$= \frac{(20.9 - \%O_{2e})}{20} \times \text{flow} \quad (3)$$

This is known as the Weir equation (221) and assumes that Vi=Ve, which is only true when the Respiratory Quotient (RQ) -the ratio of CO_2 produced to the ratio of O_2 used -is 1, and this is seldom the case (error 1). However the volume of nitrogen and inert gases in inspired air always equals the volume of nitrogen and inert gases in exhaled air. Returning to equation 1 and re-writing:

$$\text{metabolic rate} = Vi \times \frac{20.9}{100} - Ve \times \frac{\%O_{2e}}{100} \times 5 \quad (4)$$

It is apparent that assuming Vi=Ve when in fact Ve<Vi (RQ<1)

will underestimate the volume of oxygen inspired, and will under-estimate the metabolic rate (error 1).

However the calorific equivalent of oxygen also varies depending upon the fuel being oxidised: fat has an RQ of 0.71 and a calorific equivalent of 4.69 Kcal/L and protein an RQ of 0.81 and a calorific equivalent of 4.60 Kcal/L. As the RQ falls the calorific equivalent of oxygen falls, and assuming the calorific equivalent to be 5, as in equation (4), will over-estimate the metabolic rate (error 2).

Under normal circumstances these two errors cancel out and the de Weir equation gives an accurate assessment of metabolic rate.

The most widely used formula for calculating BMR is that of de Weir (221).

$$BMR = 1.44\ (3.941\ VO_2 + 1.106\ VCO_2) - 2.17\ (UN)$$

where BMR = Basal Metabolic Rate in Kcal/24 hours

VO_2 = Oxygen consumption in ml/min

VCO_2 = Carbon dioxide production in ml/min

UN = Total urinary nitrogen excretion in grams/24 hours

Several researchers have shown that omitting to measure urinary nitrogen results in an error of less than 2% (222) so that:

$$BMR = 1.44\ (3.941\ VO_2 + 1.106\ VCO_2)$$

Under steady state conditions $VCO_2 / VO_2 = RQ$ and by substituting $(RQ \times VO_2)$ for CO_2 the formula becomes

$$BMR = 1.44\ (3.941\ VO_2 + 1.106[RQ \times VO_2])\ Kcal/24\ hours$$

$$BMR = 1.44\ VO_2\ (3.94 + 1.11\ RQ)\ Kcal/24\ hours$$

If the RQ is 0.7 (when fat is being predominantly oxidised) then assuming a patient has an oxygen uptake of 250ml/min:

$$BMR = 1.44 \times 250\ (3.94 + [1.11 \times 0.7]) = 1698\ Kcal/24\ hours$$

If the RQ is actually 1.0 then assuming the same patient has an oxygen uptake of 250ml/min

BMR= 1.44 x250 (3.94 +[1.11 x 1.0]) = 1818 Kcal/24 hours

The usual figure for RQ in clinical practice, after an overnight fast, is in the region of 0.80-0.85. Assuming a value of 0.85 then

BMR= 1.44 x250 (3.94 +[1.11 x 0.85]) = 1758 Kcal/24 hours

This is approximately 3% different from the value which would be obtained with an RQ of 0.7 or 1.0. Errors of this magnitude have no clinical significance and by using estimated RQ values, the necessity to measure VCO_2 is avoided simplifying all measurements.

DOUBLY LABELLED WATER TECHNIQUE

The Doubly Labelled Water (DLW) technique for calculating energy expenditure entails the administration of two stable isotopes, ^{2}H and and ^{18}O, in the form of labelled water (ie $^{2}H_2O$ and $H_2{}^{18}O$) and is based on the observation that oxygen in respired CO_2 is in equilibrium with oxygen in body H_2O through the action of carbonic anhydrase (223). After mixing with body water ^{2}H is lost from the body as water whereas ^{18}O is lost both as water and carbon dioxide. The difference in the rate of excretion of the two isotopes from the body is therefore due to CO_2 production from which energy expenditure can be calculated (224). Thus whereas indirect calorimetry provides a factorial computation of total energy expenditure, by extrapolation from a brief measurement of BMR, the use of DLW allows calculation of integrated estimates of total energy expenditure over fixed periods.

The DLW method was described in 1955 (224), used for the study of energy expenditure in small animals from then (225), and applied to man by Schoeller and van Santen in 1982 (226). The DLW technique has been demonstrated to have an accuracy to within 2%-5% of CO_2 production rates calculated by whole-body indirect calorimetry (227-230).

The initial dilution of either isotope in body water reflects the body water pool size and therefore allows estimation of body composition (see **Calculations**, page 83).

Methodology

Patients presented ten days prior to surgery and, after passing a baseline urine sample to determine background enrichment, received 75 mg ^{18}O per Kg body weight (8.7 atom% ^{18}O, Delta Isotopes, Crewe, UK) mixed with deuterium (25 mg ^{2}H per Kg body weight, 99.8 atom percent excess, MSD isotopes, Montreal, Canada). Patients were allowed home and daily urine samples (20 ml) were collected until surgery.

A second identical dose of DLW was administered six hours after surgery with residual levels of oxygen and hydrogen enrichment being calculated from the pre-surgery isotope decay curves. Daily morning 20 ml urine samples were collected for a further 10 days following surgery.

A final dose of deuterium alone (10 mg ^{2}H per Kg body weight, 99.8 atom percent excess) was administered on the tenth postoperative day and 4 accurately timed urine samples obtained in the subsequent eight hours, to be analysed if deemed necessary for a third and final determination of total body water (TBW).

Patients kept a daily diary before and after surgery to record both dietary intake and activity levels.

^{18}O Analysis

All urine samples were analysed for ^{18}O enrichment by continuous flow isotope ratio mass spectrometry (CF-IRMS) (231). 1.5 ml samples of urine were equilibrated with CO_2 (18.5 ml of 10% CO_2) in a

vacutainer at 25°C for 72 hours. 1 ml gas samples (100 uL CO_2) were then injected into a CF-IRMS (Roboprep-CN Sample Convertor, Europa Scientific, Crewe, UK) interfaced to an MM602 IRMS (VG Isogas, Middlewich, UK) via a septum inlet where the gas was in turn dried, purified by gas chromatography and bled into the IRMS. The mass ratio 46/44+45 was measured automatically and the instrument calibrated against distilled water standards of known ^{18}O enrichment. The coefficient of variation for replicate urine samples was less than 0.2%. Accuracy was within 1% over the calibrated range (0-350 ppm above background).

Deuterium (^{2}H) analysis

Baseline urine samples, samples showing maximum ^{18}O enrichment and those every second day from those samples, were analysed for deuterium enrichment by conventional dual batch inlet gas source IRMS, after reduction to hydrogen gas over depleted uranium (232).

The vacuum rig for the preparation of hydrogen and deuterium gas from urine specimens has two components, the first for distilling water from urine and the second for reducing water vapour to hydrogen gas. Urine is heated to vaporise the water which eventually freezes into a cold trap. Non-condensible gases are pumped away to leave a pure water sample. During this process the uranium furnace is heated to 800°C and the hydrogen gas collection vessels are evacuated and cooled with liquid nitrogen. Water from the initial urine specimen warms, vaporises and passes through to the uranium furnace where it is reduced by the uranium to release hydrogen/deuterium gas which adheres to the activated carbon in cold hydrogen gas collection vessels.

Each sample was analysed with reference to a calibrated hydrogen working standard and the internal precision of deuterium analysis at

natural abundance was 0.05 parts per million (1 SD).

Calculations

The fractional turnover rate of each isotope for both pre and post surgery phases was equivalent to the gradient of the plot of the natural logarithm of the baseline-corrected isotope enrichment against time in days.

Total body water (TBW) was estimated by extrapolating the ^{18}O enrichment of the first five samples from the dose time to zero time. TBW at the time of surgery was calculated in a similar fashion after calculating by extrapolation the residual isotope from the first ^{18}O dose. It was assumed that ^{18}O overestimated TBW by 1% (226).

Fat free mass (FFM) was calculated assuming constant hydration: FFM = TBW/0.73 assuming that lean tissue contains 73% water and fat tissue negligible amounts (233). The fat mass (FM) was calculated by the difference in body mass (BM) and FFM, ie FM= BM-FFM.

Changes in baseline enrichment of either isotope during the experimental period could introduce significant error. Variations in the ^{2}H baseline due to fluid source variation was more likely than changes in ^{18}O baseline. However, analysis of ^{2}H natural abundance in urine of patients undergoing the same surgical procedure and receiving fluids from the same source, but not receiving deuterium, showed only minor variations (142 sd 2 parts per million).

As there was some evidence of deviation from a simple regression line in both isotopes, especially after surgery, it was considered desirable to compare the regression of only the ^{18}O samples equivalent to each ^{2}H sample analysed, rather than analysis of the full ^{18}O data set. The probability of the regression coefficient for ^{18}O was typically <0.05 for five points and <0.0005 for the full data set.

Energy expenditure was calculated using the formula of Lifson and Mclintock which includes a 4% correction for isotope fractination assuming that 50% of water loss is insensible (224,234):

$$r\ CO_2 = \frac{N}{2(1.04)} (k_O - k_D) - 0.015\ k_D\ N$$

$r\ CO_2$ = moles of CO_2 per day

N = the size of the body water pool in moles

2 = a constant equating two atoms of oxygen in each molecule of CO_2 with one in each molecule of water

k_o = the fractional turnover rate of oxygen

k_h = the fractional turnover rate of hydrogen

1.04 and 0.015 are correction factors for isotopic fractionation assuming that 50% of water output is lost as vapour.

Energy expenditure was calculated from Weir's fromula (221), explained on Page 80, by converting moles of CO_2 to the volume of CO_2 in litres ($VCO_2 = rCO_2 \times 22.4$) and by assuming a mean RQ of 0.85 over the whole study periods (see Chapter 13).

PART 7: STATISTICAL METHODS

The statistical methods are described in each experimental chapter.

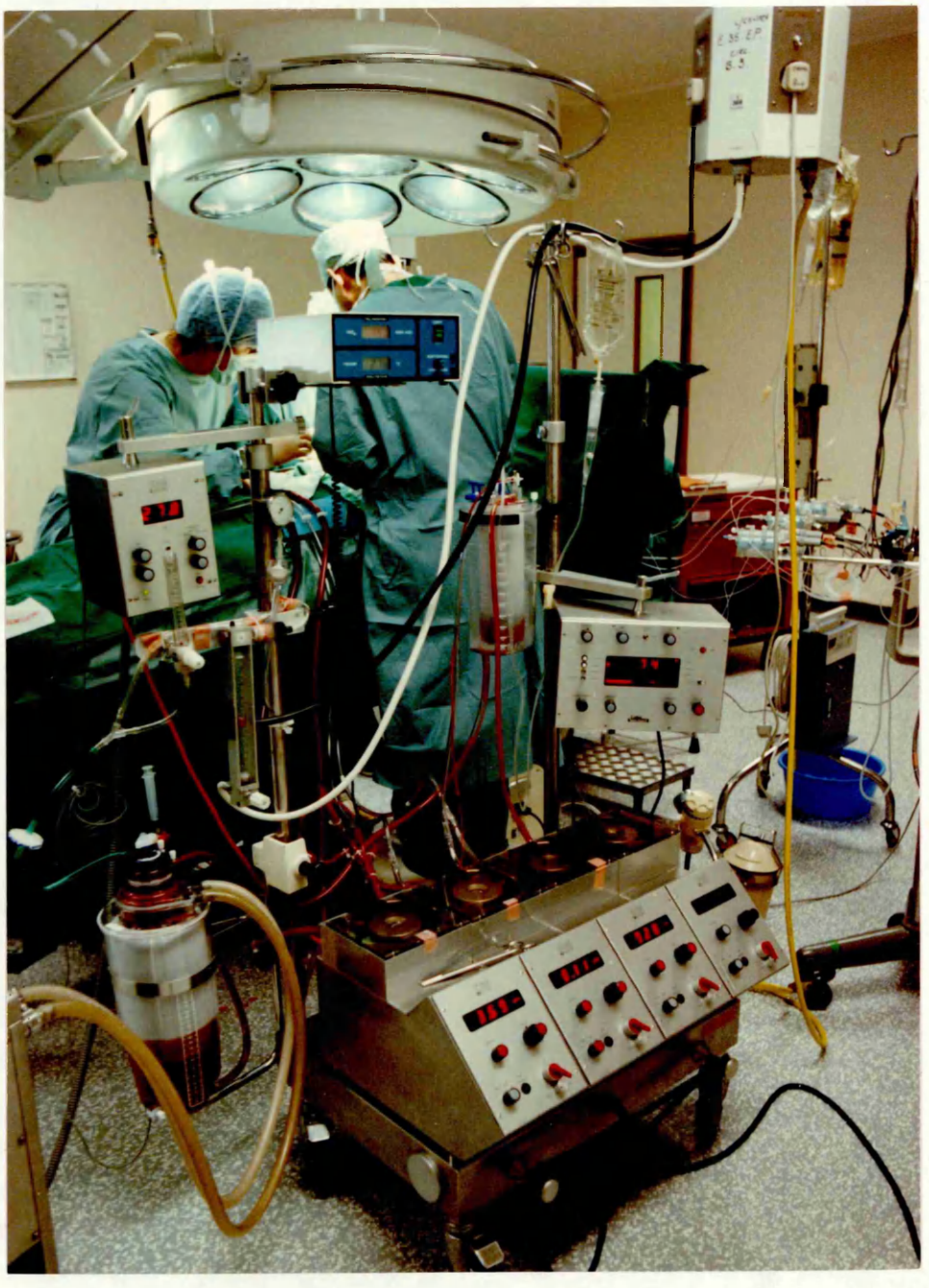


FIG 3.1 Operative setting for cardiopulmonary bypass

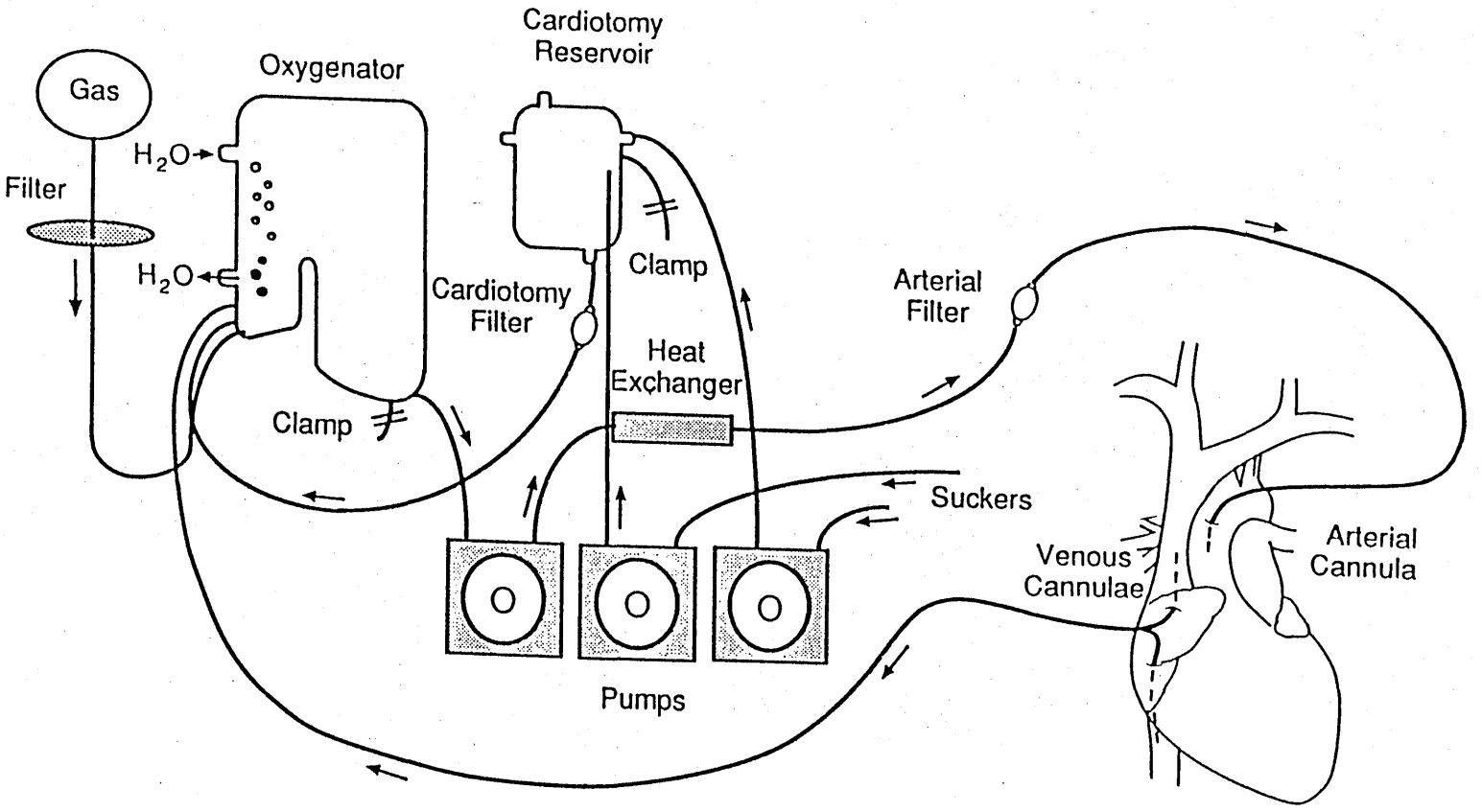
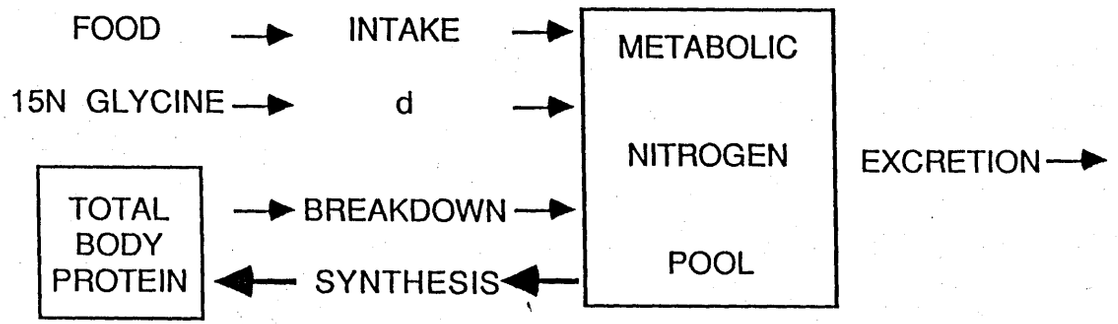


FIG 3.2 PRINCIPLES OF EXTRACORPOREAL PERFUSION

PROTEIN TURNOVER

THEORETICAL CONSIDERATIONS AND CALCULATIONS



$Q = d/E$ Q: NITROGEN TURNOVER

d: QUANTITY OF ISOTOPE INFUSED

E: ISOTOPIC ENRICHMENT OF CHOSEN URINARY END PRODUCT

$\underline{Q = I + B = S + E}$

$\therefore$ IN FASTING STATE : BREAKDOWN $= Q$
: SYNTHESIS $= Q - E$

FIG 3.3 Theoretical assumptions for protein turnover studies.

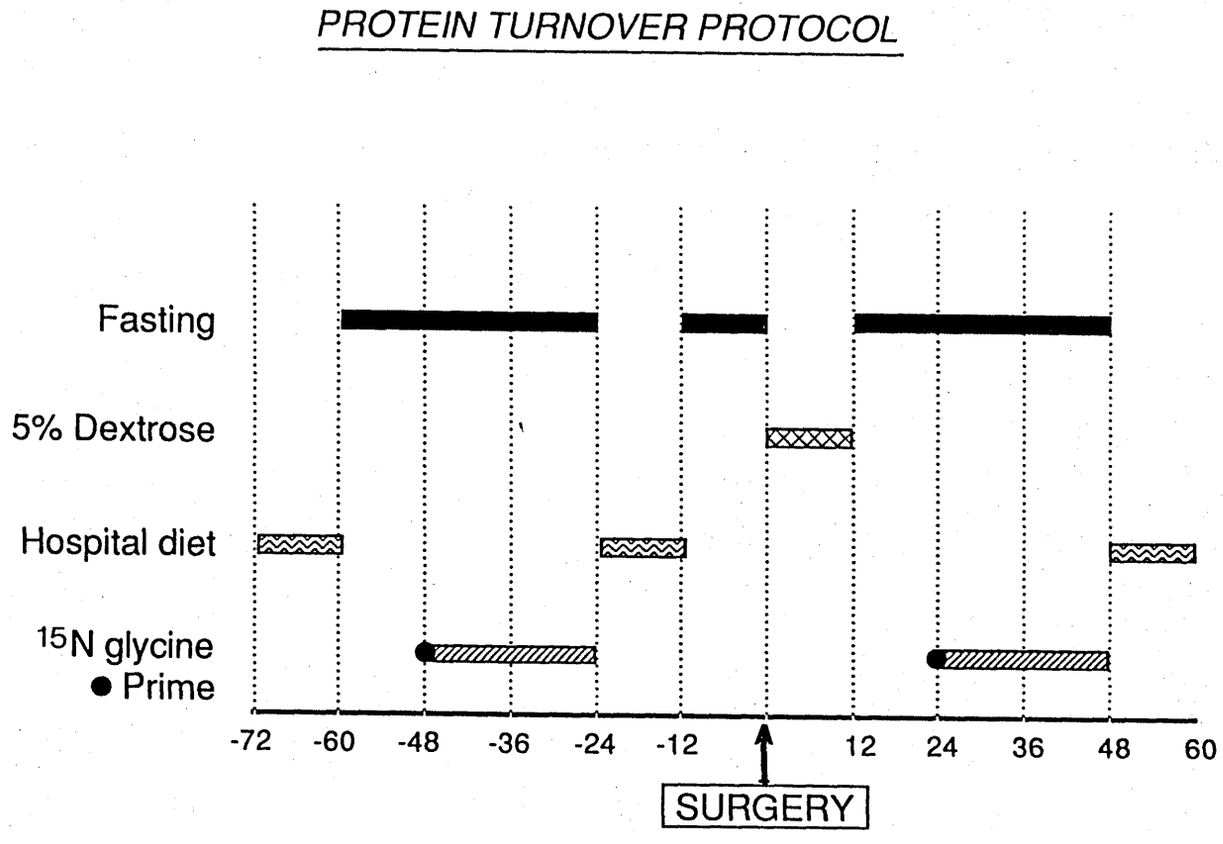


FIG 3.4 Protocol for protein turnover studies with reference to operation and nutritional intake.

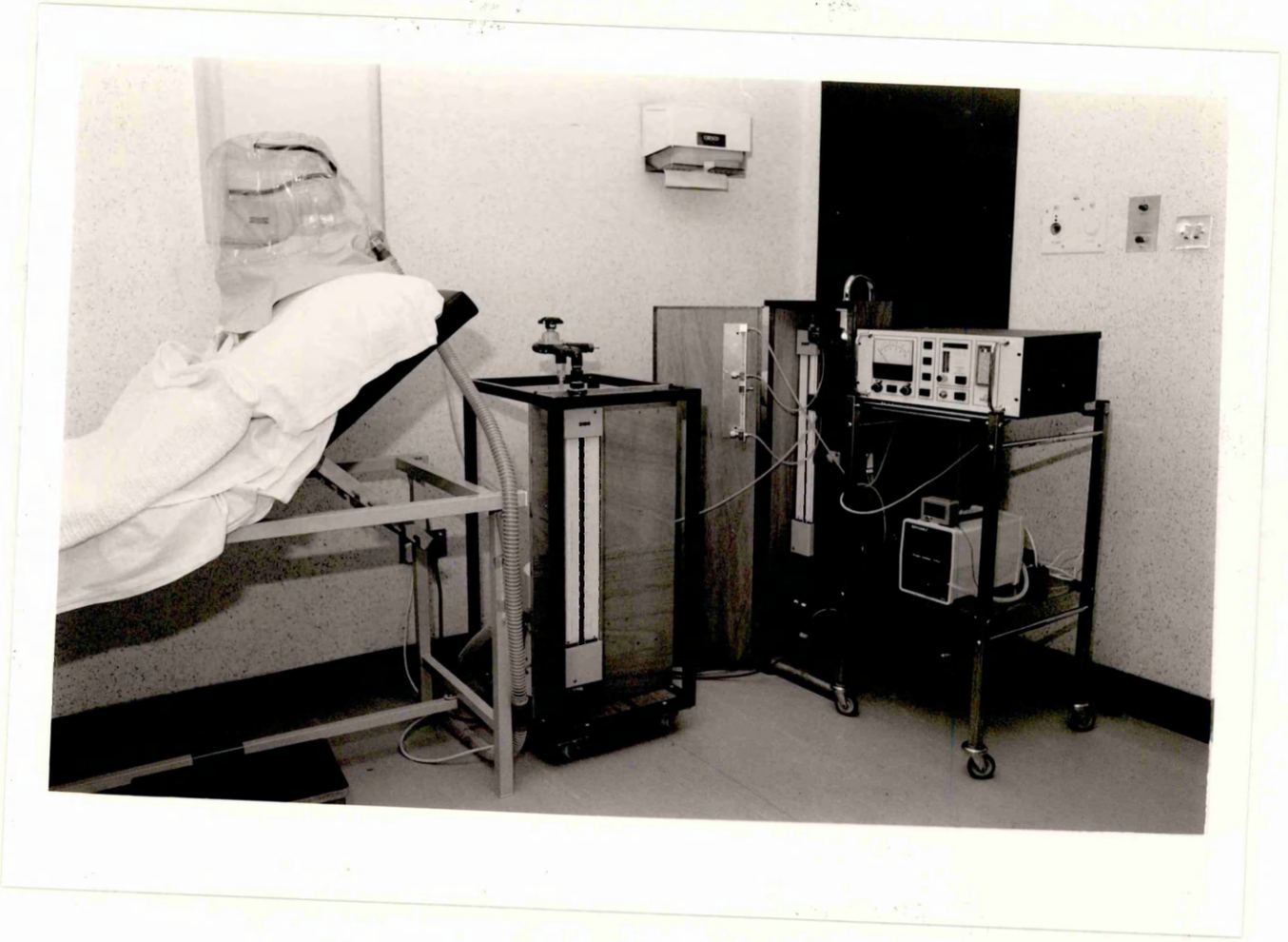


FIG 3.5 Indirect calorimetry equipment used in the studies.

CHAPTER 4

NUTRITIONAL ASSESSMENT OF CARDIAC SURGICAL PATIENTS

CHAPTER 4

NUTRITIONAL ASSESSMENT OF CARDIAC SURGICAL PATIENTS

Let me have men about me that are fat,
Sleek-headed men, and such as sleep a-nights
Yond Cassius has a lean and hungry look;
He thinks too much: such men are dangerous.

William Shakespeare (1564-1616). Julius Caesar I. ii. 192.

INTRODUCTION

Pre-existing nutritional status is one of the major determinants of the magnitude of the catabolic response to surgery (60,235). Although obesity is a recognised risk factor for ischaemic heart disease, there is little objective evidence regarding the nutritional status of patients undergoing coronary artery surgery. Biochemical and anthropometric assessment was performed in a group of unselected male patients admitted for elective coronary revascularisation.

PATIENTS and METHODS

Sixty one consecutive, unselected, preoperative male patients admitted for elective coronary artery surgery over a 3 month period underwent biochemical and anthropometric assessment of nutritional status. All patients studied were otherwise healthy and, in particular, patients with diabetes mellitus, endocrine abnormalities, lipid disorders, anaemia and renal or hepatic impairment were excluded.

All blood samples for the nutritional screen were withdrawn between 0800 and 1000 hours and the biochemical assessment consisted

of 32 parameters including urea and electrolytes (sodium, potassium, chloride, bicarbonate, urea and creatinine), blood glucose, calcium and adjusted calcium, phosphate, liver function tests (bilirubin, alkaline phosphatase, aspartate transaminase, alanine transferase, gamma glutamyl transferase), proteins (albumin, globulin, transferrin, C-reactive protein), trace metals (magnesium, zinc, copper, iron), and vitamins (A, E, C, B1, B2, B6, B12, plasma and red cell folate).

Anthropometric assessments were performed in 59 of these patients by one experienced observer (Ms. Rosemary Richardson, Research Dietitian, Dept. of Biochemistry, Royal Infirmary, Glasgow). The parameters recorded in addition to age were height, weight, percentage ideal body weight, mid-arm circumference (MAC) and triceps skin fold thickness (TST). Arm muscle circumference (AMC) was calculated from the latter two measurements as described in Chapter 3. The patients were all weighed in light attire (pyjamas) and MAC was measured in the dependent and nondominant arm at the mid point between the acromion and the olecranon processes. TST was measured at the same level using Harpenden calipers (British Indicators, Holtain Ltd, Bryberian, Pembrokeshire, UK) and the mean value calculated from three readings.

Standards for weight/height, AMC, TST and serum albumin were obtained from a generally accepted source (214,215). Moderate depletion is defined as 60% to 90% of standard, and severe depletion 60% of standard and below. A serum albumin between 28 g/L and 35 g/L indicates moderate depletion and below 28 g/L severe depletion.

RESULTS

The patients studied were all within the age range 34-64 years (mean age 53.3 years, SD 6.4 years, SE 0.9 years) with uncomplicated

ischaemic heart disease. In particular, patients with diabetes mellitus and/or evidence of hepatic or renal disease were excluded.

All patients were receiving regular anti-anginal medication consisting of a beta-blocker, calcium antagonist and nitrate, alone or in combination. In addition 10 patients had been prescribed a modest dose of diuretic (frusemide, 40 mg/day), seven a vasodilator and three digoxin. Twenty three patients were receiving "triple therapy" (beta-blocker, calcium antagonist, nitrates), 14 a beta blocker and nitrate, 10 a calcium antagonist and nitrate and eight a beta blocker and calcium antagonist. Six patients were receiving single anti-anginal therapy (three beta-blockers, two calcium antagonists and one nitrates). It was in this latter group that there was usually concomitant administration of vasodilators or diuretics.

NUTRITIONAL SCREEN (Table 4.1)

The nutritional screen consisted of 32 biochemical parameters including urea and electrolytes and the results are summarised in Table 4.1, indicating the number of patients with a value above or below the limits of the normal reference range. The mean value for all biochemical indices was within the normal reference range.

The majority of patients had normal urea and electrolytes although in six patients (10%) the plasma chloride was slightly elevated (108-109 mmol/L).

No patient had a plasma albumin below 35 g/L although six patients had a total globulin concentration between 19 and 21 g/L (lower limit of reference range 22 g/L). Two patients had modestly reduced serum transferrin concentrations (respectively 1.9 and 1.7 g/L), one of whom also had a low serum iron. In neither patient was there any other clinical or anthropometric evidence of malnourishment.

In most patients the plasma concentrations of total proteins, calcium, phosphate and random glucose were within the normal reference range.

Liver function tests were normal in most patients with the exception of gamma glutamyl transferase which was elevated in 24 of 60 (40%) patients. An isolated elevation in gamma glutamyl transferase concentration is often a reflection of recent alcohol intake. Four patients had slightly elevated bilirubin concentrations (23-24 umol/L). A fifth patient had a serum bilirubin level of 43 umol/L accompanied by a high C-reactive protein concentration (60 mg/L), a high concentration of serum copper (30.5 umol/L), and a low serum concentration of iron (6.5 umol/L) consistent with an ongoing inflammatory response. There was, however, no obvious focus of inflammation on clinical history or examination. This patient had a stormy postoperative course due to low cardiac output and eventual hospital discharge was delayed until the 12th postoperative day (against a mean postoperative stay of nine days).

C-reactive protein was minimally elevated in another 15 patients lying between 10-20 mg/L (upper limit of normal reference range 10 mg/L) but in none of these patients was there any other evidence of infection or inflammation.

Serum magnesium, iron and copper were within the normal reference range for most patients. In contrast serum zinc concentration was modestly reduced in nine patients (15%). As 80 % of serum zinc is bound to albumin, a molar ratio can be calculated as explained in Chapter 7. Assuming albumin has a molecular weight of 69,000 then the zinc:albumin molar ratio should be within the range 0.0236-0.0226 (normal reference range of zinc of 12-18 umol/L and albumin 35-55 g/L). Nineteen patients in this study had a

zinc:albumin molar ratio less than 0.020.

The mean concentration of each plasma vitamin was within the normal reference range although a considerable number of patients had elevated plasma concentrations of vitamin A and E. Plasma vitamin A was elevated in 19 patients (31%), of whom only three also had a simultaneously elevated retinol binding protein concentration. Plasma vitamin E concentration was elevated in 27 patients (44%), of whom two-thirds had a plasma cholesterol or triglyceride concentration above the upper limit of the normal reference range.

There was an impairment in vitamin B2 status (six patients; 10%) and in vitamin B6 status (five patients; 8%) as defined by increased activation in vitro of the red blood cell enzymes glutamase reductase and aspartate transaminase, respectively. Vitamin C concentration was low in five patients (20%) while plasma and red cell folate concentrations were normal in all patients, except one.

ANTHROPOMETRIC ASSESSMENT (Table 4.2, Table 4.3)

The results of the anthropometric measurements are presented in Table 4.2 and summarised in Table 4.3. 90%-120% of predicted values was considered to be normal, moderate depletion was 60% - 90% of standard and severe depletion 60% of standard and below (214,215). Values greater than 120% of predicted were considered to indicate obesity.

The mean percentage ideal body weight (IBW) was 113.7% (SD 11.6%). Percentage IBW figures revealed that 72% of patients had a body weight within normal limits and a further 29% were obese (Table 4.3).

The mean arm muscle circumference (AMC) was 106% (SD 8.2%) of predicted and percentage AMC indicated that 93% of patients were

within the normal range and a further 7% obese (Table 4.3).

The mean triceps skinfold thickness (TST) was 90% (SD 27.7%) of predicted and percentage TST implied that almost 60% of patients were malnourished, one third severely so, while 29% of patients were within the normal range and 14% obese (Table 4.3).

DISCUSSION

The clinician's awareness of the importance of nutritional status has been reinforced by three related events over the last 15 years. Firstly, in the early to mid 1970s a series of publications drew attention to the poor nutritional status of many hospitalised patients, implying that up to 50% of patients in medical and surgical wards were malnourished (155-157). Secondly, there was growing awareness of an increased morbidity and mortality in malnourished hospital patients (152-154) initially reported by Studley in 1936 in patients undergoing peptic ulcer surgery (9). Finally, some workers reported that nutritional intervention had a favourable effect on outcome in malnourished patients (182,183) and, although this was not a universal observation (184,185), there is some evidence that nutritional support, commenced prior to surgery in high risk patients, may reduce postoperative morbidity (186).

There were two cogent reasons for determining nutritional status in cardiac surgical patients. Cuthbertson, in the 1930's, recognised that preceding nutritional status was a major determinant of the magnitude of the metabolic response to injury (60) and this has been reiterated recently (235). Indeed the cachectic patient faced with an acute stress may be better adapted to semi-starvation in contrast to the nutritionally replete patient faced with an acute illness. It therefore follows that before an investigation into the effects of

cardiopulmonary bypass and hypothermia on the metabolic response to surgery, the nutritional status of the population should be clearly defined. A failure to quantify the nutritional status of patient populations explains, in part, conflicting reports regarding the benefit or otherwise of some therapeutic modalities and this is exemplified in the conflicting evidence regarding the benefits of nutritional support (182-186).

Secondly, most assessments of nutritional status in cardiac surgical patients have been in patients with "cardiac cachexia" which may accompany end stage valvular heart disease (158-160). The pathogenesis of cardiac cachexia was first described in the classic reports of Pittman and Cohen (161,162) where cardiac failure caused relative tissue hypoxia and anorexia but a concomitant increase in the metabolic rate due to increased catecholamine secretion. Despite the paucity of objective information regarding the nutritional status of patients undergoing elective coronary artery surgery, these patients are generally considered to be "overnourished" (164).

Biochemical and anthropometric measurements are commonly used to define nutritional status. A broad spectrum of biochemical parameters has been advocated, and although no single indicator has consistently proved superior to any other, albumin and transferrin have been most widely used (236). More recently albumin and transferrin have again been recommended, along with weight loss, as providing a quick, inexpensive and reliable method of preoperative identification of high-risk patients (237). In the current study not one of 61 patients had a serum albumin less than 35 g/L and only two a serum transferrin less than 2 g/L.

Of the 32 biochemical parameters measured in 61 patients the majority were within the normal reference range. Modest elevations in

C-reactive protein were common and not associated with a complicated postoperative recovery as previously reported (135), although the one patient with a significantly elevated CRP (60 mg/L) did have a stormy postoperative recovery.

There was a mild elevation in gamma-glutamyl transferase in 40% of patients which, in the absence of other abnormalities of liver function tests, is commonly an indication of recent alcohol intake (238).

Fifteen percent of patients had serum zinc concentrations immediately below the lower limit of the normal reference range. As at least 80% of serum zinc is albumin bound, calculation of a zinc:albumin ratio should allow more accurate estimation of the "true" serum zinc concentration. The use of this ratio demonstrated low zinc:albumin molar ratios in 19 patients in this study. There is circadian variation in serum zinc concentration with highest concentrations in the morning (239), and all blood samples in the current study were withdrawn between 0800-1000. It is interesting to note that in a recent nutritional survey of 157 patients in a general surgical ward in Glasgow, 68% had a low serum zinc concentration of whom approximately one third had concomitant anthropometric or biochemical evidence of malnutrition (240).

A significant number of patients had elevated concentrations of Vitamin A (31%) and E (44%) even when respective concentrations of retinol binding protein, cholesterol and triglycerides were considered. Vitamins A and E are considered to have antioxidant properties and low concentrations have been implicated in the aetiology of both ischaemic heart disease and malignancy (241). The current results were unexpected as both vitamins have been reported to be low in Scottish patients with ischaemic heart disease (240).

Whether any of these biochemical abnormalities may be related to drug therapy is uncertain. Diuretics are known to increase urinary excretion of trace metals (239) but only two of the 10 patients receiving diuretics in this study had a modestly reduced zinc concentration. The vitamin abnormalities may be related to disturbance of the plasma lipoprotein profile which can occur with frequent use of B-blockers (242,243).

The most commonly used anthropometric data in the clinical setting are height, weight, ideal body weight (expressed as %), mid arm circumference, arm muscle circumference and triceps skin fold thicknness (244). One problem in interpretation of anthropometric data in hospital patients is that standard tables are based on healthy populations in various parts of the world and may therefore nutritionally misclassify an elderly European population (245).

The most surprising observation in the current study was the contrast between percentage IBW and AMC which both implied that most patients were within the ideal body weight range or obese, while percentage TST suggested that two-thirds of the patients were malnourished (and one-third severely so). All measurements were made by the same experienced research dietitian (RR) as errors in anthropometric measurements can occur with different observers (246). It is interesting that Abel and colleagues (164) describing the anthropometric data in 100 cardiac surgical patients (91 of whom had coronary artery disease) found TST below normal in 29% and implied that this represented genuine malnourishment. Ruiz and colleagues have drawn attention to sources of error in the measurement of TST in obese patients (247) and this is the most likely source of error in the current study, particularly as there was no other biochemical or anthropometric evidence of malnutrition in these patients. Harries

and co-workers have suggested that MAC is the most reproducible arm measurement since it does not depend on estimation of TST which is prone to errors in the obese where landmarks are obscured (248).

SUMMARY and CONCLUSIONS

A nutritional assessment, using biochemical and anthropometric parameters, was made in 61 male patients admitted for elective coronary artery surgery.

In the majority of patients most biochemical indices were within the normal reference range although modest elevations in CRP and gamma glutamyl transferase were common. Low concentrations of serum zinc were common even after correction for albumin binding.

A surprising observation was the frequency of high concentrations of vitamins A and E which have previously been reported to be reduced in patients with ischaemic heart disease. It has been suggested that this may be due to altered vitamin binding to lipids as a consequence of drug therapy.

There was a discrepancy in nutritional assessment between anthropometric indices: percentage IBW and AMC suggested the majority of patients were nutritionally replete or even "overnourished". In contrast, percentage TST impied that 60% of patients were malnourished and it was concluded, in the absence of any other biochemical or anthropometric evidence of malnutrition, that this presumably reflected errors in the measurement of TST which are particularly prone to occur in the obese.

In conclusion routine biochemical and anthropometric nutritional screening is not justified in this population and the tendency for percentage TST to nutritionally misclassify these patients should be recognised.

	REFERENCE RANGE		MEAN	(SD)	NUMBER OF OUTLIERS ABOVE	BELOW
SODIUM	135-145	mmol/L	139.9	(2.2)		1
POTASSIUM	3.5-5.0	mmol/l	4.3	(0.32)		3
CHLORIDE	97-107	mmol/l	103.6	(3.0)	6	
BICARBONATE	23-30	mmol/l	25.8	(2.05)		2
UREA	2.5-8.0	mmol/l	5.7	(1.1)	2	
CREATININE	40-130	mmol/l	97.7	(13.5)		
RANDOM GLUCOSE	<10	mmol/l	6.0	(1.3)		
CALCIUM	2.2-2.6	mmol/l	2.4	(0.1)	1	1
ADJUSTED CALCIUM	2.2-2.6	mmol/l	2.4	(0.1)		
PHOSPHATE	0.7-1.4	mmol/l	1.0	(0.16)		2
BILIRUBIN	3-22	umol/L	13.0	(6.0)	5	
ALKALINE PHOSPHATASE	80-280	U/L	161.7	(41.3)		1
ASPARTATE TRANSAMINASE	12-48	U/L	27.4	(5.9)		
ALANINE TRANSFERASE	3-55	U/L	27.3	(14.0)	3	
GAMMA GLUTAMYL TRANSFERASE	<36	U/L	40.8	(25.4)	24	
ALBUMIN	35-55	g/L	43.2	(3.1)		
GLOBULIN	22-33	g/L	25.4	(3.2)		6
TRANSFERRIN	2-4	g/L	2.8	(0.4)		2
C REACTIVE PROTEIN	<10	mg/L	9.2	(7.6)	16	
MAGNESIUM	0.7-1.0	mmol/L	0.8	(0.06)		1
ZINC	12-18	umol/L	13.8	(1.9)		9
COPPER	15-25	umol/L	19.2	(2.9)	1	2
IRON	8-30	umol/L	17.9	(4.8)		1
VITAMIN A	1.0-2.8	umol/L	2.6	(0.9)	19	
VITAMIN E	14-39	umol/L	38.3	(10.6)	27	
VITAMIN C	11-114	umol/L	28.2	(23.8)		13
VITAMIN B1	<25%	activation	8.1%	(6.2%)	1	
VITAMIN B2	<60%	activation	36.1%	(26.6%	6	
VITAMIN B6	<150%	activation	87.8%	(37.9%	5	
VITAMIN B12	150-730	pg/ml	399.2	(140.2	2	
SERUM FOLATE	2.2-11.4	ng/ml	4.6	(1.5)		1
RED CELL FOLATE	106-614	pg/ml	310	(87)		

TABLE 4.1 Summary of biochemical nutritional screen in 61 patients indicating number of patients with value outside reference range.

ANTHROPOMETRIC DATA IN 59 MALE PATIENTS

	AGE	HT (cm)	WT (Kg)	%IBW	TSF (mm)	%TSF	AMC (mm)	%AMC		AGE	HT (cm)	WT (Kg)	%IBW	TSF (mm)	%TSF	AMC (mm)	%AMC
1	51	183	87	116	10.5	84	26.7	105	31	48	163	67	112	9.5	76	26	103
2	54	165	79	127	13	104	28.9	114	32	51	168	87	137	13.5	108	26.3	104
3	41	175	73	105	15.5	124	24.6	97	33	54	173	79	116	13	104	26.9	106
4	48	170	74	111	9.5	76	26.5	105	34	61	180	87	118	8	64	24.5	97
5	50	178	89	125	14	112	27	107	35	54	163	59	98	7	56	25.8	102
6	57	173	64	94	7.5	60	23.1	91	36	51	180	100	136	14	112	30.1	119
7	52	175	98	141	22	176	27.6	109	37	50	183	84	107	12	96	29.2	115
8	55	175	79	114	16	128	23	91	38	59	170	76	116	15	120	27.3	108
9	58	170	83	126	10	80	27.4	108	39	55	173	82	120	13	104	30.4	120
10	61	173	80	118	9.5	76	25.5	101	40	60	178	71	124	13.5	108	28.8	114
11	59	163	67	112	14	112	23.6	93	41	51	175	77	111	12.5	100	27	107
12	59	165	73	118	6	48	28	110	42	44	178	76	107	9	72	28.2	114
13	62	168	66	104	7	56	26.8	106	43	48	165	64	108	8	64	27.5	104
14	61	165	73	117	14	112	24.1	95	44	62	173	68	105	14	112	26.6	105
15	34	180	81	110	11	88	28.5	113	45	57	175	76	114	10.5	84	26.7	106
16	47	180	98	132	11	88	30.5	121	46	56	173	73	112	14	112	26.6	105
17	64	163	64	106	9	72	25.2	100	47	51	175	100	145	18	144	31.8	126
18	51	168	67	105	7	56	27.8	110	48	61	168	71	112	11	88	30.5	121
19	46	163	63	105	8	64	27	107	49	58	170	80	121	18	144	28.3	112
20	49	188	80	101	7	56	26.3	104	50	47	163	57	95	6	48	25.1	99
21	57	170	68	102	8	64	26	103	51	48	164	60	108	10	80	23.9	94
22	52	170	77	117	7	56	23.8	94	52	65	178	70	98	11.5	92	23.4	92
23	64	168	61	96	7	56	24.3	96	53	62	170	83	125	7	56	27.8	110
24	52	183	80	106	8	64	28	111	54	59	173	78	115	8	64	26.5	105
25	56	170	72	108	9	72	24.6	98	55	58	175	90	130	22	176	23.6	93
26	50	163	70	150	8.5	68	25.8	102	56	62	163	69	156	9	72	27	107
27	41	180	73	99	11	88	28	110	57	63	168	66	104	7	56	24.3	96
28	50	170	83	125	14	112	25.1	99	58	54	170	76	115	10	80	23.9	94
29	57	183	85	113	12	96	25.2	110	59	66	178	82	115	13	104	25.4	100
30	52	157	98	145	18	144	27.3	100									

TABLE 4.2 Anthropometric data in 59 male patients.

	<60%	60%-90%	90%-120%	>120%
% IDEAL BODY WEIGHT			42 (72%)	17 (29%)
% TRICEPS SKINFOLD THICKNESS	11 (19%)	23 (39%)	17 (29%)	8 (14%)
% ARM MUSCLE CIRCUMFERENCE			55 (93%)	4 (7%)

TABLE 4.3 Summary of anthropometric data in 59 male patients.

CHAPTER 5

MEASUREMENT AND PREDICTION OF BASAL METABOLIC RATE

CHAPTER 5

MEASUREMENT AND PREDICTION OF BASAL METABOLIC RATE

"Physical fitness goes with mental fitness. It goes with energy."

John F Kennedy (1917-1963). Interview January 13, 1963.

INTRODUCTION

Determination of energy expenditure may be desirable in surgical practice both from the clinical and research point of view. Energy requirements can be estimated from the basal metabolic rate (BMR), measured by indirect calorimetry (132) or from predictive formulae based on body composition (249-251). There is no report in the literature of the BMR in a cardiac surgical population.

In this study the BMR was measured in 20 patients with ischaemic heart disease by indirect calorimetry and compared to that predicted by the Harris-Benedict (249), Cunningham (250) and Bogardus formulae (251).

PATIENTS and METHODS

20 patients admitted for elective coronary artery surgery or coronary angiography were studied. All patients had isolated ischaemic heart disease and in particular diabetes, hyperlipidaemia, anaemia and endocrine disease were excluded. No patient had undergone a change in body weight of >10% in the six months preceding the study. All patients were taking anti-anginal medication consisting of a beta-blocker and/or calcium antagonist and/or nitrate.

Anthropometric data, including height, weight, and skinfold thickness, measured in four areas (biceps, triceps, supra-iliac and

subscapular) were collected in all patients. Measurement of skinfold thickness allows estimation of lean body mass as described by Durnin and Womersley (214,252).

The basal metabolic rate (BMR) was determined by four methods:

1) Indirect calorimetry as detailed in Chapter 3
2) The Harris-Benedict formula based on weight, height and age: males= 66.473 + 13.752 (Wt) + 5.003 (Ht)- 6.755 (A) (249)
3) The Cunningham formula based on lean body mass:
   males= 501.6 + 21.6 (LBM) (250)
4) The Bogardus formula based on lean body mass:
   males= 489 + 22.8 (LBM) (251)

where A = age in years
Wt = body weight in kilograms
Ht = height in centimetres
LBM = lean body mass (fat free mass)

STATISTICAL ANALYSIS

The BMR, measured by indirect calorimetry, was compared to the BMR predicted by the formulae using a Wilcoxon sign rank test. Linear regression analysis was performed using the method of least squares and correlation (r) coefficients determined. Linear regression equations are in the form :

$$y = a + bx$$

where a is the intercept on the y axis
b is the gradient of the line.

RESULTS

The mean age of the 20 patients undergoing indirect calorimetry was 55 (SD 6), similar to the mean age of the other groups described in this thesis (Table 5.1).

Anthropometric data, including height, weight, and skinfold thickness measured in four areas (biceps, triceps, supra-iliac and subscapular) is given in Table 5.2. The mean percentage body fat, derived from the sum of the skinfold thickness by a standard method (214,252), was 26.4% (SD 4.2%). The mean TST of 12 mm was 96% of predicted and similar to the population described in Chapter 4.

BMR measured by indirect calorimetry and calculated by the predictive formulae is summarised in Table 5.2. The mean BMR measured by indirect calorimetry was 1545 Kcal/day and 1678 Kcal/day when calculated from the Harris-Benedict formula. The Harris-Benedict formula overestimated measured BMR, by a mean of 9.5%, in 16 of the 20 patients ($p=0.003$ by Wilcoxon sign rank test). The mean BMR calculated from Cunningham's formula was 1785 Kcal/day which overestimated measured BMR by a mean of 17.7% in 19 of the patients ($p=0.00009$ by Wilcoxon sign rank test). The mean BMR calculated from the formula proposed by Bogardus was 1843 Kcal/day which was 20.7% greater than the mean measured BMR. This formula overestimated the measured BMR in all patients ($p=0.0001$ by Wilcoxon sign rank test).

Linear regression equations for measured BMR against calculated BMR, by the three formulae, are illustrated in Figs 12.1, 12.2 and 12.3. The regression equations are in the form $y = a + bx$, where a is the intercept on the y axis and b the slope:

Harris-Benedict: $y= 888.4 + 0.510x$ ($p=0.004$, $r=0.609$; $r^2=37\%$)

Cunningham : $y= 1203 + 0.376x$ ($p=0.009$, $r=0.680$; $r^2=46\%$)

Bogardus : $y= 1227 + 0.399x$ ($p=0.009$, $r=0.682$; $r^2=46\%$)

The BMR values derived from the predictive formulae correlated in a highly significant manner with the BMR measured by indirect calorimetry. The correlation coefficients were low, however, suggesting that while the predictive formulae provide a good mean estimate for the group as a whole they are less useful in predicting BMR for the individual.

DISCUSSION

There has been a resurgence of interest in measuring energy expenditure in the clinical situation, over the last decade, since the classic studies of Dubois (133) and Harris Benedict (249) in the first quarter of this century. This renaissance has been prompted by efforts to elucidate the pathophysiology of changes in body composition in terms of energy imbalance in obesity, malnutrition, cancer cachexia and following trauma. From the clinical standpoint estimation of energy requirements is helpful in determining optimal feeding regimens when nutritional support is desirable (253). Inadequate provision of calories fails to prevent loss of body mass while hyperalimentation may produce a number of metabolic problems such as increased oxygen consumption, carbon dioxide production, lipogenesis and hepatic dysfunction (254).

A number of methods have been used to estimate energy requirements in surgical patients but the "gold standard" is still indirect calorimetry which measures the BMR. BMR is strongly correlated with body composition and accounts for up to 75 % of energy expenditure in sedentary adults (255).

BMR can be measured with a fixed indirect calorimetry system (as in the current study) or a mobile "metabolic cart". Both systems require considerable technical expertise and are confined to

relatively few centres. Even when physically available, some patients- particularly after surgery -are unable to tolerate the nose clips or ventilated hoods associated with the procedure (256).

Consequently a number of formulae for predicting energy expenditure, based on body composition, have been developed (249-251). The formulae all presuppose that use of anthropometric parameters can reflect the metabolically active portion of the body. The most commonly used formula in clinical practice is the Harris-Benedict formula (249) which was derived from indirect calorimetry measurements in 239 healthy individuals. That study assumed that measurement of oxygen utilisation and carbon dioxide production could be used to indirectly calculate the rate of thermogenesis and that 24 hour energy expenditure could be predicted from such measurements made over 5-15 minute periods in the early morning. This formula takes account of height, weight and age but does not attempt to directly estimate lean body mass (LBM).

On the basis that it is the active protoplasmic mass which largely determines oxygen consumption, a number of formulae have been developed which relate BMR to LBM (250,251). Cunningham re-analysed the data of Harris-Benedict and showed that the most important component dictating BMR was LBM and that age and sex were of little importance (250). Bogardus independently reached the same conclusion from studies on 130 American Indians and proposed a formula (251) similar to that of Cunningham.

Durnin and Womersley demonstrated that the logarithm of skinfold thickness measurements, which anthropometrically estimate subcutaneous fat, bear a linear relationship to the fat mass of the body as, calculated by body densitometry measurements (214,252). The relationship remains valid even in the obese (252) and permits easy

calculation of LBM (LBM = body weight - fat mass) in the clinical setting. In the current study, measurements of TST were similar to those in the population described in Chapter 4.

The BMR values derived from the predictive formulae correlated in a highly significant manner with measured BMR. The correlation coefficients were low, however, implying that the predictive formulae provide a good mean estimate for the group as a whole but are less predictive for the individual. The current study demonstrated that measured BMR is considerably lower than that estimated by the three predictive equations. The measured BMR was 9.5% lower than that predicted by the Harris-Benedict formula and almost 20% lower than that predicted by the Cunningham and Bogardus formulae. The discrepancy amongst the predictive formulae is accounted for by the fact that the former is based on weight and the latter two on LBM. As patients with ischaemic heart disease tend to be overweight with an excess fat mass (see Chapter 4), then the measured BMR appears even lower when accounted for by LBM as opposed to body weight.

There are two possible explanations for the discrepancy between measured and predicted BMR in this population. It is possible that the predictive formulae overestimate BMR and indeed Daly and colleagues reported that the Harris-Benedict formula overestimated BMR in 127 healthy volunteers by a mean of 12.3% (257). The estimation of LBM in the cardiac surgical patients was based on a well-established anthropometric technique which remains valid regardless of body fat content (214,252). It is therefore surprising that the two formulae based on LBM, the Cunningham and Bogardus formulae, and derived independently from different populations, both overestimate BMR to such a similar extent.

This observation therefore raises the possibility of a true

reduction in BMR in a population with ischaemic heart disease. The autonomic nervous system, and in particular, the sympathetic component, are involved in regulation of energy balance and fat metabolism (258,259) and administration of beta adrenoreceptor agonists has been shown to promote weight loss (260). It is possible that chronic beta-blockade therapy produces a reduction in BMR. If so, this is of considerable epidemiological importance in view of the widespread use of such therapy for ischaemic heart disease and hypertension. Currently an age and sex matched population is being recruited by the Department of Physiology at Glasgow University to answer this question.

SUMMARY and CONCLUSIONS

In this study the basal metabolic rate was measured in 20 patients with ischaemic heart disease by indirect calorimetry and compared to that predicted by the Harris-Benedict (249), Cunningham (250) and Bogardus formulae (251).

BMR, measured by indirect calorimetry, was 10-20% lower than that estimated by the predictive formulae. The discrepancy between measured and predicted BMR was greatest when BMR was related to LBM rather than weight.

It is not known whether the low values for measured BMR in patients with ischaemic heart disease is a genuine phenomenon, perhaps related to beta-blockade therapy, or due to unsuitability of the predictive formulae in this population. This point underlines the importance of using the most suitable predictive formula for a particular group of patients even if this necessitates the development of a specific formula for that group.

PATIENT	AGE	HEIGHT (cm)	WEIGHT (Kg)	SKIN FOLD THICKNESS (mm) biceps	triceps	iliac	scapula	SUM	%FAT	FFM (Kg)
1	55	168	76.5	6.5	11.5	20.0	15.0	53	25.5	56.9
2	48	174	83.0	4.5	7.0	14.0	13.0	38.5	21.0	65.6
3	40	168	77.5	6.0	9.0	16.0	12.0	43.0	22.5	60.1
4	56	173	70.7	5.0	7.0	13.0	10.0	35.0	20.8	56.0
5	54	172	92.5	2.0	19.0	22.0	17.0	70.0	31.6	63.3
6	56	175	70.3	5.0	10.0	12.0	8.0	35.0	20.8	55.7
7	58	172	76.3	6.0	8.0	16.0	15.0	45.0	24.7	57.5
8	62	175	82.0	1.0	14.0	18.0	19.0	62.0	29.7	57.6
9	61	175	69.5	5.0	8.0	13.0	10.0	36.0	21.3	54.7
10	53	171	75.2	7.0	8.0	16.0	13.0	44.0	24.0	57.2
11	42	178	89.5	6.0	17.0	24.0	30.0	77.0	26.2	66.1
12	55	177	86.6	0.0	10.0	20.0	13.0	53.0	27.0	63.2
13	58	174	97.5	9.0	20.0	25.0	28.0	82.0	34.0	64.4
14	56	162	63.5	6.0	10.0	12.0	13.0	41.0	23.0	49.0
15	56	168	83.4	8.0	18.0	20.0	26.0	72.0	32.0	56.7
16	57	171	79.2	5.0	13.0	18.0	18.0	54.0	27.5	57.4
17	62	171	77.0	8.0	14.0	20.0	17.0	59.0	28.9	54.7
18	60	179	83.5	0.0	14.0	18.0	18.0	60.0	29.2	59.1
19	60	178	85.9	6.0	10.0	16.0	15.0	47.0	25.4	64.1
20	47	180	103.0	7.0	15.0	30.0	35.0	87.0	32.3	69.7
MEAN	55	173	81.1	5.0	12.0	18.0	17.3	54.7	26.4	59.5
SD	6	4	9.7	2.6	4.1	4.7	7.2	16.1	4.2	5.0
SEM	1	1	2.2	0.6	0.9	1.0	1.6	3.6	0.9	1.1

TABLE 5.1 Anthropometric data in 20 patients undergoing indirect calorimetry.

Patient	MEASURED BMR (Kcal/ day)	HARRIS-BENEDICT PREDICTED	(%DIFF)	CUNNINGHAM PREDICTED	(%DIFF)	BOGARDUS PREDICTED	(%DIFF)
1	1510	1567	(-6.6)	1731	(-14.6)	1786	(-18.3)
2	1733	1754	(-1.2)	1918	(-10.6)	1984	(-14.8)
3	1235	1702	(-37.8)	1800	(-45.7)	1856	(-50.0)
4	1645	1525	(+7.3)	1712	(-4)	1766	(-7.3)
5	1684	1834	(-8.9)	1867	(-10.8)	1931	(-14.6)
6	1241	1530	(-23.2)	1704	(-37.3)	1758	(-41.6)
7	1456	1584	(-8.8)	1743	(-19.7)	1799	(-23.5)
8	1708	1651	(+3.4)	1746	(-2.2)	1802	(-5.5)
9	1353	1486	(-9.8)	1683	(-24.4)	1736	(-28.3)
10	1520	1598	(-5.1)	1736	(-14.2)	1792	(-17.9)
11	1704	1904	(-11.7)	1928	(-13.1)	1995	(-17.0)
12	1900	1771	(+6.8)	1867	(+1.7)	1930	(-1.5)
13	1780	1886	(-5.9)	1892	(-6.2)	1956	(-9.8)
14	1414	1372	(+3.0)	1560	(-10.3)	1606	(-13.5)
15	1333	1676	(-25.7)	1725	(-29.4)	1781	(-33.6)
16	1486	1626	(-9.4)	1742	(-17.2)	1798	(-21.0)
17	1323	1562	(-18.1)	1683	(-27.2)	1736	(-31.2)
18	1448	1705	(-17.7)	1779	(-22.8)	1836	(-26.7)
19	1715	1733	(-1.0)	1886	(-9.9)	1950	(-13.7)
20	1720	2066	(-20.1)	2007	(-16.6)	2077	(-20.7)
MEAN	1545	1678	(-9.5)	1785	(-17.7)	1843	(-20.7)
SD	194	163	(11.6)	108	(11.3)	114	(12.7)
SEM	43	34	(2.6)	24	(2.5)	25	(2.7)

TABLE 5.2 Measured BMR compared to predicted BMR.

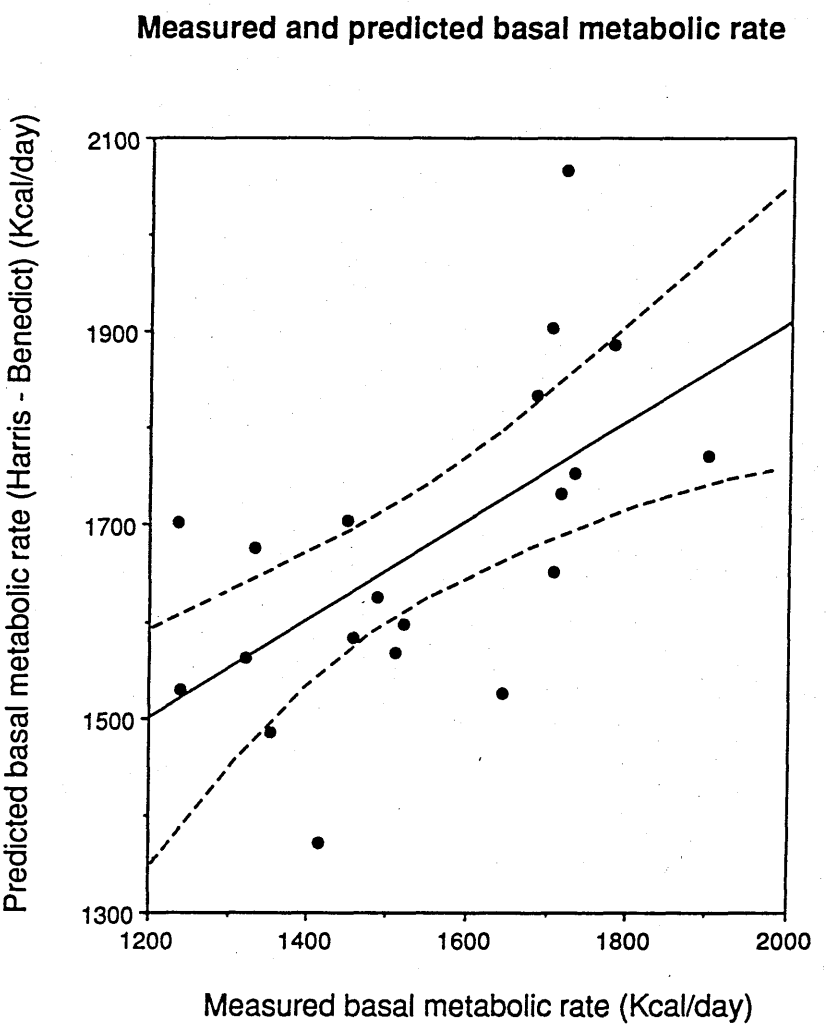


FIG 5.1 Measured and predicted (Harris-Benedict formula) BMR illustrating 95% confidence limits (dotted lines).

Regression equation (solid line): y= 888.4 + 0.510x

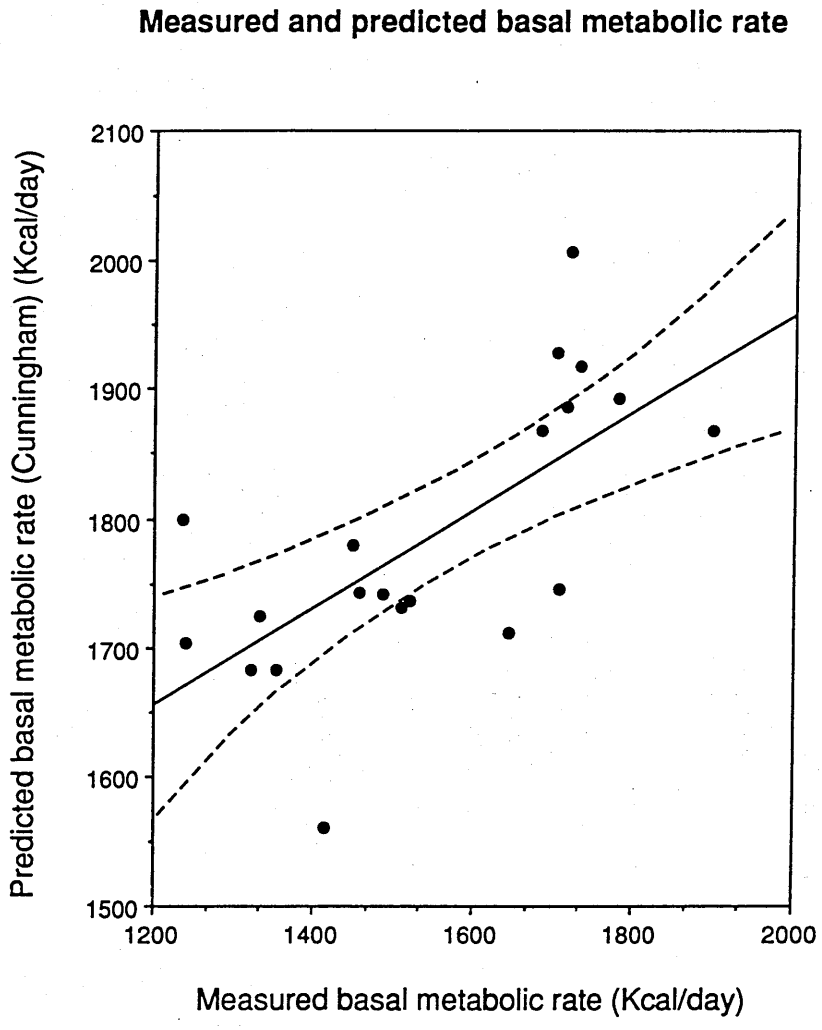


FIG 5.2 Measured and predicted (Cunningham formula) BMR illustrating 95% confidence limits (dotted lines).

Regression equation (solid line): y= 1203 + 0.376x

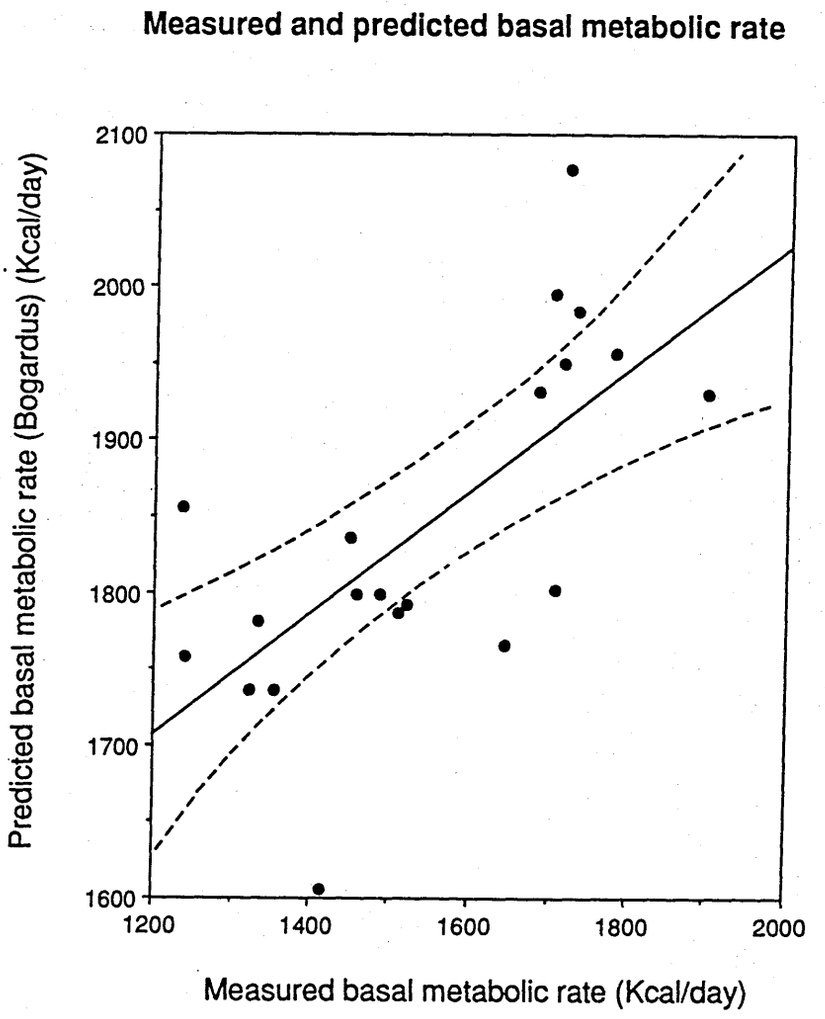


FIG 5.3 Measured and predicted (Bogardus formula) BMR illustrating 95% confidence limits (dotted lines).

Regression equation (solid line): y= 1227 + 0.399x

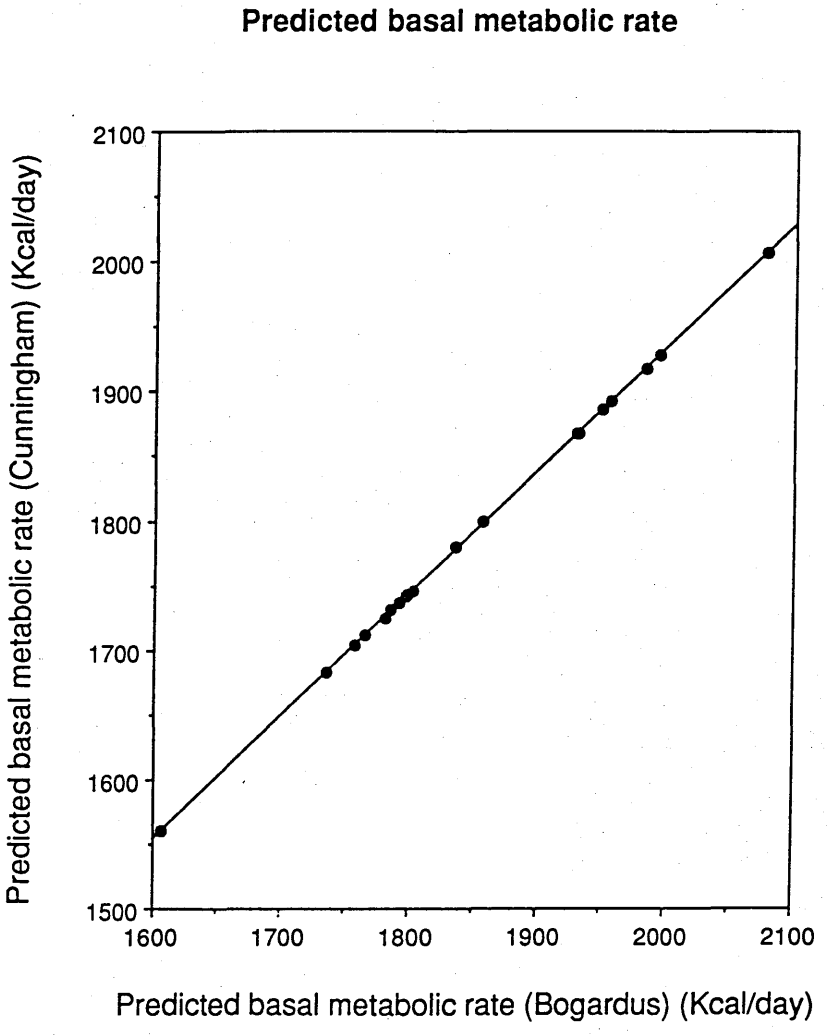


FIG 5.4 Predicted metabolic rate: Bogardus formula v Cunningham formula.

Regression equation (solid line): y= 37.88 + 0.948x

CHAPTER 6

CORRECTION FOR HAEMODILUTION

CHAPTER 6

CORRECTION FOR HAEMODILUTION

Blood is thick, but water's thin.

Sir William Schenwick Gilbert (1836-1911). Iolanthe.1.

INTRODUCTION

Alterations in the concentrations of plasma proteins, especially albumin, have important therapeutic (261) and prognostic significance (262). Moreover, comparative studies of the effects of surgical trauma may require evaluation of the magnitude of the acute phase response of plasma proteins (263,264). Interpretation of such changes may however be complicated by changes in fluid balance.

The effect of haemodilution on the concentration of plasma constituents, due to perioperative transfusion of fluids, can be corrected by a number of formulae (263-265) based on alterations in the packed cell volume (PCV). Alpha-2 macroglobulin may also be useful in elucidating the effects of haemodilution as its concentration appears to remain constant despite surgical injury (256). Cardiac surgery, involving cardiopulmonary bypass, provides an exacting test of the ability of any formula to correct for marked haemodilution as a large volume of fluid is added to the circulation in a relatively short period of time. The efficacy of four correctional formulae to adjust for the effects of haemodilution in patients undergoing coronary artery surgery and to correct for the effects of dilution in a closed, <u>in vitro</u>, system was examined.

PATIENTS and METHODS

The PCV and plasma concentrations of albumin, alpha-2 macroglobulin and caeruloplasmin were measured in four patients undergoing coronary artery surgery. Blood was withdrawn prior to anaesthesia, one minute after the commencement of cardiopulmonary bypass and subsequently at two, 12, 24, 48, and 72 hours and on the seventh postoperative day. All blood samples were withdrawn when the patient had been in the supine position for at least one hour.

Full details of the surgical procedure and perioperative fluid management are detailed in Chapter 3. Essentially the extracorporeal circuit was primed with 2 litres of Hartmann's solution (containing 8,000 units of sodium heparin, 50 mmol of sodium bicarbonate, 55 mmol of mannitol, 15 mmol of potassium chloride and 750 mg of cefuroxime) which was added to the circulation at the commencement of cardiopulmonary bypass. A few minutes later 1 litre of cardioplegic solution (1 litre of Ringer's Lactate containing 16 mmol of potassium chloride, 34 mmol of magnesium chloride and 1 mmol of procaine hydrochloride) was infused into the aortic root to produce flaccid paralysis of the myocardium.

This situation was simulated, <u>in vitro</u>, by adding 0, 1, 2, 3, 4 and 5 ml of a 2:1 mixture of priming fluid and cardioplegic solution to six lithium heparin tubes, each containing 5 ml of blood from a volunteer, and again measuring the concentrations of albumin, alpha-2 macroglobulin and caeruloplasmin.

All samples from a given patient were assayed within the same batch for all analytes. Plasma albumin and alpha-2 macroglobulin concentrations were measured by immunoturbidimetry (Baker, Encore R) using sheep anti-human albumin antisera (Scottish Antibody Unit, Carluke) and goat anti-human alpha-2 macroglobulin antisera (Atlantic

Antibodies). Caeruloplasmin was measured by an immunoturbidimetric method on an Encore^{R} centrifugal analyser (Baker Instruments) using antisera obtained from Atlantic Antibodies, Scarborough Main, USA.

The PCV of each sample was measured by microcentrifugation.

The correctional formulae employed were:-

Normalised concentration (of plasma protein) =

Formula A: Measured concentration X $\frac{\text{initial PCV}}{\text{new PCV}}$ (263)

Formula B: Measured concentration X $\frac{\text{[1-new PCV]}}{\text{[1-initial PCV]}}$ (264)

Formula C: Measured concentration X $\frac{\text{[1-new PCV] X initial PCV}}{\text{[1-initial PCV] X new PCV}}$ (265)

Formula D: Measured concentration X $\frac{\text{initial [A-2 macroglobulin]}}{\text{new [A-2 macroglobulin]}}$ (266)

RESULTS

Alterations in PCV from the in vitro study are shown in Fig 6.1. The decrease in PCV with haemodilution was similar to that expected from arithmetical calculations as demonstrated in Fig 6.2.

Measured and corrected plasma albumin and caeruloplasmin concentrations, from the in vitro study, are shown in Figs 6.3 and 6.4. Formulae A and B fail to correct for haemodilution and their inability to do so increases with the volume of diluent added. Formulae C and D correct the diluted albumin and caeruloplasmin concentrations back to the pre-dilution value at all levels of haemodilution.

In vivo, immediately after the commencement of CPB bypass, there was a 41% decrease in the PCV (Fig 6.5) from 44% to 26%. This is of a

magnitude that would be expected by the addition of 3 litres of crystalloid to a 5 litre vascular volume as shown in Fig 6.2. The PCV returned towards the preoperative value by 12 hours but decreased again during the subsequent 24 to 72 hour period.

In vivo changes in measured and corrected plasma albumin and caeruloplasmin concentration are illustrated in Figs 6.6 and 6.7. In the early bypass period formula A underestimates the pre-bypass plasma albumin concentration by 28% but plasma caeruloplasmin concentration by only 3%. Formula B underestimates pre-bypass plasma albumin and caeruloplasmin concentrations by, respectively, 40% and 30%. Formulae C and D correct for the immediate effects of haemodilution, at the onset of bypass, but subsequently overcorrect. This overcorrection is apparent by two hours, increases to a peak at 12 hours, and is still apparent by the third postoperative day.

DISCUSSION

The aim of this study was to determine the most appropriate method of correcting plasma protein concentrations for the effect of haemodilution, so that concomitant or subsequent *non-dilutional* changes, resulting from surgical trauma, could be detected. The ability to correct for the effects of haemodilution is, however, more than an academic exercise as the plasma albumin concentration may be used as an indicator of the need to provide albumin supplements (261), while sequential changes in plasma albumin in critically ill patients have been reported to have prognostic value (262). Furthermore postoperative alterations in the acute phase response proteins can be used to quantify surgical trauma (263,264).

Apart from the effects of fluid transfusion, a large number of factors influence the concentrations of plasma proteins under normal

circumstances. These include posture, exchange with proteins in the the interstitial space, synthesis and catabolism. Approximately 10 % of the intravascular pool of albumin is replaced by synthesis or catabolism each day, while almost ten times this amount is exchanged with albumin in the tissue spaces (267). The transcapillary escape rate of albumin increases within a few hours of surgery (268) and plasma albumin concentrations usually fall by approximately 10% within three days of uncomplicated surgery taking up to three weeks to return to preoperative values (266). In contrast Skillman has demonstrated that 10-20% of extravascular albumin can shift to the intravascular space within 12 hours of haemorrhage (269). These situations are further complicated in malnourished patients where a low plasma albumin concentration may reflect an expanded extracellular fluid space (270).

In such complex clinical situations, an aid to interpretation of changes in the concentrations of plasma proteins would be valuable and, in particular, should enable direct comparison with the concentration prior to fluid transfusion.

Correction for haemodilution is of paramount importance in cardiac surgery because of the large volumes of fluid involved. Haemodilution is an integral component of the cardiopulmonary bypass technique and counteracts the increase in blood viscosity and vasoconstriction which accompany hypothermia, as well as improving renal function and reducing blood requirements (271). At the beginning of cardiopulmonary bypass, the patient's vascular system is effectively increased by the volume of the extracorporeal circuit primed with 2 litres of crystalloid solution. A further 1 litre of cardioplegic solution is infused into the aortic root a few minutes later (as detailed in Chapter 3) and this solution rapidly returns to

the systemic circulation via the coronary arteries and coronary sinus unless vented.

Three existing formulae (A,B,C) were used to correct for haemodilution. Formula A is the most widely used correctional formula for haemodilution, eg Ref 252, although no justification of its efficacy could be found in the literature. It has presumably been adapted from a formula quoted by Gregersen in 1944 to correct for concentrations in the intensity of dyes in plasma : $D_c = D_t \times P_o/P_t$ where D_c and D_t are respectively the corrected and observed dye readings and P_o and P_t are respectively the initial and final concentrations of a protein in plasma (272). Formula B has been used to correct for the effects of haemodilution in other models of the acute phase response and is based on computer predictions of the in vivo effects of haemodilution (264). Formula C is based on simple arithmetical principles which take account of the differential rates of alteration in the concentration of plasma constituents compared to the elements of whole blood (265). Formula D was constructed on the rationale that alpha-2 macroglobulin is slowly metabolised and is not subject to transcapillary leakage in view of its large molecular weight (800,000). Indeed Werner and Cohnen have reported that alpha-2 macroglobulin remains relatively constant after surgery (266).

In the current study there was a marked drop in the PCV at the onset of bypass (Fig 6.5), as would be expected when the intravascular volume was effectively expanded by 2 to 3 litres of prime and cardioplegic solution. The magnitude of the fall in PCV (41%) was similar to that predicted from the in vitro experiment, as demonstrated in Fig 6.2, when 3 ml of crystalloid fluid was added to 5 ml of whole blood.

By 12 hours the PCV had returned towards, but did not reach,

preoperative values as the prime fluid was excreted as urine or lost to the interstitial spaces. A subsequent fall in PCV between 24 and 72 hours results in part from a continued blood loss through traumatised tissues but also from a "dilutional anaemia" arising from a relative hypervolaemia as fluid returns to the vascular volume from the interstitial tissues (273).

Formula A undercorrected for the effects of haemodilution both in vivo and in vitro. The inability of this formula to correct for haemodilution in vitro increased with the volume of diluent added and can be explained by its failure to take account of the differential rates of decrease in the concentration of plasma constituents and elements of whole blood as illustrated in Fig 6.2. The inability of Formula A to correct for haemodilution in vivo is not simply due to surgical blood loss which entails the loss of whole blood and therefore would not, at least initially, affect the concentration of plasma constituents. Using Formula A there was a 23% decrease in plasma albumin concentration after the onset of CPB but only a 3% fall in plasma caeruloplasmin at the same time, suggesting that differences in the magnitude of decreases in the protein concentrations cannot be entirely explained by dilution. Loss of albumin, through transcapillary leakage, is an early component of the acute phase response and has been reported after cardiac surgery (268). The large molecular weight of caeruloplasmin (160,000), in comparison to albumin (69,000), may prevent a similar loss.

Using Formula A, in vivo, albumin and caeruloplasmin concentrations return towards pre-bypass levels by 12 hours. This is due to a combination of factors, including loss of intravascular water through urinary excretion and to the interstitial space, and may also be due to redistribution of albumin from the interstitial to the

intravascular space as discussed in Chapter 7. With Formula A there is very little change in caeruloplasmin concentration over the first 12 hours of study, as would be expected for this acute phase protein whose plasma concentration does not normally increase until 24 hours after tissue injury (see Chapter 7).

Between 24 to 72 hours after cardiac surgery, a decrease in plasma albumin concentration of around 25% occurs (274). There was a decrease in measured plasma albumin concentration of approximately 20% during this period but after application of Formula A there was only a 2% to 4% fall in plasma albumin in the week following surgery. Similarly the use of Formula A after 24 hours resulted in substantially greater increases in plasma caeruloplasmin than would otherwise be expected as discussed in Chapter 7.

Formula B manifestly fails to correct for haemodilution both in vitro (Figs 6.3 and 6.4) and in vivo (Figs 6.6 and 6.7). The magnitude of error increased with progressive dilution and this formula was even more inaccurate than Formula A.

The in vitro study showed that Formulae C and D provided the most accurate correction for the effects of haemodilution. Regardless of the volume of diluent added, these formulae adequately corrected albumin and caeruloplasmin concentration back to pre-dilutional values as shown in Figs 6.3 and 6.4. The same formulae, when applied in vivo, provided an immediate (ie one minute) correction of the plasma concentration of albumin and caeruloplasmin back to preoperative values but subsequently overcorrected for the effects of haemodilution. This overcorrection was apparent by two hours and peaked at 12 hours, at which time Formulae C and D overestimated predicted plasma albumin concentrations, by 11% and 30% respectively, assuming there had been no true change in the intravascular mass of

albumin. It could be argued that if there is a predominant loss of plasma water from the circulation due to increased transcapillary permeability, which accompanies the response to trauma (268), then Formulae C and D do reflect true alterations in the concentration of plasma albumin. Formulae C and D erroneously imply, however, that plasma albumin concentration remained elevated until at least the third postoperative day, by which time plasma albumin concentration has decreased by at least 10% (266,274).

The inability of Formulae C and D to correct for haemodilution *in vivo* demonstrates again that the correlations between PCV and plasma constituent concentration in a closed *in vitro* model do not hold *in vivo* where alterations in the concentration of plasma proteins occur independently of changes in PCV. *In vivo*, while red blood cells are confined to the vascular space (5 litres), proteins can be distributed to the interstitial space (12 litres) and water to the intracellular volume (28 litres).

In summary there is no correction formula which consistently temporally corrects for the effects of haemodilution *in vivo*. Formulae C and D provide the best correction for haemodilution in the *in vitro* situation and for the very early *in vivo* period but subsequently produce a marked overcorrection. Despite its limitations, Formula A is the most easily applicable formula when correction for considerable haemodilution is necessary and allows comparison with the large body of literature which has previously used this formula. Formula A will subsequently be used in this thesis when correction for haemodilution is necessary in the first 24 hours following CPB, after which no formula will be used. As the inaccuracies of Formula A will consistently apply to both temperature groups this will not invalidate comparative studies between the groups.

SUMMARY and CONCLUSIONS

The ability of four formulae (three based on PCV and one on alpha-2 macroglobulin) to correct for postoperative dilutional changes in plasma albumin and caeruloplasmin was assessed in four patients undergoing coronary artery surgery. CPB is a stringent test of the efficacy of the formulae to correct for haemodilution as a large volume of fluid is added to the circulation in a relatively short period of time. Plasma albumin was measured eight times in the perioperative period in the four patients and the clinical situation was mimicked *in vitro* by adding various volumes of diluent to whole blood. None of the formulae consistently corrected for predicted changes in plasma albumin concentration *in vivo*; two of the formulae corrected for *in vitro* changes in plasma albumin but the same formulae produced considerable over-correction when applied *in vivo*.

1. There is no simple arithmetical formula, based on PCV, which can accurately and consistently correct for the effects of haemodilution *in vivo*.
2. Alterations in the concentrations of plasma proteins during CPB, and in particular albumin, may not be totally accounted for by dilution but also reflect different volumes of distribution.
3. When a correction factor has to be applied to account for large volume changes, Formula A should be used; despite its limitations, particularly in the early post bypass period, it provides the least inaccurate correction for haemodilution and allows comparison with the existing body of literature. From 24 hours no correction factor is necessary.

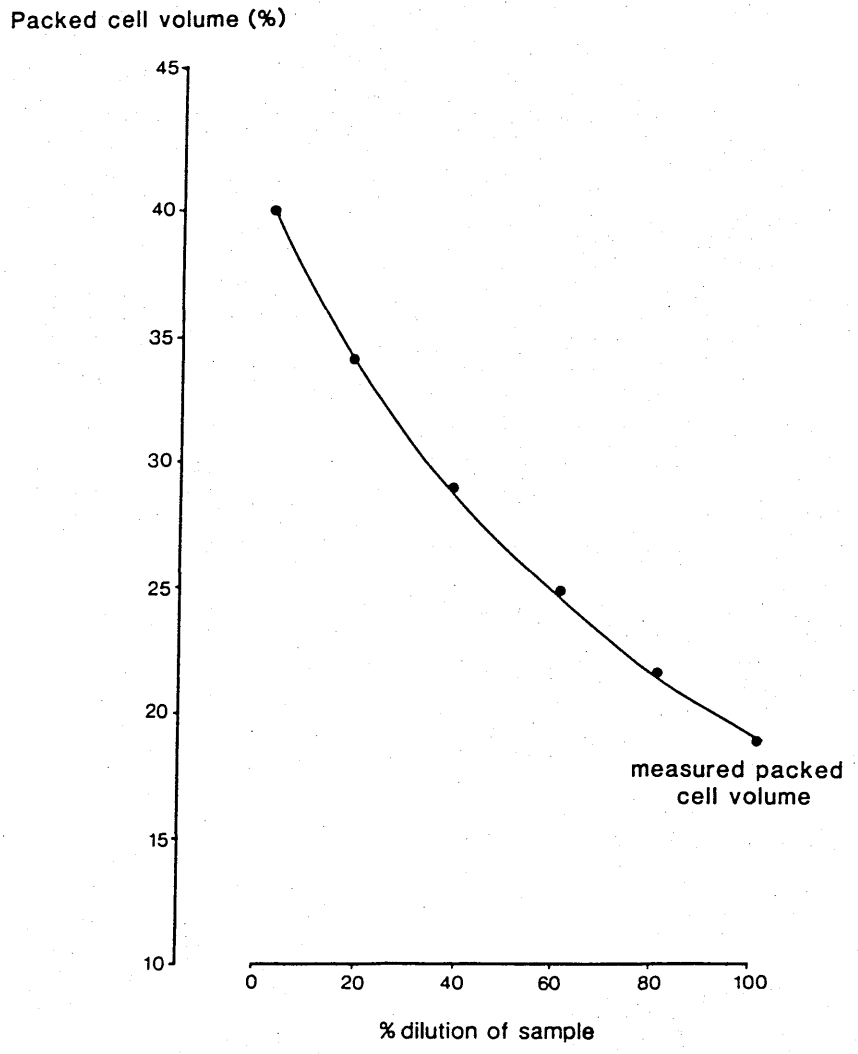
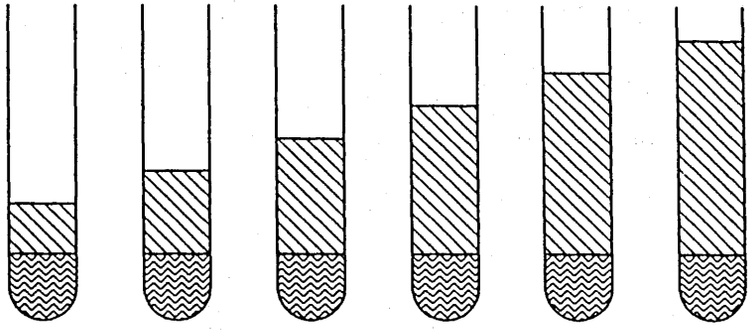


FIG 6.1 Alteration in packed cell volume from the in vitro study.



Added volume	0 ml	1 ml	2 ml	3 ml	4 ml	5 ml
Total volume	5 ml	6 ml	7 ml	8 ml	9 ml	10 ml
Red cell volume	2 ml	2 ml	2 ml	2 ml	2 ml	2 ml
Packed cell volume (Haematocrit)	40%	33.3%	28.6%	25%	22.2%	20%
% decrease in Haematocrit		16.7%	28.5%	37.5%	44.5%	50%
Plasma volume	3 ml	4 ml	5 ml	6 ml	7 ml	8 ml
Concentration of plasma constituent	100%	75%	60%	50%	42.9%	37.5%
% decrease in plasma constituent conc.		25%	40%	50%	57.1%	62.5%

FIG 6.2 Diagrammatic explanation of the differential percentage fall in the PCV and plasma constituent concentration at various levels of dilution.

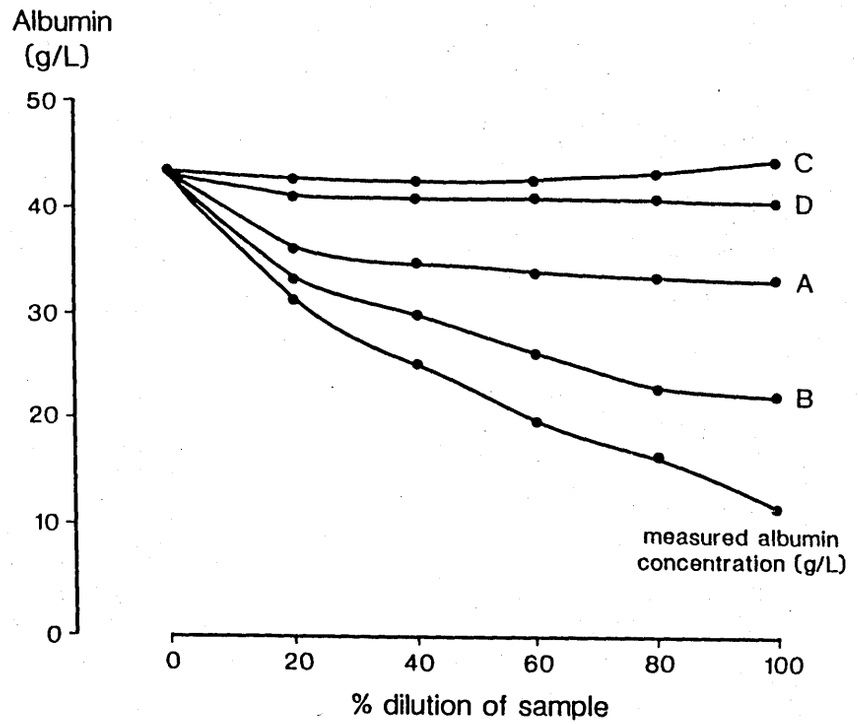


FIG 6.3 Correction of serum albumin by four formulae (as detailed in the text) from the in vitro study.

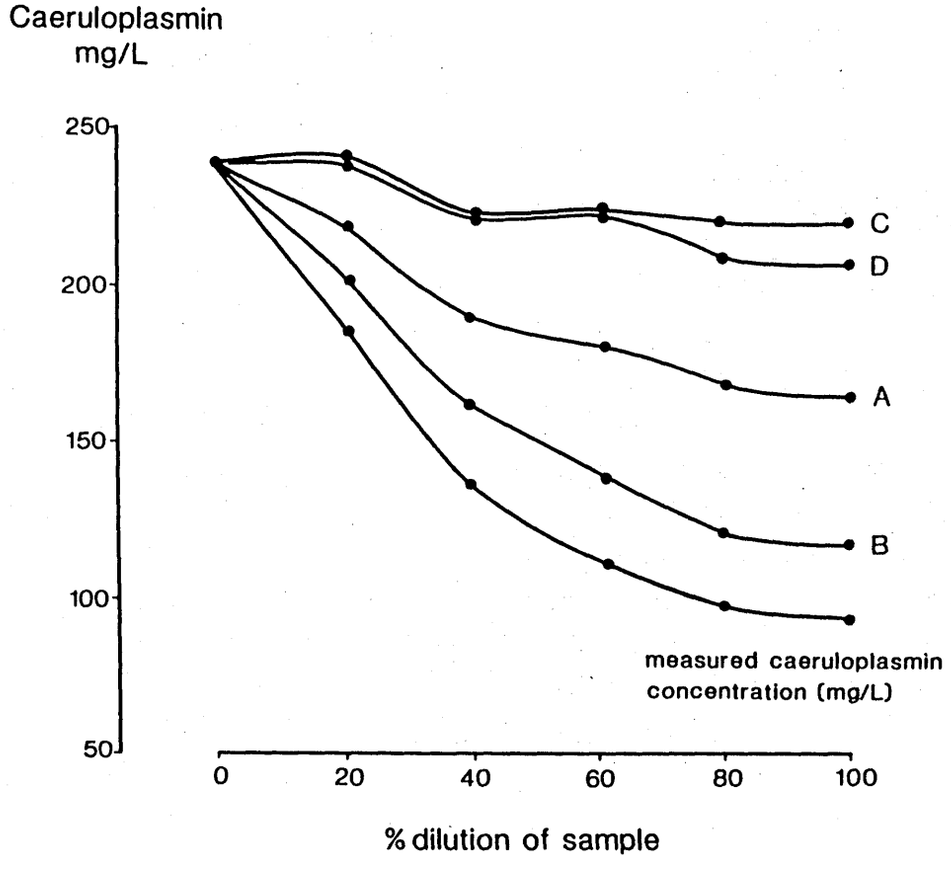


FIG 6.4 Correction of serum caeruloplasmin by four formulae (as detailed in the text) from the in vitro study.

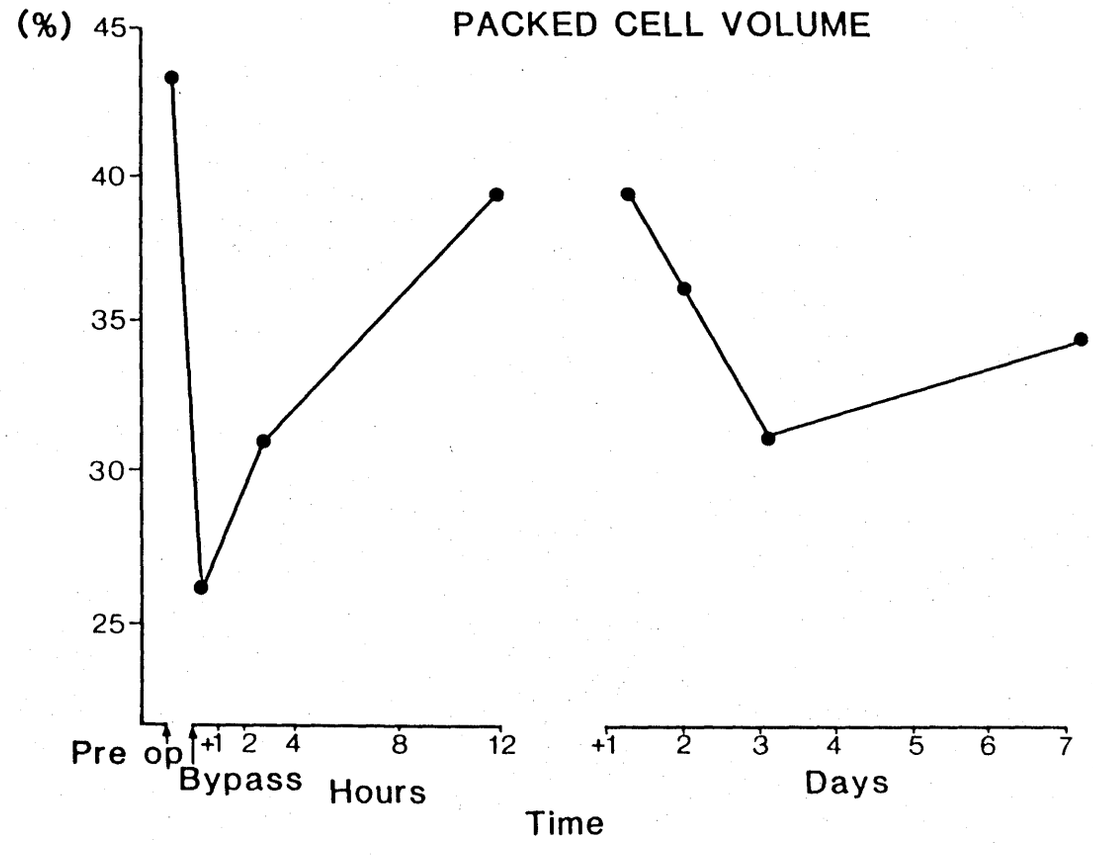


FIG 6.5 Mean alteration in PCV in 4 patients from the in vivo study.

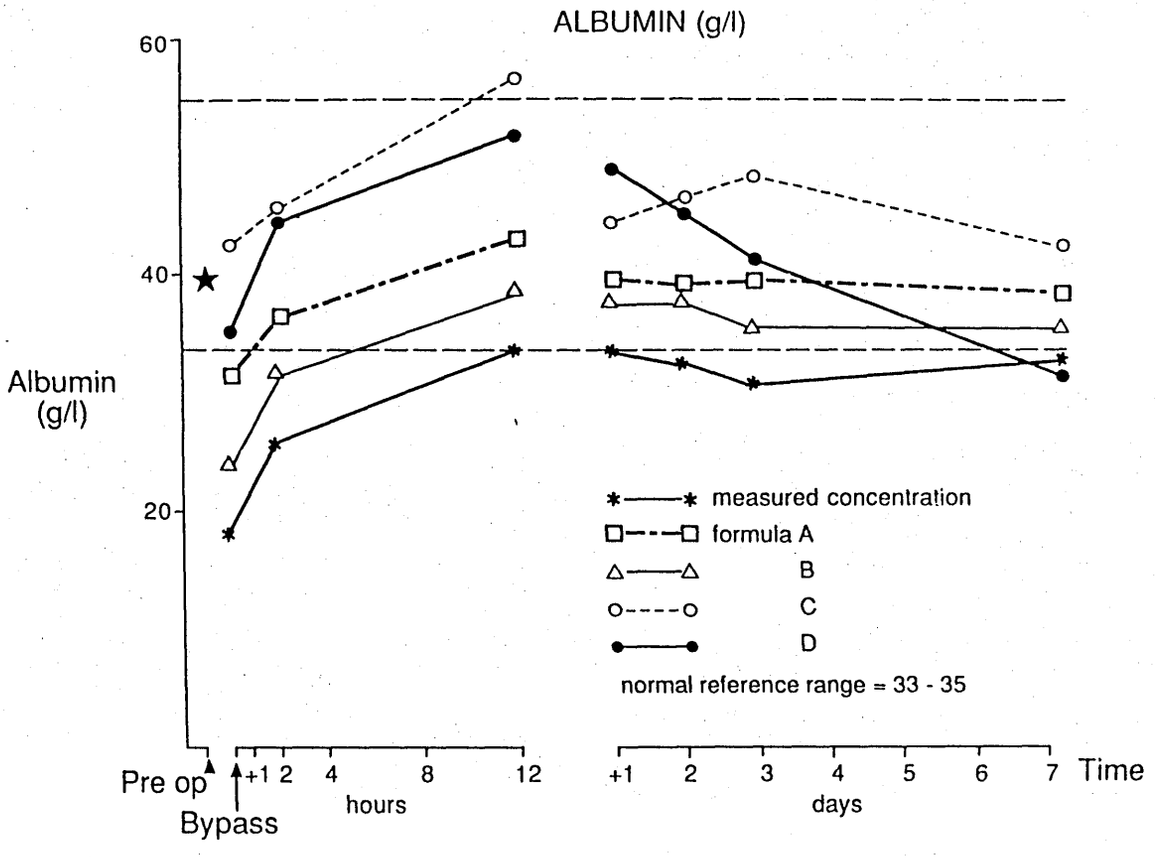


FIG 6.6 Correction of mean serum albumin concentration in four patients by four formulae (as detailed in the text) from the in vivo study.

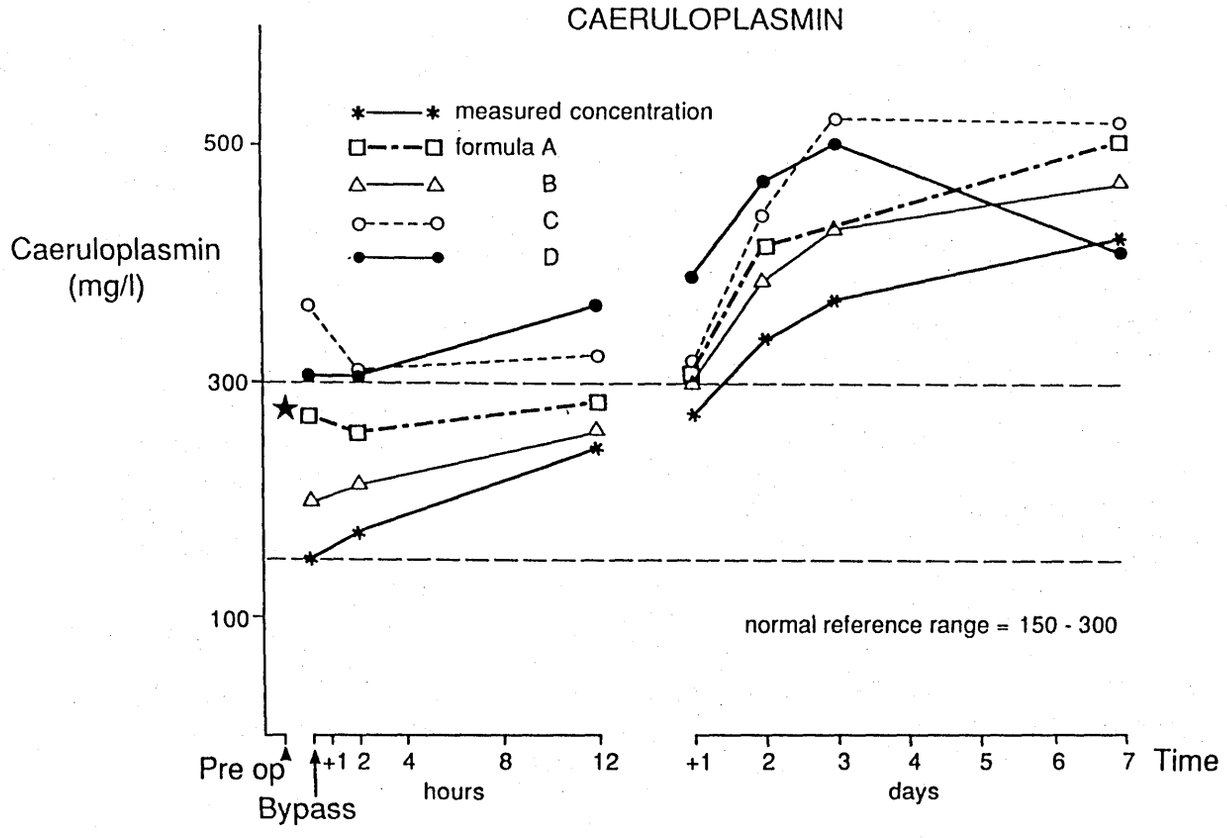


FIG 6.7 Correction of mean serum caeruloplasmin concentration in four patients by four formulae (as detailed in the text) from the in vivo study.

CHAPTER 7

THE ACUTE PHASE RESPONSE. PART 1: PLASMA PROTEINS

CHAPTER 7

THE ACUTE PHASE RESPONSE. PART 1: PLASMA PROTEINS

"The mind likes a strange idea as little as the body likes a strange protein, and resists it with similar energy."

Wilfred Trotter (1872-1939). Collected papers. "Has the intellect a function ?"

INTRODUCTION

The acute phase response is a stereotyped response to tissue injury characterised by typical alterations in the concentrations of certain plasma proteins and trace metals. The response is an attempt to redress the disturbed homeostasis which follows tissue injury and the magnitude of the response is proportional to the degree of tissue trauma (263,264). The acute phase response to cardiac surgery is poorly documented and it is not known if systemic hypothermia at the time of injury is capable of modifing the response. The aim of this chapter was to document the acute phase protein response to CPB and the effects of two levels of intraoperative hypothermia on this response.

PATIENTS and METHODS

Twenty male patients undergoing elective coronary artery surgery were randomised to an intraoperative blood temperature of 28^{o}C or 20^{o}C, as detailed in Chapter 3.

Blood samples were obtained two days before operation, on the morning of operation, and on skin incision. Further samples were obtained one minute after the commencement of total cardiopulmonary

bypass (TIME '0') and at 30, 60, 90, 120 minutes, 3, 5, 7, 9, 12, 15, 24, 36 hours and on the second, third and seventh postoperative days. All samples, except the first, were obtained with the patients in the supine position for at least one hour.

Control of perioperative fluid balance is detailed in Chapter 3 and correction for haemodilution was based on a simple PCV correction: corrected concentration = measured concentration x $\frac{\text{initial PCV}}{\text{new PCV}}$ as described in Chapter 6. Samples obtained after 15 hours were not corrected for haemodilution.

BIOCHEMICAL ANALYSES

The samples were immediately centrifuged and the serum stored for analysis of C-reactive protein, albumin, prealbumin, transferrin, ferritin and caeruloplasmin.

C-reactive protein was measured by Fluorescence Polarisation immunoassay using an Abbott TDX^R analyser and Abbott reagents. The between batch coefficient of variation was <5% across the working range of the assay.

Albumin, transferrin, prealbumin and caeruloplasmin were analysed by immunoturbidimetric methods on an Encore^R centrifugal analyser (Baker Instruments). Antisera for albumin and transferrin were obtained from the Scottish Antibody Production Unit (Carluke, Scotland, UK), for prealbumin from Serotec^R (Oxford, England, UK) and for caeruloplasmin from Atlantic Antibodies (Scarborough Main, USA). The between batch coefficients of variation across the working range of the assays were respectively <8%, <8%, <12% and <10%.

Ferritin was measured by immunoradiometric assay using kits supplied by RIA-GNOST^R (Mabing, West Germany). The detection limit of

the assay is 1 mg/L and the between batch coefficient of variation <15% across the working range of the assay.

Statistical analysis was based on a paired t-test to compare serial differences within the same group and an unpaired t-test to compare differences between groups. Differences were considered statistically significant when the probability of their arising by random sampling error was less than 1 in 20 ($p<0.05$) and highly significant if this probability was less than 1 in 100 ($p<0.01$).

RESULTS

The clinical and operative features of the two groups are listed in Table 7.1. The mean plasma protein concentration at various time points is given in Table 7.2 and percentage changes in concentration at the same time points in Table 7.3. There was no significant difference in the groups with respect to age or anthropometric indices as judged by height, weight and body surface area. The 20°C group received significantly more grafts than the 28°C group, resulting in a non-significant increase in cross clamp and bypass time in the colder group.

There was no significant difference between the two groups in perioperative fluid requirements in terms of crystalloids or blood transfusion.

There were decreases in the concentrations of most plasma constituents, of up to 10%, between the two preoperative samples although this was not statistically significant for any analyte. There was a further fall in the concentration of most plasma constituents, except ferritin and caeruloplasmin, between the second preoperative sample and that taken at skin incision but this was statistically significant only for the decrease in packed cell volume

(PCV) in both groups (Table 7.3).

There were significant decreases in the PCV and the plasma concentration of albumin and transferrin but not prealbumin, ferritin or caeruloplasmin one minute after the start of bypass (Table 7.3).

PCV (Table 7.3, Fig 7.1). There was a significant decrease in the PCV in both groups prior to CPB. The PCV decreased again one minute after the onset of CPB by 33% in the 28°C group and 39% in the 20°C group. In both groups the PCV returned towards preoperative values by 15 hours but decreased again between 24 and 72 hours. Despite some recovery after transfusion the PCV was still 15% to 20% lower than preoperative values in both groups at day 7 ($p<0.01$).

C-REACTIVE PROTEIN (Table 7.3, Fig 7.2). CRP was detected in the preoperative blood sample in eight of the 10 patients in the 28°C group (14,12,23,11,11,18,20,13 mg/L) and in six of the 10 patients in the 20°C group (27,10,12,11,16,15 mg/L). CRP subsequently became undetectable (<10 mg/L) in the majority of patients until seven hours in the 28°C group (detectable in nine out of 10 patients) and 12 hours in the 20°C group (detectable in nine out of 10 patients). CRP levels rose exponentially after seven hours in both groups reaching a peak at 72 hours. The first significant rise in the mean CRP concentration in the 28°C group was at nine hours and in the 20°C group at 12 hours. The rise was more marked and the peak value greater in the 28°C group but not significantly so ($p= 0.09$). The CRP concentration peaked at three days and remained significantly elevated at seven days.

ALBUMIN (Table 7.3, Fig 7.3). Albumin concentration decreased by approximately 10% in both groups immediately after the commencement of CPB. In the 20°C group albumin concentration then increased but continued to fall in the 28°C group reaching a minimum concentration at 30 minutes, by which time it was significantly lower than in the

20°C group (31 g/L v 36 g/L :$p< 0.05$). In both groups albumin concentration returned towards pre-bypass concentrations by 15 hours but decreased again on the first postoperative day and declined to minimum values on the third postoperative day. At day seven the albumin concentration was still 25% lower than the preoperative concentration in both groups ($p<0.05$).

TRANSFERRIN (Table 7.3, Fig 7.4). In both groups of patients plasma transferrin decreased by 11% after the onset of bypass. Transferrin concentration returned to preoperative values by 15 hours but fell to 70% of preoperative concentrations between 24 and 48 hours. There was no significant difference between the groups and on day seven the transferrin concentration was 20% below the preoperative concentration.

PREALBUMIN (Table 7.3, Fig 7.5). There was no significant change in prealbumin concentration in any sample between the preoperative period and that taken at 15 hours in either group. Prealbumin concentrations fell significantly on the first postoperative day and continued to decline to minimum values on the third postoperative day. At day seven the prealbumin concentration in both groups was still less than 60% of preoperative values. There was no significant difference between the two groups.

CAERULOPLASMIN (Table 7.3, Fig 7.6). There was no fall in plasma caeruloplasmin concentration prior to or immediately after the onset of CPB. Caeruloplasmin concentrations increased from 24 hours and continued to increase until day seven when they were 30% higher than baseline concentrations ($p<0.01$). There was no significant difference between the groups.

FERRITIN (Table 7.3, Fig 7.7). In contrast to most other plasma constituents, there was no decrease in ferritin concentration pre or

post bypass but an increase which continued throughout the perioperative period. At day seven the plasma ferritin concentration was more than three times the preoperative concentration in both groups and still rising. The increase in ferritin concentration was more rapid in the 28°C group over the first five hours but this did not reach statistical significance.

DISCUSSION

The acute phase response is an early, complex and non-specific response to tissue injury resulting from trauma, infection or malignancy. The magnitude of the response is approximately proportional to the severity of tissue injury and can therefore be used to quantify tissue trauma (275,276). The response has been well described following general surgical procedures but poorly documented following CPB and it is not known whether the response can be modified by hypothermia.

The term "acute phase" was introduced by Abernethy and Avery when describing the appearance of a protein (C-reactive protein) in the sera of patients with acute febrile illnesses which was not normally detectable (277). The acute phase response is believed to be an attempt to redress disturbed homeostasis after tissue injury (67) and its magnitude is proportional to the degree of tissue injury (275,276). The response is initiated by a number of cytokine peptides released from macrophages and other cell types activated by tissue injury. Amongst the most important cytokines controlling the acute phase response are interleukin-1 (62), interleukin-6 (63) and tumour necrosis factor (64). The response constitutes typical changes in the concentrations of certain plasma proteins and trace elements and is one component of a myriad of physiological and biochemical changes

including fever, leukocytosis, increased secretion of hormones and loss of nitrogen occurring after trauma.

The liver plays a key role in the acute phase response. It increases the synthesis of all the major acute phase proteins, which have an integral role in host defence and tissue repair (eg. C-reactive protein, ferritin, caeruloplasmin), while sacrificing production of those which act primarily as transport proteins (albumin, transferrin and prealbumin). Lebreton coined the term "negative acute phase reactants" to describe the reduction in the plasma concentrations of these latter proteins (278). Furthermore, the liver is probably responsible for the dramatic changes in the plasma concentrations of iron and zinc in the early stages of the acute phase response (see Chapter 8).

Cardiac surgery involves a unique form of tissue trauma combining the additional insults of CPB and hypothermia with conventional surgical tissue injury. CPB initiates "whole body inflammation" through stimulation of vasoactive substances in blood as it passes through the extracorporeal circuit (5,6) but the systemic metabolic consequences are poorly defined. The aims of this study were i) to characterise the effects of CPB on the acute phase response to conventional surgical insult and ii) to determine whether the response was modified by a more profound level of intraoperative hypothermia.

All blood samples, except the first, were obtained with the patient in the supine position for at least one hour to exclude postural fluid shifts which can alter the plasma volume by up to 15% (267). There was a non-significant fall in the concentration of most plasma constituents of up to 10% between the two preoperative samples, consistent with an increase in plasma volume which occurs after an

overnight rest in the supine position (267).

The effect of anaesthetic agents on plasma protein concentrations was considered by comparing alterations in the concentrations of plasma proteins between the second preoperative sample (taken approximately two hours before surgery) and that obtained after anaesthesia but immediately prior to skin incision. There were variable decreases in the concentrations of most plasma constituents between these samples which was in part due to administration of intravenous fluids, as witnessed by significant falls in the PCV, in both groups over this period. Although the magnitude of the decreases was variable for different analytes (Table 7.3), it was similar in both temperature groups for the same analyte. The variable decreases in the plama concentration of some proteins, accompanied by a simultaneous increase in the plasma concentrations of ferritin and caeruloplasmin, are presumably due to the effects of anaesthetic agents on hepatic metabolism of the latter.

The differing magnitudes of decreases in the concentrations of plasma proteins immediately after the onset of CPB (Table 7.3) cannot be explained simply by haemodilution or inadequacy of the correction formula, otherwise similar magnitudes of decreases would be equally expected for all plasma constituents. The largest decreases, of around 10%, were observed in the concentrations of plasma albumin (mol wt: 69,000) and transferrin (mol wt: 90,000) but no such decrease was observed for prealbumin (mol wt: 52,000), caeruloplasmin (mol wt: 160,000) or ferritin (mol wt: 500,000).

PCV. The maximum fall in PCV in both groups of 33% and 39%, soon after the onset of CPB, is similar to the arithmetically predicted fall in PCV of 37.5% when 3 litres of fluid is added to a fixed vascular volume of 5 litres as explained in Chapter 6. The

recovery in PCV during CPB and the early postoperative period is due to excretion of the priming fluid as urine and loss into the interstitial spaces (267), explaining the frequently observed peripheral oedema and weight gain after cardiac surgery (279). The fall in PCV between 24 and 72 hours is due in part to continued blood loss into traumatised tissue but also a relative hypervolaemia as fluid returns from the interstitial spaces causing a "dilutional anaemia" (273).

CRP. CRP epitomises the acute phase response. It is normally present in trace quantities in plasma but its concentration begins to rise within eight hours of tissue trauma to reach a maximum level at 48-72 hours. CRP synthesis is controlled by the cytokine peptides, although its exact role remains undetermined, it is believed to bind to ligands (micro-organisms and modified host tissues) enhancing phagocytosis and increasing the activity of the immune system (67,280). The magnitude of the CRP response is proportional to the degree of tissue trauma (275,276). There is a significantly greater, but delayed, rise in CRP in cardiac surgical patients compared to those undergoing cholecystectomy (281). The peak rise in CRP was on the third postoperative day as previously reported for cardiac surgery (134), although some workers have reported the maximum increase on the second postoperative day (135). There was a trend towards a slower and smaller peak rise in the colder temperature group ($p= 0.09$) in the current study, with the first significant rise in the mean CRP concentration occurring three hours earlier in the 28°C group.

ALBUMIN. There was a greater decrease in albumin concentration 30 minutes after commencement of CPB in the 28°C group ($p<0.05$) than in the 20°C group. Fleck has reported an increase in the permeability of small blood vessels in the very early stages of the acute phase

response and suggested that such a mechanism could explain early perioperative decreases in the plasma concentration of low molecular weight proteins such as albumin and transferrin (268). Attenuation of increased permeability, in the colder temperature group, could explain the smaller fall in plasma albumin and transferrin in this group after 30 minutes of CPB. It does not explain , however, the lack of change during CPB in the plasma concentration of prealbumin whose molecular weight is less than that of albumin and transferrin. Prealbumin is bound to retinol binding protein and vitamin A on an equimolar basis and the dynamics of this complex may be different from the solitary protein molecule.

The restoration of plasma albumin towards preoperative levels by 15 hours presumably reflects alterations in its distribution, between the extra- and intravascular spaces, as such changes are too rapid to be explained by alterations in rates of synthesis or breakdown. In addition to loss of "water" through urine and to the interstitial space, as described earlier, two further mechanisms operate to restore plasma albumin concentration towards normal. Using radio-labelled tracers (^{125}I) Beattie and colleagues demonstrated that during CPB albumin is transferred from the interstitial space to the intravascular volume, at a rate of 0.2 g/minute, and accompanies the loss of clear fluid during CPB (279). During a pump run of approximately 80 minutes, 16 grams of albumin (equivalent to 40% of the potentially rapidly exchangeable pool) would be transferred from the interstitial to the vascular compartment. Secondly Wasserman and colleagues reported that haemorrhage in dogs resulted in an early extra- to intravascular transfer of albumin followed by increased synthesis of new albumin and a reduction in the catabolism of existing albumin (282). These findings were substantiated in man by Skillman

and co-workers who submitted 13 volunteers to an acute blood loss, of approximately 15% of the circulating volume (269). They found a net increase in total plasma albumin, of between 10%-20% within 12 hours, and attributed this increase largely to a shift in albumin from the interstitial to intravascular space (269). It has been postulated that a consequent decrease in the interstitial mass of albumin leads to a decrease in the interstitial concentration of hepatic lymph which stimulates increased albumin synthesis by the liver (283). It is possible that the acute dilutional effects of CPB on plasma protein concentration may mimic some effects of haemorrhage.

The decrease in plasma albumin in the week following CPB is similar to that previously reported after cardiac surgery (274,279) and of a greater magnitude than that following other major surgical procedures (266,284,285). This decrease in plasma albumin following surgical trauma has been exhaustively investigated and attributed to decreased synthesis (286), increased metabolism (287), and redistribution to the interstitial space (261). Although these mechanisms are relevant to all surgical procedures, two additional mechanisms may be involved after cardiac surgery: (i) redistribution of fluid from the extravascular space to the intravascular volume, causing post-perfusion hypervolaemia (273), as witnessed by a fall in the PCV a few days after cardiac surgery and, (ii) denaturation of plasma proteins during CPB which causes physical destruction and increased plasma clearance (288,289).

PREALBUMIN and TRANSFERRIN. The carrier proteins prealbumin and transferrin, like albumin, may be considered as "negative" acute phase reactants. Changes in their plasma concentration follow a similar time course to albumin with minimum concentrations on the third postoperative day. The relatively short half-lives of prealbumin (2

days) and transferrin (eight days) in comparison to albumin (20 days) has led to widespread acceptance of these proteins as markers of nutritional status. The magnitude of the falls in prealbumin and transferrin, respectively 50% and 30% by day 3, are of a similar magnitude to that reported for other operations such as thyroidectomy and cholecystectomy (266,284,285). At day seven the plasma concentrations of albumin, transferrin and prealbumin were still respectively reduced by 25%, 20% and 45% compared to preoperative concentrations. This magnitude of reduction is greater than expected for most other operations (as above) and the only comparable situations to produce similar changes at seven days are gastrectomy (290) and severe traumatic injury (291). Neither the prealbumin nor the transferrin response was modified by a more profound level of intraoperative hypothermia.

CAERULOPLASMIN. In addition to its function as the principal transport protein of plasma copper, caeruloplasmin is an important scavenger of oxygen-derived free radicals (80) and stimulator of the immune system (79). Plasma caeruloplasmin increased steadily from the first postoperative day and was still increasing at seven days, by which time it was approximately 30% greater than baseline concentration. Caeruloplasmin is well characterised as an acute phase reactant protein whose concentration rises relatively slowly following injury. The increases in caeruloplasmin observed in this study were of a greater magnitude than those reported for other major surgical procedures (266,284,285). The caeruloplasmin response was not affected by the different intraoperative temperatures.

FERRITIN. Serum ferritin concentrations are an indication of body iron stores in normal subjects (292) but are also influenced by inflammation (70) and surgery (71). The physiological significance of

post traumatic increases in plasma ferritin is uncertain, although it has been postulated that ferritin may be important in augmenting the oxidative metabolism of circulating neutrophils thereby contributing to host defences (293). The plasma ferritin concentration was significantly increased within 60 minutes of the beginning of CPB and preceded the rise in CRP. This is presumably due to the release of ferritin from the reticuloendothelial stores as it is too rapid to be accounted for by increased synthesis. The magnitude of the ferritin response is proportional to the severity of tissue injury. Two-fold increases in serum ferritin occur three days after arthroscopy (71) while six-fold increases have been described four days after hysterectomy (294). Plasma ferritin levels had still not reached peak levels seven days after surgery in our patients, at which time they were almost 300% above baseline concentrations. There was no significant difference in the ferritin response in the two groups.

SUMMARY and CONCLUSIONS

The plasma protein component of the acute phase response was determined in 20 male patients undergoing elective coronary artery surgery and randomised to an operative blood temperature of 28°C or 20°C. The plasma protein changes observed were typical of the acute phase response with increases in plasma CRP, caeruloplasmin and ferritin and decreases in plasma albumin, transferrin and prealbumin. The magnitude of changes in concentration was greater than those described for other operations. With the exception of a greater fall in plasma albumin in the 28°C group and a trend towards a slower increase in CRP in the 20°C, group a more profound level of intraoperative hypothermia did not influence the protein component of the acute phase response to CPB.

	28°C	20°C	
NUMBER OF PATIENTS	10	10	
AGE	54(2)	53(1)	NS
HEIGHT (cm)	168(2)	170(2)	NS
WEIGHT (Kg)	81(4)	76(2)	NS
BODY SURFACE AREA (m^2)	1.93(0.04)	1.87(0.03)	NS
BYPASS TIME (mins)	85(6)	97(9)	NS
CROSS CLAMP TIME (mins)	48(5)	59(7)	NS
NUMBER OF GRAFTS	2.8(0.25)	3.7(0.25)	<0.05
LOWEST BLOOD TEMPERATURE (°C)	28(1)	20(1)	<0.001

Table 7.1 Clinical and operative features of the two temperature groups.

	-2Hours	Skin	Bypass	15 hrs	day 1	day 2	day3	day 7
28°C	44(1)	39(1)	26(1)	39(1)	39(2)	36(1)	32(1)	35(1)
PCV (%)								
20°C	41(1)	38(1)	23(1)	36(1)	37(1)	35(1)	34(1)	35(2)
28°C	40(2)	37(3)	34(2)	39(2)	33(2)	33(1)	29(1)	31(2)
ALBUMIN (g/L)								
20°C	40(1)	38(1)	34(1)	41(2)	33(2)	31(1)	29(1)	30(1)
28°C	271(13)	263(17)	258(22)	268(19)	214(15)	149(11)	118(12)	158(14)
PREALBUMIN (mg/L)								
20°C	272(12)	268(12)	261(13)	268(14)	214(8)	162(9)	135(6)	150(12)
28°C	3(.1)	2.7(.2)	2.4(.2)	2.9(.1)	2.4(.1)	2.1(.1)	1.9(.1)	2.3(.1)
TRANSFERRIN (g/L)								
20°C	2.9(.2)	2.8(.1)	2.5(.1)	2.9(.2)	2.3(.1)	2.1(.2)	2.2(.1)	2.4(.1)
28°C	99(23)	110(37)	107(29)	212(54)	237(66)	207(58)	229(43)	302(64)
FERRITIN (mg/L)								
20°C	99(16)	101(19)	96(17)	199(37)	179(33)	225(46)	246(55)	336(79)
28°C	297(12)	296(28)	309(15)	298(17)	277(15)	338(13)	360(11)	396(16)
CAERULOPLASMIN (mg/L)								
20°C	315(13)	326(16)	335(22)	310(67)	294(12)	337(9)	373(16)	416(7)
28°C	12(2)	11(2)	-	77(16)	133(10)	219(13)	260(25)	81(18)
C-REACTIVE PROTEIN (mg/L)								
20°C	14(5)	13(6)	-	50(7)	111(13)	206(13)	236(16)	105(30)

TABLE 7.2 MEAN (SE) CONCENTRATIONS OF SERUM PROTEINS AT VARIOUS TIME POINTS.

		Skin	Bypass	15 hrs.	day 1	day 2	day3	day 7
	28°C	-11%	-33%	-11%	-11%	-14%	-27%	-20%
		(0.004)	(0.004)	(0.05)	(0.03)	(0.004)	(0.004)	(0.004)
PCV								
	20°C	-7%	-39%	-12%	-10%	-15%	-17%	-15%
		(0.008)	(0.004)	(0.004)	(0.004)	(0.004)	(0.004)	(0.004)
	28°C	-8%	-8%	-3%	-17%	-18%	-28%	-22%
		(NS)	(0.06)	(NS)	(0.07)	(0.01)	(0.009)	(0.05)
ALBUMIN								
	20°C	-5%	-11%	+3%	-17%	-22%	-27%	-25%
		(NS)	(0.008)	(NS)	(0.01)	(0.006)	(0.006)	(0.01)
	28°C	-3%	-3%	+2%	-21%	-45%	-56%	-42%
		(NS)	(NS)	(NS)	(0.01)	(0.01)	(0.009)	(0.009)
PREALBUMIN								
	20°C	-1%	-3%	0%	-21%	-41%	-50%	-45%
		(NS)	(NS)	(NS)	(0.01)	(0.006)	(0.006)	(0.009)
	28°C	-4%	-11%	+4%	-14%	-25%	-32%	-18%
		(NS)	(NS)	(NS)	(0.009)	(0.009)	(0.006)	(0.008)
TRANSFERRIN								
	20°C	-3%	-11%	0%	-21%	-28%	-26%	-17%
		(NS)	(0.04)	(NS)	(0.009)	(0.006)	(0.006)	(0.006)
	28°C	+11%	-3%	+114%	+139%	+109%	+131%	+205%
		(NS)	(NS)	(0.006)	(0.006)	(0.006)	(0.006)	(0.006)
FERRITIN								
	20°C	+2%	-5%	+101%	+81%	+127%	+148%	+239%
		(NS)	(NS)	(0.006)	(0.006)	(0.006)	(0.006)	(0.009)
	28°C	0%	+4%	0%	-7%	+14%	+21%	+33%
		(NS)	(NS)	(NS)	(NS)	(0.02)	(0.01)	(0.006)
CAERULOPLASMIN								
	20°C	+3%	+3%	+2%	-7%	+7%	+22%	+32%
		(NS)	(NS)	(NS)	(NS)	(0.03)	(0.01)	(0.006)

TABLE 7.3 PERCENTAGE CHANGES IN CONCENTRATIONS OF SERUM PROTEINS AT VARIOUS TIME POINTS AGAINST BASELINE AT -2 HOURS (p value in parenthesis).

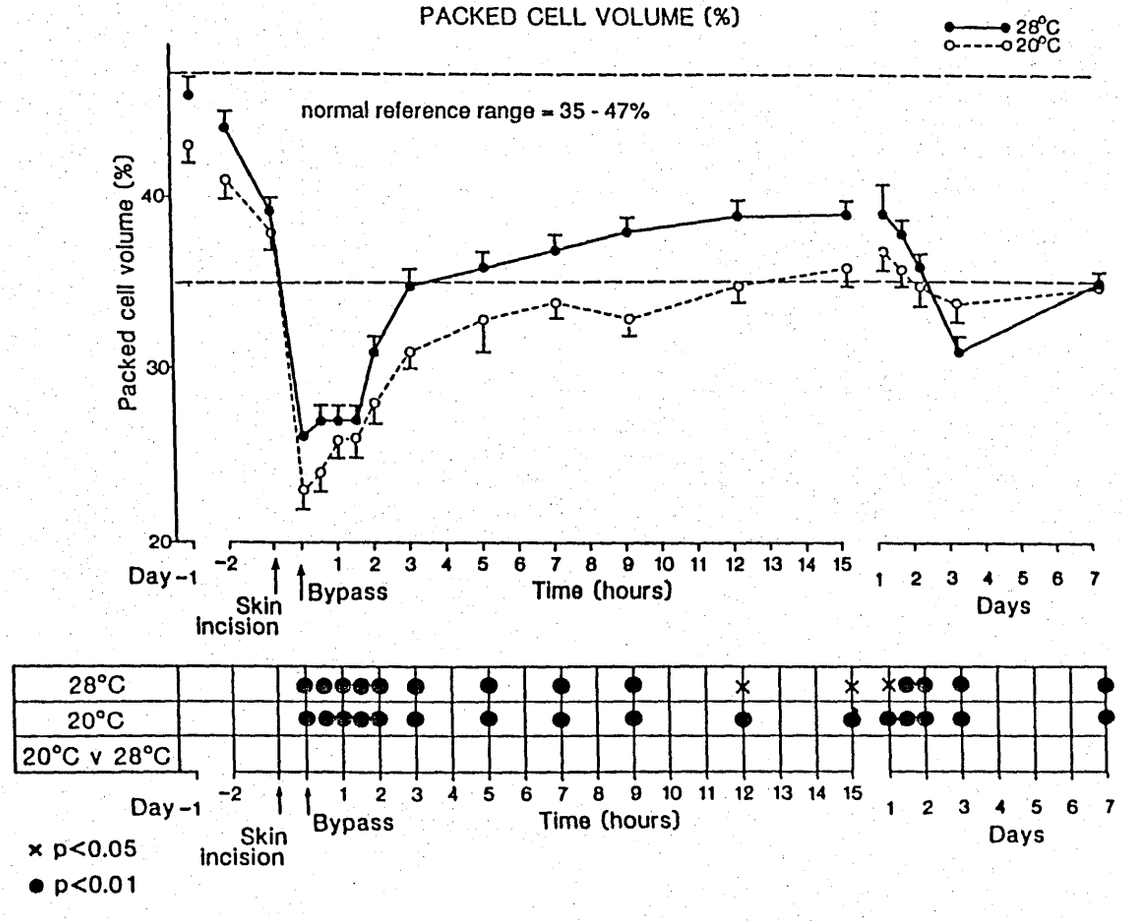


FIG 7.1 Mean (SE) changes in Packed Cell Volume in the two temperature groups.

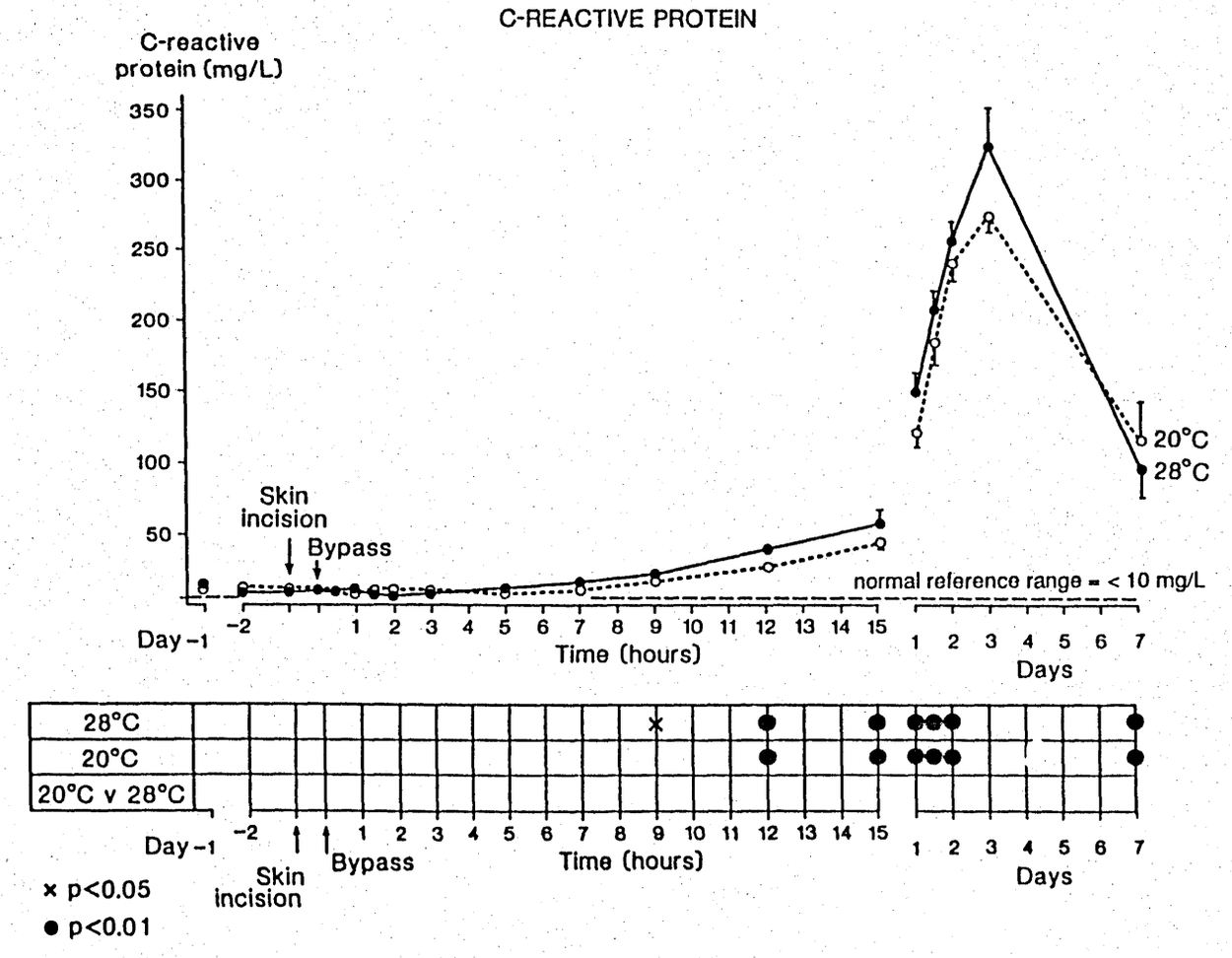


FIG 7.2 Mean (SE) changes in serum CRP in the two temperature groups.

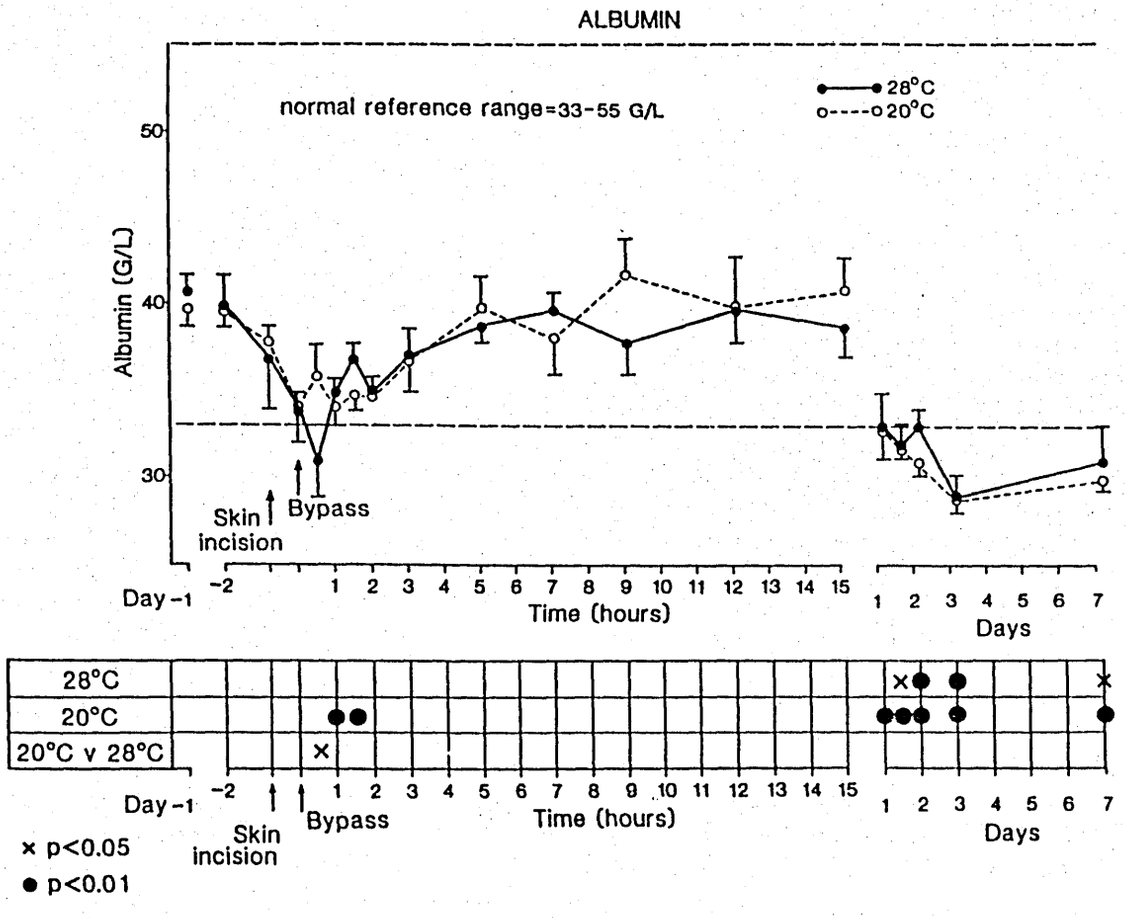


FIG 7.3 Mean (SE) changes in serum albumin in the two temperature groups.

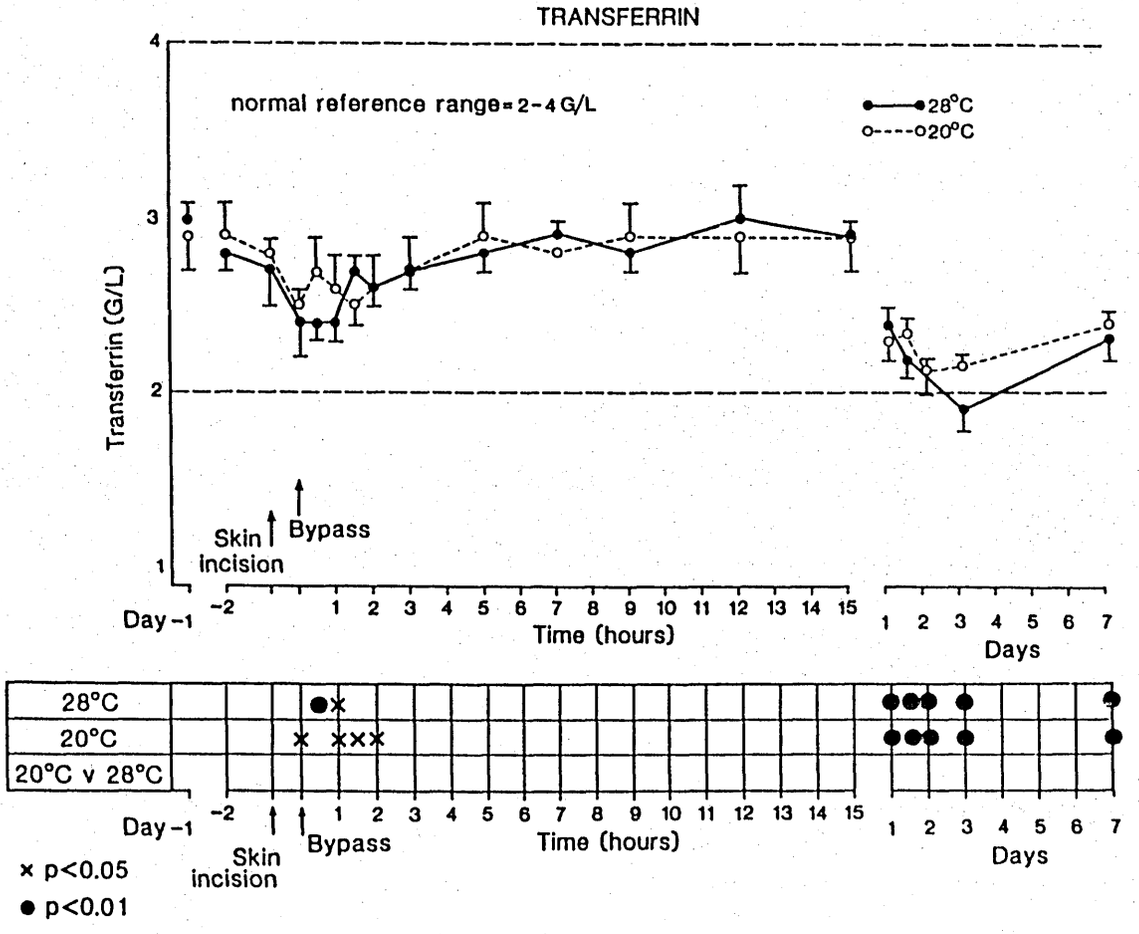


FIG 7.4 Mean (SE) changes in serum transferrin in the two temperature groups.

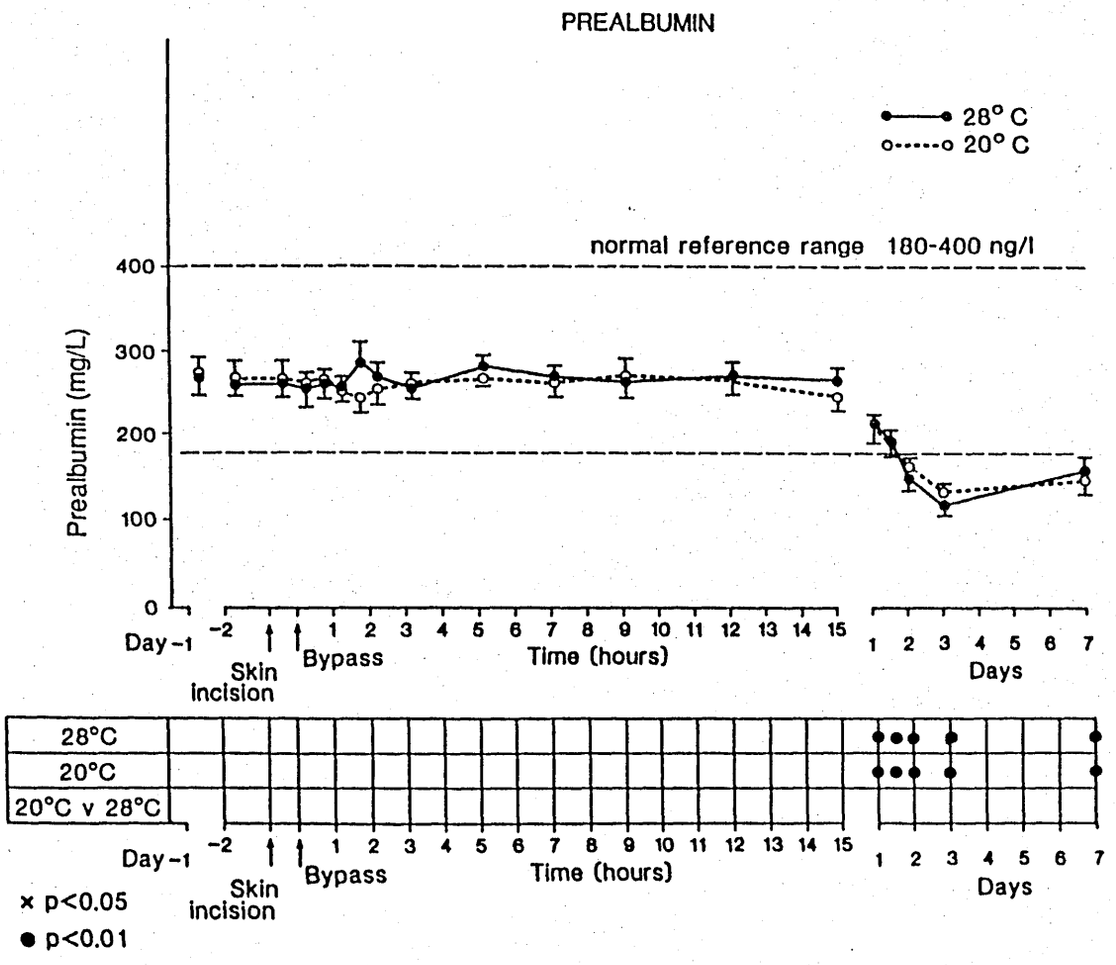


FIG 7.5 Mean (SE) changes in serum prealbumin in the two temperature groups.

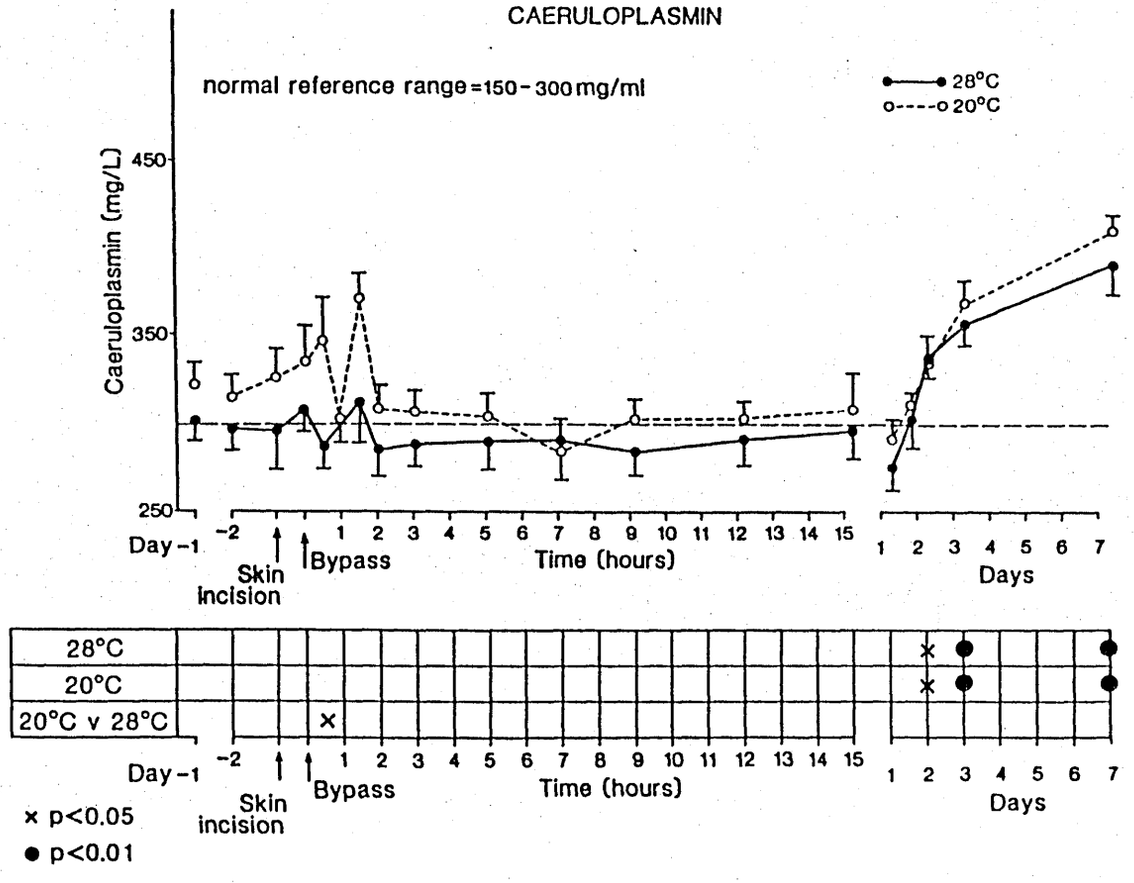


FIG7.6 Mean (SE) changes in serum caeruloplasmin in the two temperature groups

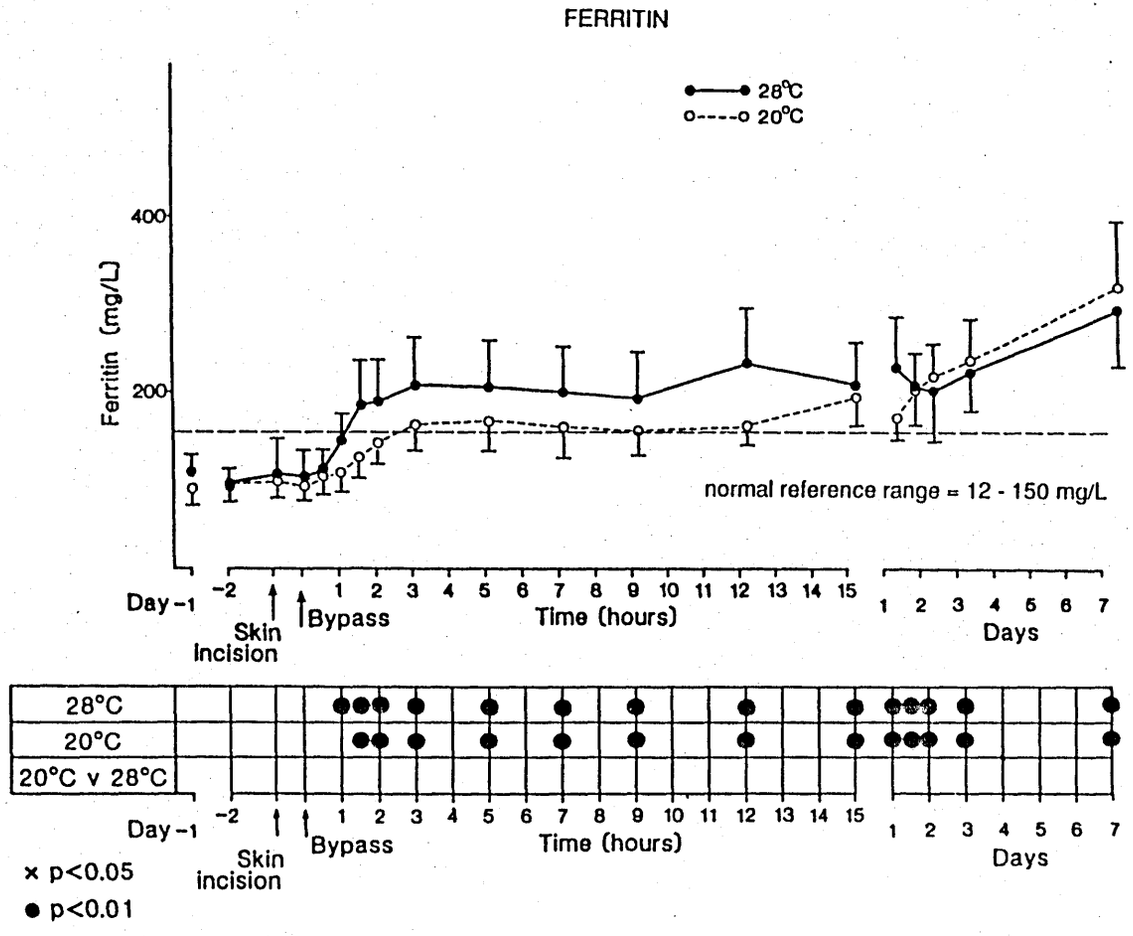


FIG 7.7 Mean (SE) changes in plasma ferritin in the two temperature groups.

CHAPTER 8

THE ACUTE PHASE RESPONSE. PART 2:

TRACE METALS AND THEIR PROTEIN BINDING RATIOS

CHAPTER 8

THE ACUTE PHASE RESPONSE. PART 2:

TRACE METALS AND THEIR PROTEIN BINDING RATIOS

"......; it can only be carried out through blood and iron".

Otto Von Bismarck (1815-1898). Speech, Reichstag. 14th May 1872.

INTRODUCTION

Alterations in the serum concentrations of the trace elements iron, zinc and copper are an integral component of the acute phase response following tissue injury (295). Classically there is a rapid and profound decrease in the serum concentrations of iron and zinc within hours of tissue injury but a slower and more sustained rise, over days, in the serum concentration of copper.

The effects of CPB on serum iron have been described previously (136) but the effects of CPB on the serum concentrations of zinc and copper are unknown. Iron, zinc and copper are transported in plasma bound to their respective carrier proteins, transferrin, albumin and caeruloplasmin. The binding of these metals to their transport proteins is independent of changes in the concentration of the binding protein and the effects of surgery on the affinity of these trace metals for their transport proteins has not been described. Furthermore there is no information on the potential of intraoperative hypothermia to modify any of these responses.

PATIENTS and METHODS

Twenty male patients undergoing elective coronary artery surgery were randomised to an operative blood temperature of 28°C or 20°C.

The patients and timing of blood samples are described in Chapter 6 and operative techniques in Chapter 3.

Iron was measured by standard automated colorimetric analysis using ferrozine as the colour reagent. Zinc and copper were measured by flame atomic absorption spectrophotometry. The techniques for measurement of proteins are described in Chapter 7.

In addition to measurement of their serum concentrations, the binding of the trace metals to their carrier proteins was calculated, as a molar ratio, as follows:

$$\frac{\text{trace metal serum concentration} \times \text{molecular weight of carrier protein}}{\text{carrier protein (serum concentration)}}$$

$$\text{Iron:transferrin molar ratio} = \frac{\text{Fe (umol/L)} \times .09}{\text{transferrin (G/L)}}$$

$$\text{Zinc:albumin molar ratio} = \frac{\text{Zn (umol/L)} \times 0.069}{\text{albumin (G/L)}}$$

$$\text{Copper:caeruloplasmin molar ratio} = \frac{\text{Cu (umol/L)} \times 160}{\text{caeruloplasmin (mg/L)}}$$

assuming transferrin has a molecular weight of 90,000, albumin 69,000 and caeruloplasmin 160,000. More recent estimates of the molecular weight of transferrin would be approximately 78,000 but the classical figure of 90,000 will not alter the interpretation of ratios in a comparative study within and between groups.

Correction for haemodilution was based on a PCV correction, up to 24 hours, as described in Chapter 6.

Statistical analysis was based on a paired t-test to compare serial differences in the same group and a non-paired t-test to compare differences between groups. Differences were considered

significant if their probability of arising from random sampling error was less than 1 in 20 ($p<0.05$) and highly significant if this probability was less than 1 in 100 ($p<0.01$).

RESULTS

The clinical and operative features of the two groups are listed in Table 7.1. The mean serum trace metal concentrations at various time points are given in Table 8.1 and percentage changes in concentration at the same time points in Table 8.2.

IRON (Table 8.2, Fig 8.1). In both groups of patients serum iron increased after the commencement of CPB and continued to rise until five hours, when it was more than double preoperative values ($p< 0.01$). The rise was lower in the 20°C group for the duration of CPB and significantly so at 90 minutes. The serum iron concentration fell rapidly after five hours in both groups and particularly so in the 28°C group. The decrease in serum iron was greater in the 28°C group where the concentration decreased by 60% between five and 15 hours compared to a 36% fall in the 20°C group over the same period. At day seven the concentration of serum iron was 40% of the preoperative concentration in the 28°C group ($p< 0.006$) and 50% of the preoperative concentration in the 20°C group ($p= 0.02$).

IRON:TRANSFERRIN MOLAR RATIO (Table 8.2, Fig 8.2). The iron:transferrin molar ratio demonstrated an increased binding of iron to its carrier protein during the perioperative period until five hours. Significant increases in this binding ratio were apparent by 90 minutes in the 28°C group but not until three hours in the 20°C group. In the 28°C group there was an increase in the iron:transferrin binding ratio of 42% within 60 minutes of the onset of CPB compared to a 22% increase in serum iron concentration over the

same period. Changes in the binding ratio were similar but slower in the 20°C group: two hours after the start of CPB the molar binding ratio had increased by 27% compared to a 13% increase in serum iron. Compared to the 20°C group the iron:transferrin molar binding ratio was significantly higher in the 28°C group at 60 and 90 minutes after the onset of CPB.

ZINC (Table 8.2, Fig 8.3). The serum zinc concentration increased in both groups after the commencement of CPB until 90 minutes, although the rise was significant only in the 28°C group. The serum zinc concentration subsequently fell in both groups to approximately half of preoperative values by 48 hours and then recovered to appproximately 90% of preoperative concentrations by day seven.

ZINC:ALBUMIN MOLAR RATIO (Table 8.2, Fig 8.4). Changes in the zinc:albumin molar ratio preceded changes in the concentration of serum zinc. There were significant increases in this molar ratio even by the time of skin incision. The molar ratio peaked in both groups at 90 minutes ($p<0.01$) and then rapidly decreased to reach minimum values at 9-12 hours. The binding ratio subsequently increased in a sustained fashion so that in both groups it was significantly higher ($p<0.05$) than baseline values at seven days (compared to serum zinc which was lower than baseline values at day seven and significantly so in the 20°C group).

COPPER (Table 8.2, Fig 8.5). There was a significant fall in the serum concentration of copper in both groups at the onset of CPB. which remained significantly lower than baseline levels until 15 hours. At 24 hours a sustained rise in the concentration of copper began so that by day seven the concentration was 30% greater than baseline concentrations in both groups ($p<0.01$).

COPPER:CAERULOPLASMIN MOLAR RATIO (Table 8.2, Fig 8.6). The copper:caeruloplasmin binding ratio decreased by almost 20% in both groups ($p<0.01$) within 30 minutes of CPB in contrast to the more modest falls in serum copper. In both groups the molar ratio returned to baseline levels by three hours. There was no further significant change in the copper:caeruloplasmin ratio for the remainder of the study despite significant increases in the serum copper concentration.

DISCUSSION

Alterations in the serum concentrations of iron, zinc and copper are an integral component of the acute phase response, whether precipitated by trauma, infection or malignancy, and the magnitude of these changes is proportional to the severity of the stress (295-297). Typically there is a marked decrease in the serum concentrations of iron and zinc within hours of the noxious stimulus but a slower and more sustained increase in the serum concentration of copper. Alterations in the serum concentrations of iron and zinc are mediated by cytokines such as interleukin-1 which promote their uptake into liver (62). Serum copper concentrations reflect alterations in the concentration of its carrier protein caeruloplasmin to which it is almost exclusively bound (77).

Alterations in serum iron, zinc and copper should be interpreted in relation to changes in the concentration of their transport proteins. At least 80% of zinc is bound to serum albumin and the remainder to alpha-2 macroglobulin, although it is almost exclusively changes in the former which account for changes in serum zinc concentration in response to stress (296). Alterations in the serum concentrations of proteins may be due to dilution, synthesis, catabolism or redistribution and can therefore obscure independent

changes in the serum concentration of the bound trace metals. Expressing results in terms of the metal to protein binding ratio therefore allows interpretation of quantitative changes in the binding of the metal to its transport protein, irrespective of changes in protein concentration.

The aims of this study were to document the effects of CPB on

(i) the serum concentrations of iron, zinc and copper

(ii) the trace metal's affinity for its binding protein and

(iii) to determine whether the responses were modified by a more profound level of intraoperative hypothermia.

IRON. In contrast to the typical acute phase response, an increase in serum iron concentration, to a maximum at 5 hours after the commencement of CPB, preceded the typical dramatic fall after tissue injury (297). This early increase has been observed previously, following cardiac surgery, and attributed to trauma to red blood cells (136). The increase in serum iron was less during CPB in the colder temperature group, and significantly so at +90 minutes (17 umol/L v 24 umol/L: p= 0.03), implying a reduction in red cell trauma, either through a protective effect of hypothermia or possibly due to reduced exracorporeal flow rates. Serum iron subsequently fell to barely detectable concentrations as expected after major surgery (72). The profound fall in the concentration of serum iron as part of the acute phase response is mediated by cytokines such as interleukin-1 (62). The beneficial effects of hypoferraemia on host defence mechanisms, by depriving invading micro-organisms of an element essential for growth and reproduction, is well recognised (297) and has been reiterated recently (298). It is of interest that Hairston and colleagues demonstrated that serum from patients after CPB encouraged bacterial growth and attributed this to immunodepression

caused by denatured proteins (299). An alternative explanation could be that large quantities of circulating iron, during CPB, enhance bacterial growth in such serum.

IRON:TRANSFERRIN MOLAR RATIO. This ratio mimicked and exaggerated changes in the serum iron concentration. It demonstrated an increased binding of iron to transferrin over the first five hours of CPB in both groups. This occurred too rapidly to be explained by the action of interleukin-1. There were significant increases in the molar binding ratio by 90 minutes in the 28°C group and by two hours in the 20°C group. Whatever the mediator, this early increase in iron binding would be consistent with the teleological concept of host defence mechanisms ensuring that circulating iron is not readily accessible to potential pathogens. After five hours there were highly significant decreases in the binding ratio consistent with iron being stripped from transferrin and being taken up into liver (62).

ZINC. In contrast to the usual response, there was an early increase in serum zinc in both groups reaching a peak at 90 minutes. This early rise in serum zinc concentration is not specific for cardiac surgery, as a similar situation has been reported after cholecystectomy although the magnitude of the increase may be greater after cardiac surgery (281). The increase was less in the colder temperature group and significantly so at 60 minutes (13 [SE 0.8] umol/L v 15 [SE 0.5] umol/L :$p < 0.05$). The decrease in serum zinc to approximately 50% of baseline values by nine hours is typical of the acute phase response (295). This fall in serum zinc is mediated by interleukin-1 which promotes zinc uptake into the liver where it is complexed to metallothioneins involved in the production of new proteins (62). Teleologically, low serum concentrations of zinc may be another component of non-specific host defence mechanisms depriving

micro-organisms of a trace element essential for growth and replication (76).

ZINC:ALBUMIN MOLAR BINDING RATIO. Alterations in this ratio demonstrated a marked increase in the binding of zinc to albumin during CPB. Significant changes in zinc binding were apparent by the time of skin incision and are presumably due, at least in part, to the effects of anaesthesia. These changes in binding preceded significant changes in the serum concentrations of zinc, iron and the iron:transferrin molar ratio. Again changes in the zinc binding ratio occurred too rapidly to be explained by the actions of interleukin-1 and may be due to changes in hormone concentrations. The zinc:albumin molar ratio demonstrated that zinc was subsequently stripped from albumin, consistent with its uptake into liver where it is incorporated into numerous zinc metallothioneins. There was a progressive rise in the zinc:albumin molar ratio after 36 hours so that by day seven there was a significant increase in the metal:protein binding ratio compared to preoperative values, and in contrast to the serum zinc concentration which was still lower than baseline concentrations at day seven.

COPPER. As part of the acute phase response, and in contrast to serum iron and zinc, there is typically a sustained increase in the concentration of serum copper due to interleukin-1 mediated increases in caeruloplasmin synthesis (77). In the current study there were significant decreases in serum copper concentration soon after the onset of CPB which persisted until 24 hours. Serum copper subsequently increased steadily so that by seven days it was 30% above baseline values in both groups. This increase in serum copper is due to increased production of the acute phase protein caeruloplasmin as described in Chapter 7.

COPPER:CAERULOPLASMIN MOLAR BINDING RATIO. Examination of copper binding to caeruloplasmin demonstrated that copper was actively removed from its transport protein during CPB. This may be related to the role of copper both as an anti-inflammatory agent (78) and as a scavenger of oxygen free radicals (80) which are generated during CPB (300). By three hours after CPB there was no further significant change in the binding ratio of copper to caeruloplasmin and there was no obvious effect of different intraoperative temperatures on either serum copper or its binding ratio to caeruloplasmin.

SUMMARY and CONCLUSIONS

Alterations in the serum concentrations of the trace elements iron, zinc and copper, and in their binding ratios to their carrier proteins were examined in 20 male patients undergoing elective coronary artery surgery and randomised to an intraoperative blood temperature of 28°C or 20°C.

Alterations in serum iron following cardiac surgery have been described previously but not changes in serum zinc or copper. Examination of the metal:protein binding ratios allowed assessment of the affinity of the metals for their carrier proteins, irrespective of changes in the transport protein concentrations, and have not previously been described after surgical operations. The potential for intraoperative hypothermia to modify surgically induced changes in serum trace metals or their binding to carrier proteins is unknown.

Decreases in serum iron and zinc, typical of the acute phase response, were preceded by early rises. Significant alterations in their respective molar binding ratios preceded significant changes in the serum concentrations of the metals and ocurred earliest with the zinc:albumin binding ratio. Changes in this ratio therefore

constitute one of the earliest markers of the stress response, being apparent by the time of skin incision. Changes in the serum concentrations of iron and zinc and their molar binding ratios may be a useful adjunct to studies of the acute phase response as the measurements are easy to perform and the facilities to do so widely available, in contrast to the situation for cytokine peptides.

A similar overall pattern of response was observed in both temperature groups, although elevations in the serum concentrations of iron and zinc were significantly less in the colder temperature group during CPB.

	-2Hours	Skin	Bypass	15 hrs	day 1	day 2	day3	day 7
28°C	18(2)	19(3)	17(2)	18(4)	8(1)	4(1)	4(1)	7(1)
IRON (umol/L)								
20°C	15(2)	15(2)	15(2)	15(4)	13(4)	3(1)	4(1)	7(1)
28°C	58(17)	63(7)	68(7)	54(10)	26(3)	16(3)	23(4)	29(3)
IRON:TRANSFERRIN RATIO (x100)								
20°C	50(6)	51(6)	57(10)	81(17)	15(17)	14(2)	17(3)	29(5)
28°C	13(.3)	13(.7)	13(.6)	9(.7)	8(.5)	7(.3)	8(.3)	12(.5)
ZINC (umoL/L)								
20°C	13(.3)	13(.3)	14(.5)	9(.7)	8(.6)	8(.3)	9(.4)	12(.4)
28°C	23 (1)	25(2)	28(1)	15(1)	16(1)	14(1)	20(1)	28(2)
ZINC:ALBUMIN RATIO (x1000)								
20°C	23(1)	23(1)	29(2)	15(1)	17(1)	18(1)	20(1)	27(1)
28°C	19(1)	18(2)	16(1)	18(1)	17(1)	20(1)	21(1)	26(1)
COPPER (umoL/L)								
20°C	21(1)	20(1)	18(1)	19(1)	17(1)	20(1)	23(1)	27(1)
28°C	100(2))	98(2)	82(4)	95(4)	89(9)	93(2)	95(2)	104(3)
COPPER:CAERULOPLASMIN RATIO (x10)								
20°C	106(6)	99(6)	91(7)	99(7)	92(5)	94(4)	95(5)	102(4)

TABLE 8.1 MEAN (SE) CONCENTRATIONS OF SERUM TRACE METALS AND MOLAR BINDING RATIOS AT VARIOUS TIME POINTS.

		Skin	Bypass	15 hrs.	day 1	day 2	day3	day 7
	28°C	+6%	-6%	0%	-56%	-78%	-78%	-61%
		(NS)	(NS)	(NS)	(p<0.01)	(p<0.01)	(p<0.01)	(p<0.01)
IRON								
	20°C	0%	0%	0%%	-13%	-80%	-73%	-53%
		(NS)	(NS)	(NS)	(p<0.01)	(p<0.01)	(p<0.01)	(p<0.01)
	28°C	+9%	+17%	-8%	-55%	-72%	-61%	-50%
		(NS)	(NS)	(p<0.01)	(p<0.01)	(p<0.01)	(p<0.01)	(p<0.01)
IRON:TRANSFERRIN RATIO								
	20°C	+2%	+14%	+62%	-70%	-72%	-66%	-42%
		(NS)	(NS)	(p<0.01)	(p<0.01)	(p<0.01)	(p<0.01)	(p<0.05)
	28°C	0%	0%	-31%	-38%	-46%	-38%	-8%
		(NS)	(NS)	(p<0.01)	(p<0.01)	(p<0.01)	(p<0.01)	(p<0.01)
ZINC								
	20°C	0%	+8%	-31%	-38%	-38%	-31%	-8%
		(NS)	(NS)	(p<0.01)	(p<0.01)	(p<0.01)	(p<0.01)	(p<0.05)
	28°C	+9%	+22%	-35%	-31%	-39%	-13%	+22%
		(p<0.05)	(p<0.01)	(p<0.01)	(p<0.01)	(p<0.01)	(p<0.05)	(p<0.05)
ZINC:ALBUMIN RATIO								
	20°C	0%	+26%	-35%	-26%	-22%	-13%	+17%
		(NS)	(p<0.01)	(p<0.01)	(p<0.01)	(p<0.01)	(p<0.05)	(p<0.05)
	28°C	-5%	-16%	-6%	-11%	+5%	+11%	+37%
		(NS)	(p<0.01)	(NS)	(p<0.05)	(NS)	(p<0.05)	(p<0.01)
COPPER								
	20°C	-5%	-14%	-10%	-19%	-5%	+10%	+29%
		(NS)	(NS)	(NS)	(p<0.05)	(NS)	(NS)	(p<0.01)
	28°C	-2%	-18%	-5%	-11%	-7%	-5%	+4%
		(NS)	(p<0.01)	(NS)	(NS)	(NS)	(NS)	(NS)
COPPER:CAERULOPLASMIN RATIO								
	20°C	-7%	-15%	-7%	-13%	-11%	-10%	-4%
		(NS)	(NS)	(NS)	(NS)	(NS)	(NS)	(NS)

TABLE 8.2 PERCENTAGE CHANGES IN CONCENTRATIONS OF TRACE METALS AND THEIR MOLAR BINDING RATIOS AGAINST BASELINE AT -2 HOURS (p value in parenthesis).

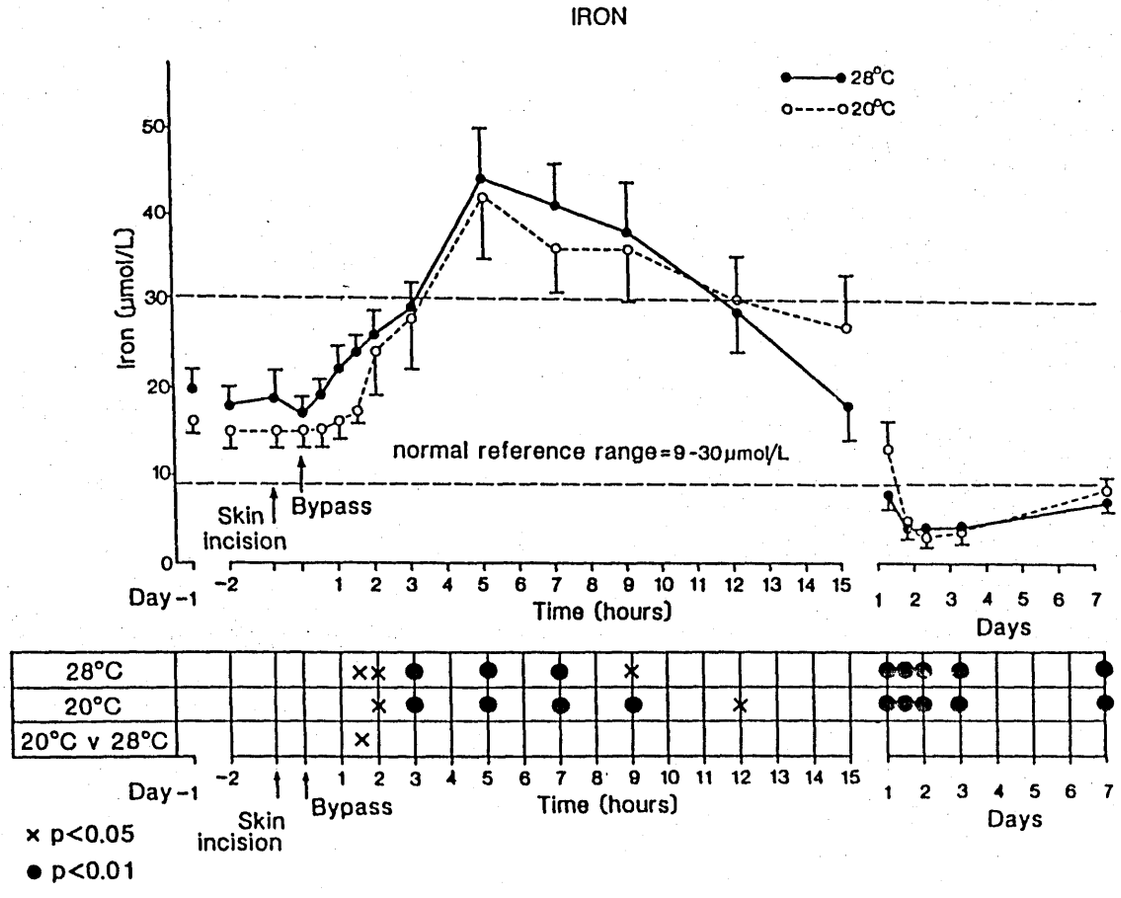


FIG 8.1 Mean (SE) changes in serum iron in the two temperature groups.

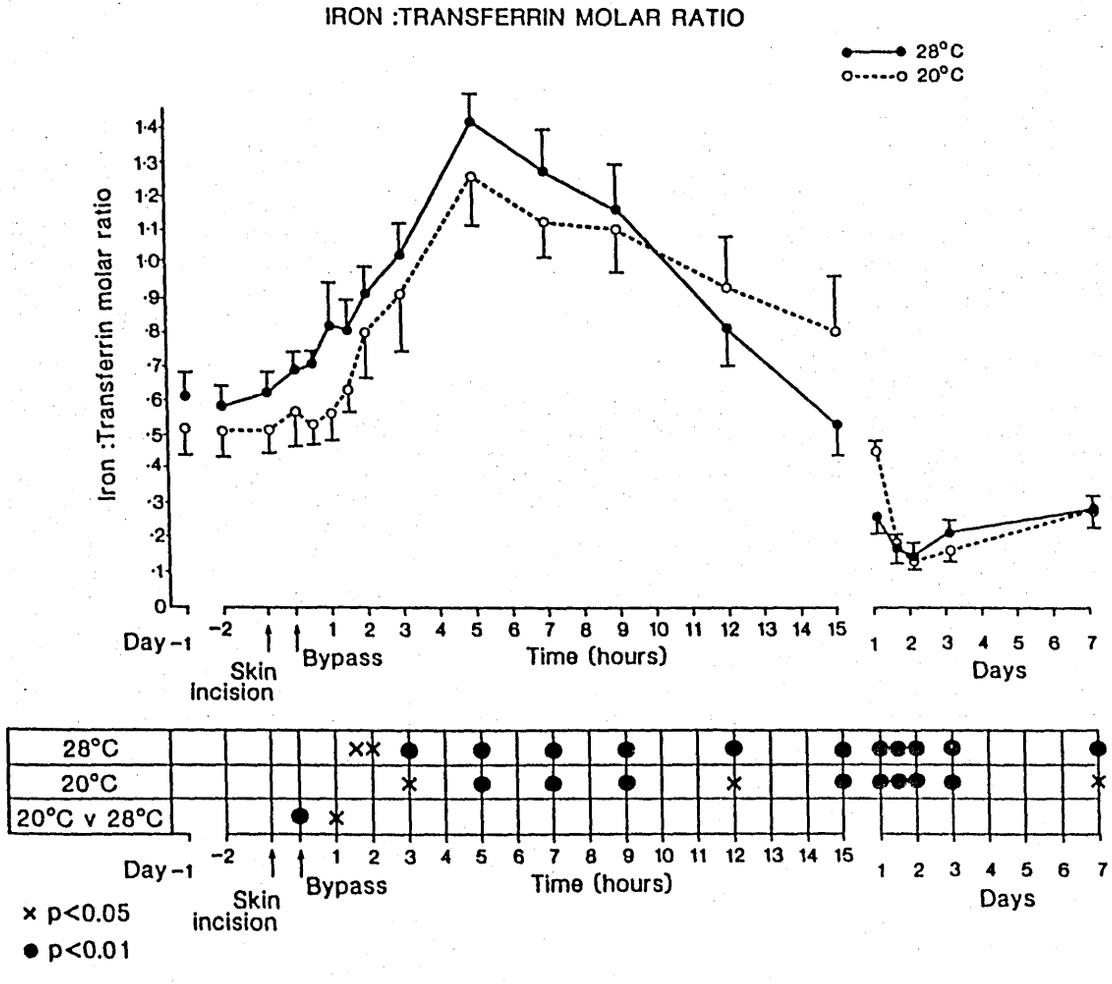


FIG 8.2 Mean (SE) changes in the iron:transferrin molar ratio in the two temperature groups.

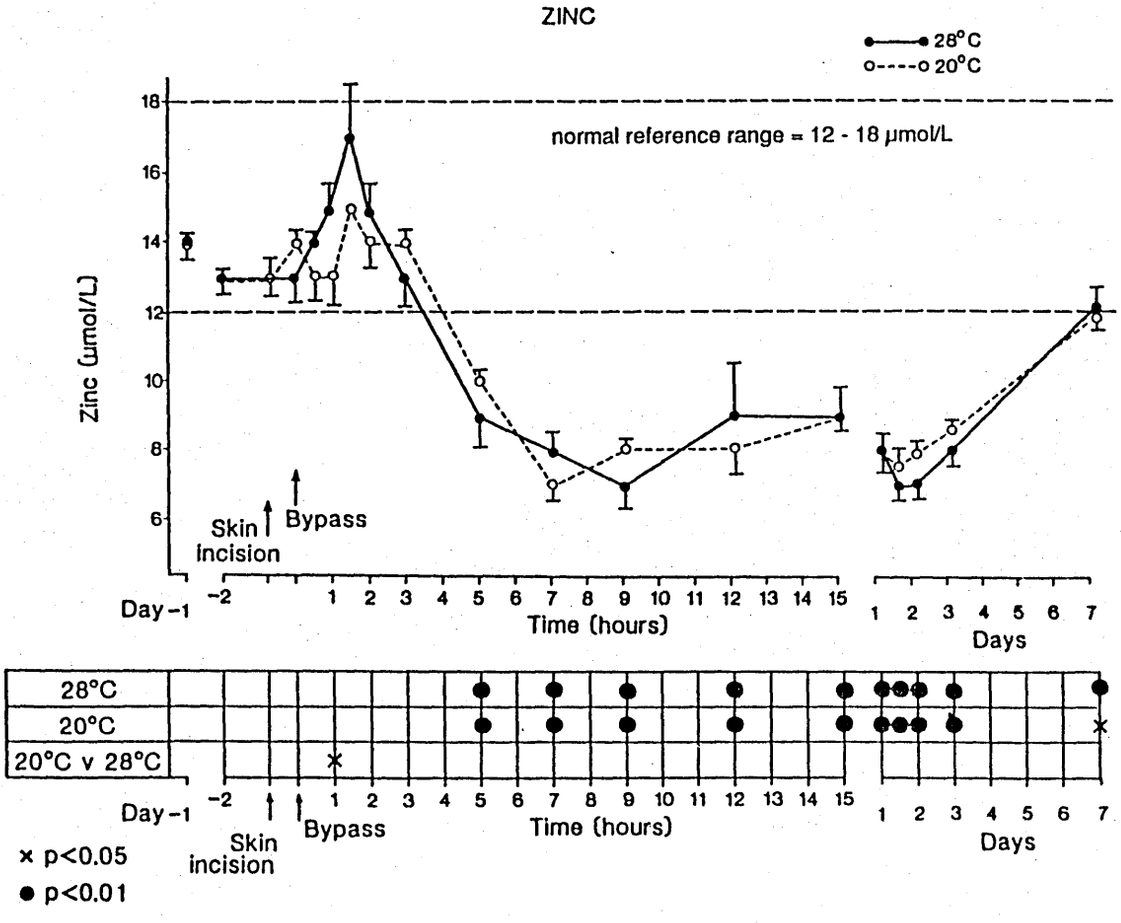


FIG 8.3 Mean (SE) changes in serum zinc in the two temperature groups.

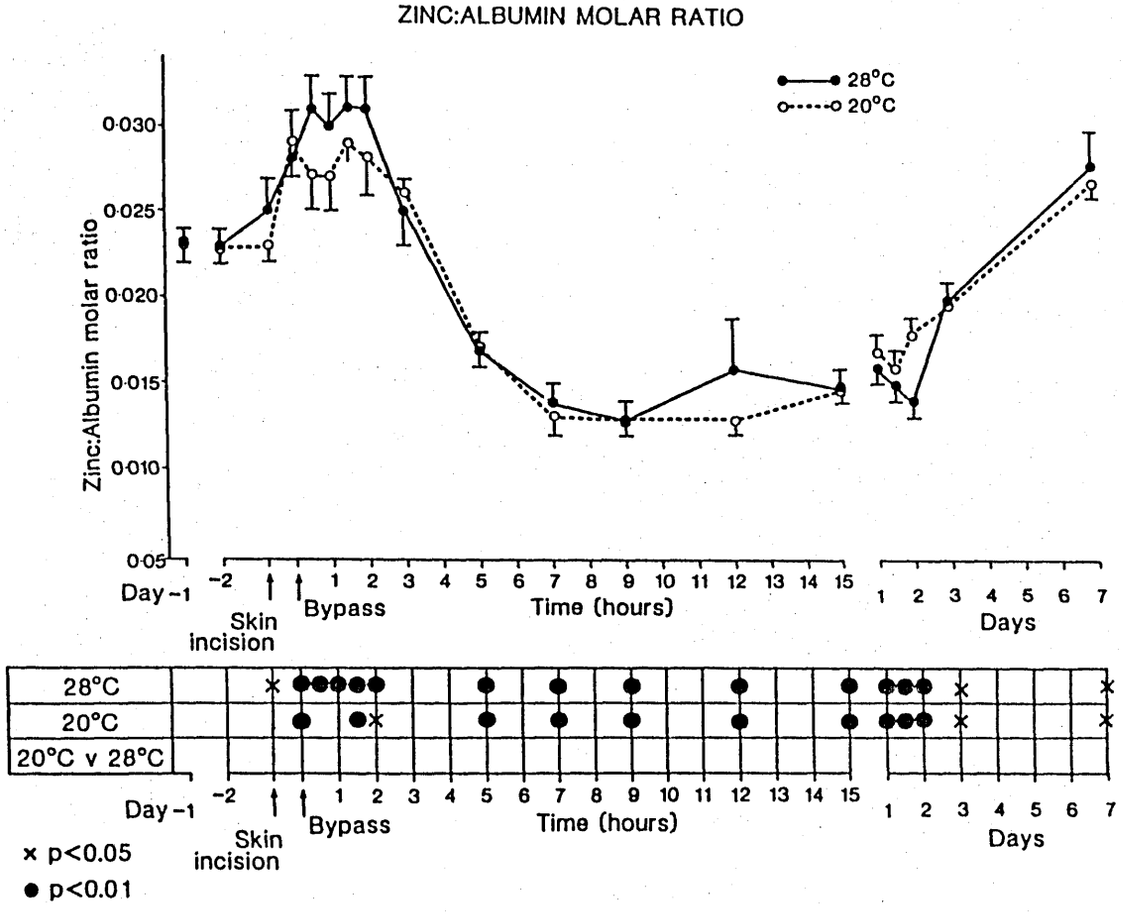


FIG 8.4 Mean (SE) changes in the zinc:albumin molar ratio in the two temperature groups.

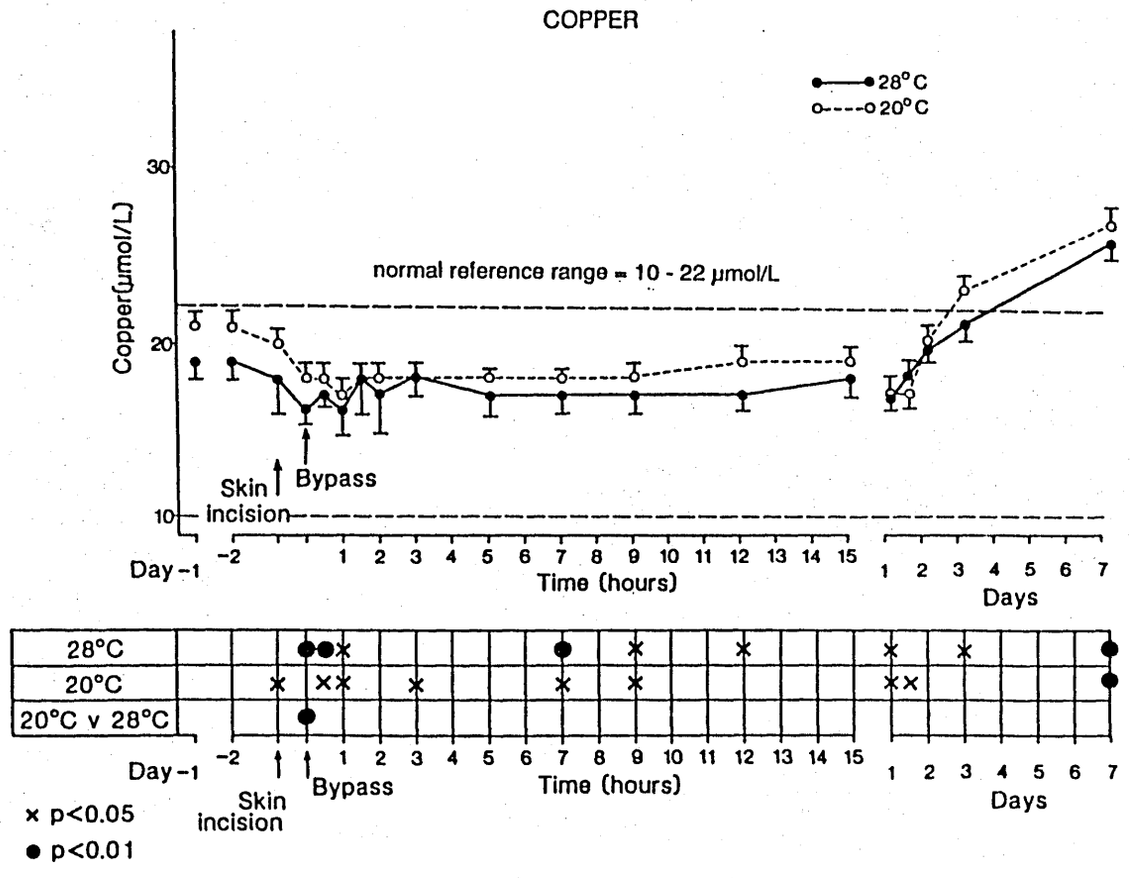


FIG 8.5 Mean (SE) changes in serum copper in the two temperature groups.

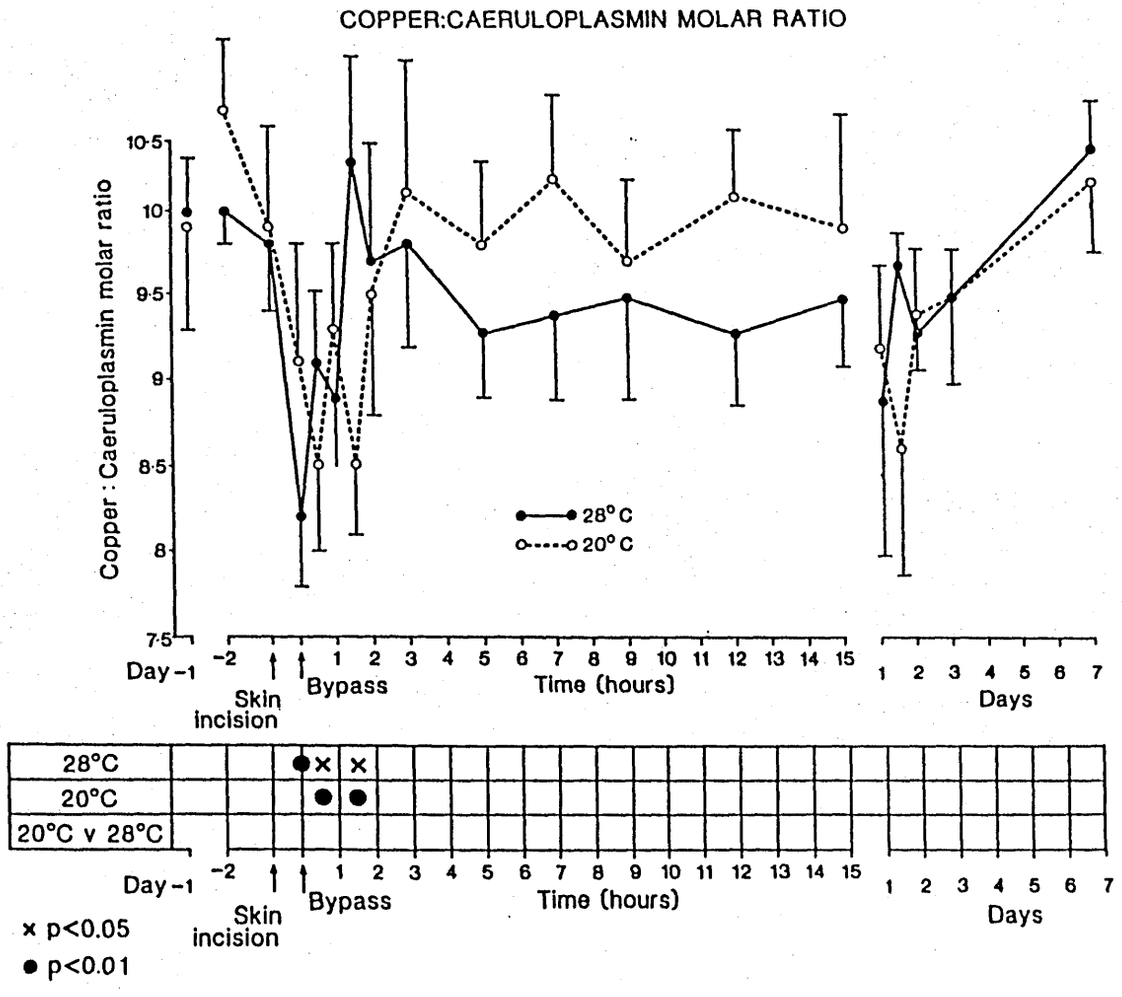


FIG 8.6 Mean (SE) changes in the copper:caeruloplasmin molar ratio in the two temperature groups.

CHAPTER 9

THE ENDOCRINE RESPONSE. PART 1: PLASMA HORMONES

CHAPTER 9

THE ENDOCRINE RESPONSE. PART 1: PLASMA HORMONES

"Somehow there is a constant and shifting balance of forces in which hormones, ferments, enzymes, memories, ideas, emotions and moods all play a part..."

Abraham Myerson (1881-1948). Speaking of Man. Foreword.

INTRODUCTION

There is an extensive literature pertaining to the endocrine response to surgery, its correlation with the magnitude of tissue injury and its manipulation by various anaesthetic agents. During cardiac surgery the endocrine response to tissue injury is additionally modified by CPB, haemodilution and hypothermia. Previous studies of the endocrine response during CPB have produced conflicting results regarding both the direction and the magnitude of changes in the plasma concentrations of hormones. Furthermore there has been no study of the effects of different levels of intraoperative hypothermia on the endocrine response to CPB and few studies have examined the plasma endocrine response in the week following cardiac surgery.

In the widest sense, the endocrine response involves a large number of hormones but this chapter is concerned with the effects of CPB and two levels of intraoperative hypothermia on the plasma concentration of the counter-regulatory hormones (cortisol, adrenaline, noradrenaline) and thyroid hormones (thyroxine, triiodothyronine, thyroid stimulating hormone). The role of the counter-regulatory hormones adrenaline, noradrenaline and cortisol, in mediating the metabolic response to trauma is discussed in Chapter 12.

This is the first prospective study to report the effect of two levels of perfusion cooling on the endocrine response to CPB.

PATIENTS and METHODS

The operative techniques are described in detail in Chapter 3 and the patients in Chapter 7.

The anaesthetic technique is of particular relevance to the endocrine response and a standard regimen was followed as described in Chapter 3. Premedication consisted of 20 mg of temazepam on the night before surgery. In the anaesthetic room, sleep was induced with midazolam and fentanyl and intubation was performed after the administration of a muscle-relaxing agent (atrocurium or pancuronium). During surgery anaesthesia was maintained with a combination of morphine, fentanyl and midazolam and boluses of atrocurium or pancuronium. The total anaesthetic dose of morphine was less than 2 mg/Kg body weight and fentanyl less than 20 ug/Kg body weight.

On return to the Cardiac Intensive Care Unit boluses of morphine (1-2 mg), midazolam (1-2 mg) and vercuronium (2 mg) were used to maintain analgesia and permit mechanical ventilation until the patient was fully rewarmed, haemodynamically stable and ready for extubation.

Statistical analysis was based on a paired t-test to compare serial differences within the same group and an unpaired t-test to compare differences between groups. Differences were considered statistically significant when the probability of their arising by random sampling error was less than 1 in 20 ($p<0.05$) and highly significant if this probability was less than 1 in 100 ($p<0.01$).

ANALYSIS OF HORMONES

The timing of blood samples is described in Chapter 6. After

measurement of the PCV the samples were centrifuged to separate plasma which was stored at -50°C for the following analyses.

CATECHOLAMINES. The assay for catecholamines was performed in the University Department of Materia Medica at Stobhill Hospital and is based on a modification of the technique described by Passon and Peuller (301). This method uses the isolated enzyme, catechol-o-methyl transferase, to transfer a radioactive methyl group from S-adenosyl-L- methionine (SAM) to an endogenous catecholamine acceptor model forming a radioactive O-methyl catecholamine derivative. In the presence of SAM (^{3}H-methyl) adrenaline is converted to ^{3}H-metadrenaline and noradrenaline to ^{3}H-normetadrenaline. This technique enables detection of circulating adrenaline and noradrenaline at the picomole level of sensitivity.

CORTISOL. Cortisol was measured by an in-house radioimmunoassay (Glasgow Royal Infirmary) using antisera obtained from the Scottish Antibody Production Unit, Carluke ML8 5ES. Separation of the assay was performed using a solid phase technique (302). The detection limit of the assay was 40 nmol/L and the between batch coefficient of variation <10% across the working range of the assay.

THYROXINE and TRIIODOTHYRONINE. Total Thyroxine (T4) and Triiodothyronine (T3) were measured by an in-house radioimmunoassay (Glasgow Royal Infirmary) using antisera obtained from the Scottish Antibody Production Unit, Carluke ML8 5ES. Separation of the assays was performed using a solid phase technique with a between batch coefficient of variation of <8%, for both hormones, across the working range of the assay.

THYROID STIMULATING HORMONE. Thyroid Stimulating Hormone (TSH) was measured by an in-house immunoradiometric assay (Glasgow Royal Infirmary) using antisera obtained from the Scottish Antibody

Production Unit, Carluke ML8 5ES. The assay used a sucrose wash step with a detection limit of 0.1 mU/L and a between batch coefficient of variation of <15% across the working range of the assay.

RESULTS

The clinical and operative features of the two groups are listed in Table 7.1. The mean plasma hormone concentrations at various time points are given in Table 9.1 and percentage changes in concentration at the same time points in Table 9.2. There was no significant difference in the groups with respect to age or anthropometric indices as judged by height, weight and body surface area. The 20°C group received significantly more grafts than the 28°C group resulting in a non-significant increase in the cross clamp and bypass time in this group.

ADRENALINE (Table 9.2, Fig 9.1). The adrenaline results are based on analysis of samples in 12 patients (six patients per group). There was no increase in plasma adrenaline prior to skin incision. Subsequently there was a significant rise in adrenaline concentration between skin incision and the sample taken one minute after onset of CPB, in both groups. In the 28°C group adrenaline continued to rise throughout CPB, reaching a maximum at the end of CPB (90 minutes). In contrast, in the 20°C group, there was no further rise in adrenaline concentration after the initial increase at the onset of CPB. The increases in adrenaline concentration during CPB were statistically significant only in the 28°C group. In both groups plasma adrenaline returned to baseline values by the third postoperative day.

NORADRENALINE (Table 9.2, Fig 9.2). The noradrenaline results are based on analysis of the same samples, as for adrenaline, in the same 12 patients (six patients per group). In both groups there was

a slight fall in noradrenaline between the two preoperative samples. Noradrenaline subsequently increased between skin incision and the onset of CPB and this was significant in both groups. In the 28°C group noradrenaline continued to increase for the duration of CPB and peaked at the end of CPB (90 minutes). In the 20°C group there was no further increase in noradrenaline concentration after the onset of CPB so that the mean noradrenaline concentration remained within the normal reference range in contrast to the 28°C group. In both groups noradrenaline decreased after five hours but did not return to baseline levels until 48 hours in the 20°C group and 72 hours in the 28°C group.

CORTISOL (Table 9.2, Fig 9.3). The cortisol response was similar in both groups over the course of the study. There was a decrease in the concentration of plasma cortisol of approximately 40% in both groups between the pre-anaesthetic sample and that obtained at skin incision. The cortisol concentration increased from its nadir at skin incision and continued to rise throughout CPB so that by three hours it was markedly increased ($p<0.05$) over preoperative concentrations. The highest plasma cortisol concentration in both groups was reached at 12 hours (being more than double baseline values). Cortisol concentrations remained elevated over baseline values in both groups until day seven; in the 28°C group this increase was greater than 50% ($p<0.05$) whereas in the 20°C group the increase was less than 20% (NS).

TRIIODOTHYRONINE (Table 9.2, Fig 9.4). There was an 11% fall in T3 by time of skin incision in both groups. At the onset of CPB T3 decreased by 25% in the 28°C group ($p=0.03$)and by 47% in the colder group ($p=0.01$), so that in the latter group T3 concentration fell below the lower limit of the normal range. There was some recovery in

T3 concentration in both groups by five hours followed by a further decrease to a nadir at 24 hours when the concentration of T3 was less than half the preoperative concentration and below the lower limit of the reference range in both groups. T3 concentrations subsequently increased slowly but by day seven were still 17% below preoperative concentrations in both groups.

THYROXINE (Table 9.2, Fig 9.5). T4 fell by 10%-15% in both groups at the onset of CPB and then returned towards preoperative values by 3 hours, before falling again to minimum values at 24 hours. T4 subsequently increased towards preoperative values by day three and continued to increase, so that by day four it was 10-15% higher than preoperative values in both groups. Changes in T4 concentration were similar in magnitude in both groups at all time points.

THYROID STIMULATING HORMONE (Table 9.2, Fig 9.6). There was an increase in TSH in excess of 20% in both groups, between the two preoperative samples. TSH increased further in both groups by skin incision and continued to rise for the duration of CPB, reaching peak levels at 90 minutes, being more than double baseline values ($p<0.01$). TSH concentration subsequently declined to preoperative values at 15 hours with a secondary small increase between 24 and 36 hours. The magnitude of the changes was similar in both groups at all time points.

DISCUSSION

Although numerous studies have addressed various aspects of the endocrine response to CPB the magnitude and the direction of the response remain controversial. Most studies to date have investigated the effects of pulsatile and nonpulsatile perfusion on the endocrine response while the effects of hypothermia have not been addressed.

CATECHOLAMINES. The catecholamines, adrenaline and noradrenaline, are important counter-regulatory hormones and their role in the catabolic response to trauma, accidental and surgical, has been studied extensively in recent years (303). In general the magnitude of increase in adrenaline and noradrenaline correlates with the severity of trauma (304,305). The evidence for elective surgical procedures is conflicting, however, with some groups documenting postoperative increases in both catecholamines (306) and others only in adrenaline (307). The duration of the catecholamine response also appears to be related to the severity of injury persisting for a few hours after elective surgery (307) and 2-3 days after severe injuries (304).

Although increased catecholamine secretion following CPB has been recognised for more than 25 years (308), the development of more sensitive assays has allowed better understanding of the catecholamine response. Plasma noradrenaline is predominantly derived from overflow from post-ganglionic nerve endings and adrenaline from the adrenal medulla (303). Myocardial ischaemia promotes a "cardiocardiac reflex" involving local release of noradrenaline from the myocardium and reflex mediated release of adrenaline from the adrenal medulla (309,310).

It has been reported that morphine in excess of 4 mg/ Kg body weight (188) and fentanyl in doses of 50-100 ug/Kg of body weight (189) can prevent increases in catecholamine secretion prior to, but not during, CPB implying that CPB is a particularly potent stimulus to catecholamine secretion (311). In the current study there was no rise in adrenaline or noradrenaline prior to skin incision although the patients received only moderate doses of morphine (1-2 mg/Kg) and fentanyl (<20 ug/Kg).

Most investigators have reported large rises in adrenaline and noradrenaline during CPB. In a particularly detailed study of 28 patients undergoing open heart surgery at 28°C, Reves and colleagues documented a ninefold rise in adrenaline and a twofold rise in noradrenaline (147). This group made two previously unreported observations: (i) the peak rise in adrenaline and noradrenaline occurred during the "cross-clamp" period (ie period of exclusion of the heart and lungs from the circulation) and, (ii) there was little difference in catecholamine concentrations in simultaneous estimates of arterial and venous catecholamine concentration.

In the current study there was a large increase in adrenaline and noradrenaline in both groups immediately after the onset of CPB. Previous workers have attributed similar findings to a compensatory increase in catecholamine secretion due to the fall in blood pressure consequent on haemodilution at the commencement of CPB (312). In the present study the increases in adrenaline and noradrenaline secretion during CPB in the 28°C group were of a similar magnitude to that reported by Reves' group and followed an identical time course reaching a peak at the end of CPB (147). These workers postulated that exclusion of the lungs from the circulation during CPB removes an important catecholamine metabolic pathway (147), while myocardial ischaemia during aortic cross-clamping stimulates reflex release of adrenaline and noradrenaline (309,310). The heart and lungs were excluded from the circulation for a longer period in the 20°C group [59 (SE 7) minutes in the 20°C group and 48 (SE 5) minutes in the 28°C group] and reduced plasma catecholamine concentrations in the 20°C group therefore imply diminished catecholamine production during the period of hypothermia. This is consistent with experimental work in dogs which has demonstrated that profound hypothermia can reduce

adrenal gland production of adrenaline, noradrenaline and cortisol (49,50) but no such similar work exists in humans.

The magnitude of the increases in plasma catecholamine concentration during CPB is similar to that after myocardial infarction, sepsis or severe injuries (313). High concentrations of plasma catecholamines generated during CPB have been positively incriminated as an important factor in the pathogenesis of postoperative hypertension (314). The consequences of perfusing the post-ischaemic heart with such high catecholamine concentrations remain to be elucidated, although Reves and colleagues reported that re-perfusion of the well protected heart with high concentrations of plasma catecholamines does not appear to produce biochemical evidence of myocardial damage (315).

CORTISOL. Plasma concentrations of cortisol have been shown by numerous investigators to be significantly elevated during and following surgery without CPB (316,317). The peak plasma cortisol concentration is usually reached about six hours after injury with a return to normal concentrations by 12 hours (318). Although an increase in the plasma concentration of cortisol is an invariable facet of the metabolic response to injury, the magnitude of the increase does not bear a simple relationship to the severity of injury. A maximum cortisol response is produced by moderate to severe trauma; with further severity of trauma there is a significant negative relationship with the plasma concentration of cortisol (319)

In contrast to the concordance of the effects of general surgical procedures on plasma cortisol concentrations, the effect of CPB has produced conflicting results (138,141,142,320,321). It was initially reported that CPB produced a fall in the concentration of plasma cortisol (138,141,320) either as a result of anaesthesia or

haemodilution, and this "adrenal hypofunction" led to widespread and empirical steroid administration during CPB. Despite evidence that CPB produces an elevation in the free (ie physiologically active) cortisol concentration (138,141), the prophylactic use of steroids has persisted in some centres (322).

There was a decrease in the plasma cortisol concentration of approximately 40% between the blood sample taken two hours prior to skin incision and that at skin incision in both groups of patients. This decrease is too large to be explained by haemodilution alone (see Chapter 6) and is presumably due to the anaesthetic agents used. The doses of morphine and fentanyl used in these patients, however, were smaller than those conventionally regarded as being able to attenuate the stress response to surgery (188,189,311). Large doses of morphine (188), alfentanil(147) and halothane (311) can suppress adrenal gland activity prior to CPB, giving rise to the concept of "stress-free" anaesthesia. This term is misleading, however, as cortisol concentration increases rapidly on surgical stimulation and continues to rise throughout CPB, as reported by Stanley and colleagues (311), and as evidenced in the present study.

Taylor and co-workers reported that pulsatile CPB produced a significant rise in plasma cortisol concentration compared to nonpulsatile CPB (142), implying physiological superiority of the former. These findings were disputed by Kono and colleagues who failed to demonstrate any intraoperative increase in plasma cortisol concentration in patients undergoing pulsatile or nonpulsatile CPB (321). In the latter study, however, no correction for haemodilution was made and the patients received large anaesthetic doses of halothane which is known to suppress adrenal gland function (323). The results of the current study are in accordance with Taylor's

findings that pulsatile CPB preserves the adrenal response to injury, as increases in the plasma concentration of cortisol occurred in both groups during CPB. Indeed considering the inadequacies of correction for haemodilution (Chapter 6), based on changes in PCV, the true adrenal response may be underestimated.

The effect of temperature on plasma cortisol concentrations has aroused considerable scientific interest since the early 1950's when the clinical potential of topical hypothermia was recognised (56,154). Swann and co-workers (324) examined the effects of topical hypothermia in patients undergoing cardiac surgery prior to the availability of CPB. They found that topical cooling was an initial stimulant of adrenal secretion which prevented further rises in plasma cortisol during the surgical procedure but did not prevent a rebound increase in cortisol secretion during rewarming. Ganong and colleagues demonstrated, by cannulation of the adrenal vein in dogs, that hypothermia resulted in a reduction in plasma cortisol concentration by reducing cortisol secretion from the gland (325). In a further elegant study in dogs, the same workers demonstrated hepatic metabolism of cortisol by temporarily excluding the liver from the circulation and showed that liver function was depressed by hypothermia (326).

There was no difference in plasma cortisol concentration during CPB between the 28°C and 20°C groups. These findings are similar to those of Kucera (327) who found no difference in plasma cortisol concentrations in children submitted to hypothermic circulatory arrest using operative temperatures between 20°C and 27-32°C. In the present study, and that reported by Kucera (327), it is feasible that cortisol production was reduced in the colder temperature group but that a concomitant depression of liver function resulted in the maintenance

of plasma cortisol concentrations.

Whether moderate or profound hypothermia attenuates the endocrine response which would be expected during normothermic CPB is a matter for speculation. In a historically controlled study Knutschen found significantly lower levels of plasma cortisol in patients operated on at 28°C compared to those operated on at normothermia during CPB but a significant reversal of this situation during rewarming (144).

A striking observation in the current study was the persistence of significantly elevated cortisol concentrations of greater than 50% in the 28°C group until day 7, in contrast to the non-significant increases of less than 20% in the 20°C group over the same time period. There are, however, no comparable studies examining the plasma steroid response after CPB until the seventh postoperative day. This observation is discussed further in Chapter 10.

THYROID HORMONES. The thyroid hormones are the major regulators of cellular metabolism and of these T3 is the most important (328,329). The classical response of thyroid hormones to a wide variety of stresses including infection, malignancy and surgical operations is a fall in T3 (accompanied by normal or elevated reverse T3), a normal or decreased T4 and normal or decreased TSH levels (330). These changes have been described as the "sick euthyroid syndrome" and attributed to a reduction in the conversion of T4 to T3 with a decrease in reverse T3 deiodination in peripheral tissues (330). A similar pattern of thyroid response occurs after general surgical procedures (331-333) and, similarly, a "low T3 T4 syndrome" has been reported after CPB (143,334-336). Free T4 is known to increase for the duration of CPB (143) mediated by the effects of heparin, either on T4 affinity for its binding hormone (337) or by

promoting its release from the large liver stores of T4 (338). During CPB there is a transient increase in free T3 which subsequently, and similarly to T3, becomes markedly reduced (143).

The present study demonstrated a decrease in T4 and T3 concentration during CPB. The magnitude of the decreases in T3 was greater in both groups than T4 (Table 8.2) being 56% for T3 and 22-27% for T4 on the first postoperative day. Throughout the postoperative period, and in comparison to preoperative concentrations, the decrease in T3 was consistently more than double the concomitant decrease in T4. These large and highly significant postoperative falls in T3 were similar in both temperature groups and comparable to those reported by Paschen and colleagues (334).

T3 is the most important hormone regulating cellular metabolism (328,329) and low levels of T3 after operation could be explained from a teleological viewpoint as an attempt to reduce energy expenditure after trauma (339). In vitro T3 increases cellular uptake of carbohydrates and amino acids (340) and reduces muscle protein catabolism in starved rats (341). Furthermore, thyroid hormones are partially responsible for regulation of myocardial contractility through an adenyl cyclase system (342) which regulates intracellular calcium (the other adenyl cyclase system is catecholamine-dependent). It is of interest that administration of T3 in dopamine-dependent shock has been reported to have a beneficial effect on arterial blood pressure (343).

There are conflicting reports regarding the TSH response to CPB. The current study demonstrated significant increases in TSH in both groups during CPB. Only one other group of workers has reported an increase in TSH during CPB (334), while many groups have reported a decrease (143,335,336). Low TSH concentrations during CPB have been

ascribed to heparin administration (344), release of large doses of glucocorticoid (345) and dopamine administration (346). The reasons for the differing results may reflect different techniques of CPB but are hard to elucidate as descriptive methods are often inadequate, eg whether CPB was pulsatile or nonpulsatile (334) and whether any correction was made for haemodilution (336). Taylor and colleagues (347) demonstrated that the pituitary gland responds to thyrotrophin releasing hormone stimulation during pulsatile CPB but not during nonpulsatile perfusion; unfortunately their study did not compare differences in unstimulated TSH secretion between pulsatile and nonpulsatile bypass.

It is well recognised that hypothermia stimulates thyroid activity in certain mammals (348) but the situation in man has produced conflicting results. Moderate reductions in core temperature, by a few degrees, in humans has been reported alternatively to stimulate (349) and to have no effect on thyroid function (350). In contrast, profound cooling (to 20^{o}C) during circulatory arrest stimulated marked increases in TSH concentrations in four of seven patients studied (351). In the current study the magnitude of the decreases in T3 was similar at all time points for the two temperature groups as were the decreases in T4. It remains unclear whether the large increases in TSH during CPB were due to the effects of perfusion cooling or simply due to pulsatile CPB itself, but a more profound level of intraoperative hypothermia did not appear to influence the TSH response.

SUMMARY and CONCLUSIONS

The plasma endocrine response to two levels of intraoperative hypothermia was studied in 20 male patients undergoing elective

coronary artery surgery. This is the first study to report the effects of two levels of intraoperative hypothermia on the endocrine response to cardiopulmonary bypass.

Pre-CPB increases in catecholamines and cortisol were less than expected and presumed to be due to the anaeshetic agents fentanyl and morphine although these were used in doses not conventionally thought to reduce the metabolic response to surgery.

The catecholamine response was similar to that previously described in the 28°C group. In both groups there was a significant increase in plasma adrenaline and noradrenaline immediately after the onset of CPB attributable to a fall in blood pressure consequent on haemodilution at this time. In the 28°C group there was a progressive rise in adrenaline and noradrenaline throughout CPB, as previously described, but these latter increases were not observed in the 20°C group.

There was a decrease in cortisol prior to CPB, presumably due to the effects of anaesthetic agents, followed by a normal/exaggerated response to surgical stress and the response was similar in both groups. It was postulated that although a more profound level of intraoperative hypothermia may reduce cortisol secretion, plasma concentrations are maintained through a concomitant attenuation of hepatic metabolism in the colder temperature group. Cortisol concentration remained significantly elevated only in the 28°C group at day seven.

There was a marked fall in T3 and T4 in both groups and decreases in the former were consistently double those of the latter. The TSH response was increased during CPB in both groups. There was no significant difference in thyroid function between the temperature groups.

	-2Hours	Skin	Bypass	15 hrs	day 1	day 2	day3	day 7
28°C	44(1)	39(1)	26(1)	39(1)	39(2)	36(1)	32(1)	35(1)
PCV (%)								
20°C	41(1)	38(1)	23(1)	36(1)	37(1)	35(1)	34(1)	35(2)
28°C	0.6(.1)	0.6(.1)	9.7(5.7)	2.1(.5)	1.5(.5)	1.1(.1)	1.2(.1)	0.9(.2)
ADRENALINE (nmol/L)								
20°C	0.5(.1)	0.5(.2)	7.6(3.2)	1.5(.4)	1.1(.1)	1.0(.2)	0.8(.2)	0.8(.2)
28°C	2.4(.4)	2.6(.4)	7.7(1.5)	6.6(1.5)	5.4(1.2)	6.0(1.1)	4.8(2.1)	4.4(1.6
NORADRENALINE (nmol/L)								
20°C	2.3(.4)	2.3(.5)	5.0(1.2)	5.0(1.4)	4.7(1.2)	3.1(.7)	4.4(1.7)	4.3(1.6
28°C	436(62)	239(33)	388(81)	1017(155)	651(68)	692(75)	663(111)	708(80
CORTISOL (nmol/L)								
20°C	503(39)	303(20)	446(55)	863(98)	754(101)	594(69)	531(32)	552(36)
28°C	1.8(.1)	1.6(.2)	1.2(.1)	1.1(.1)	0.81(.1)	1.1(.1)	1.1(.1)	1.5(.1
TRIIODOTHYRONINE (nmol/L)								
20°C	1.8(.2)	1.6(.2)	.85(.1)	1.0(.1)	0.8(.1)	1.2(.1)	1.4(.2)	1.5(.1
28°C	101(6)	97(12)	82(4)	88(6)	74(3)	88(4)	87(5)	109(8)
THYROXINE (nmol/L)								
20°C	104(8)	102(10)	91(10)	88(6)	82(10)	92(10)	100(7)	120(7)
28°C	2.3(.4)	2.6(.4)	3.2(.6)	1.4(.3)	1.5(.2)	1.7(.2)	1.9(.2)	1.6(.3)
THYROID STIMULATING HORMONE (mU/L)								
20°C	1.6(.3)	1.9(.3)	2.3(.3)	1.5(.3)	1.2(.3)	1.4(.2)	1.8(.2)	1.3(.2)

TABLE 9.1 MEAN (SE) CONCENTRATIONS OF PLASMA HORMONES AT VARIOUS TIME POINTS.

		Skin	Bypass	15 hrs.	day 1	day 2	day3	day 7
	28°C	0%	+1650%	+250%	+150%	+100%	+150%	0%
		(NS)	(0.05)	(NS)	(NS)	(NS)	(NS)	(NS)
ADRENALINE								
	20°C	0%	+1520%	+200%	+180%	+100%	+160%	0%
		(NS)	(0.05)	(NS)	(NS)	(NS)	(NS)	(NS)
	28°C	+8%	+220%	+175%	+125%	+150%	+100%	+80%
		(NS)	(0.01)	(0.05)	(0.05)	(0.05)	(NS)	(NS)
NORADRENALINE								
	20°C	0%	+117%	+117%	+100%	+34%	+70%	+70%
		(NS)	(0.05)	(NS)	(NS)	(NS)	(NS)	(NS)
	28°C	-45%	+62%	+133%	+49%	+59%	+52%	+62%
		(0.01)	(0.01)	(0.01)	(0.01)	(0.01)	(0.09)	(0.01)
CORTISOL								
	20°C	-40%	+47%	+49%	+18%	+18%	+5%	+9%
		(0.01)	(0.01)	(0.01)	(NS)	(NS)	(NS)	(NS)
	28°C	-11%	-25%	-39%	-56%	-36%	-39%	-17%
		(NS)	(0.05)	(0.01)	(0.01)	(0.01)	(0.01)	(NS)
T3								
	20°C	-11%	-47%	-44%	-56%	-33%	-25%	-17%
		(NS)	(0.01)	(0.01)	(0.01)	(0.01)	(0.01)	(NS)
	28°C	-4%	-15%	-13%	-27%	-13%	-14%	+8%
		(NS)	(NS)	(NS)	(0.01)	(NS)	(NS)	(NS)
T4								
	20°C	-2%	-11%	-15%	-22%	-12%	-4%	+15%
		(NS)	(NS)	(NS)	(NS)	(NS)	(NS)	(NS)
	28°C	+13%	+22%	-39%	-35%	-26%	-18%	-28%
		(NS)	(NS)	(NS)	(NS)	(NS)	(NS)	(NS)
TSH								
	20°C	+19%	+18%	-23%	-9%	-13%	+9%	-19%
		(NS)	(NS)	(NS)	(NS)	(NS)	(NS)	(NS)

TABLE 9.2 PERCENTAGE CHANGES IN CONCENTRATIONS OF PLASMA HORMONES AT VARIOUS TIME POINTS AGAINST BASELINE AT -2 HOURS (p value in parenthesis).

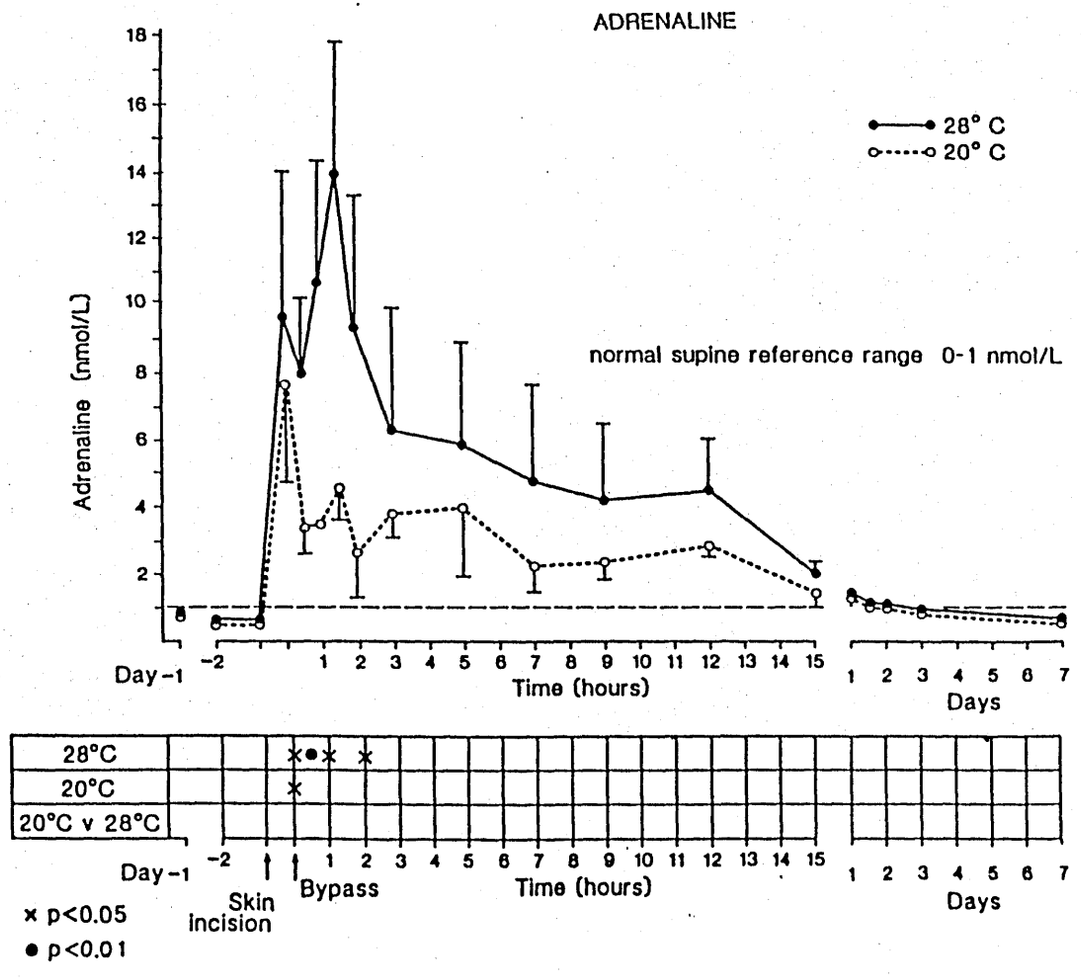


FIG 9.1 Mean (SE) changes in serum adrenaline in the two temperature groups.

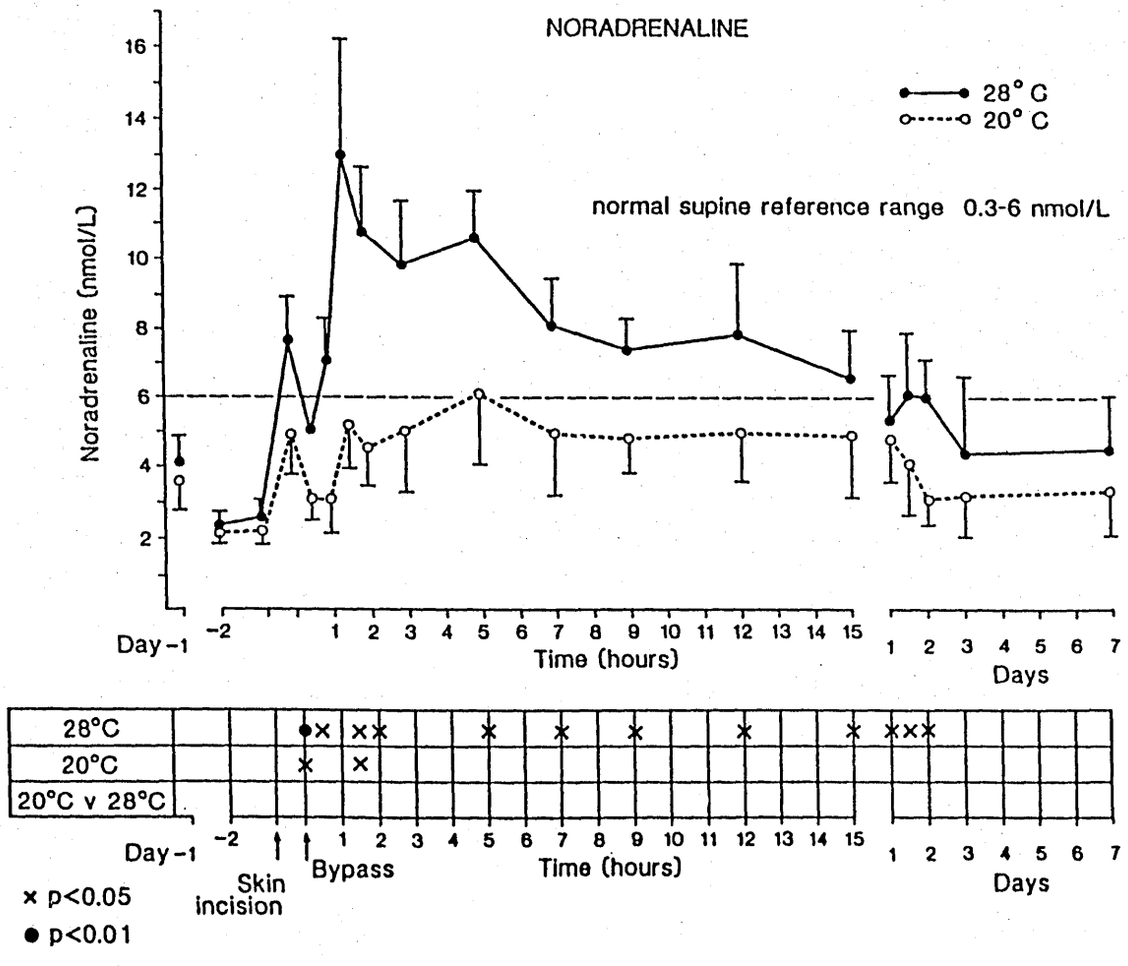


FIG 9.2 Mean (SE) changes in serum noradrenaline in the two temperature groups

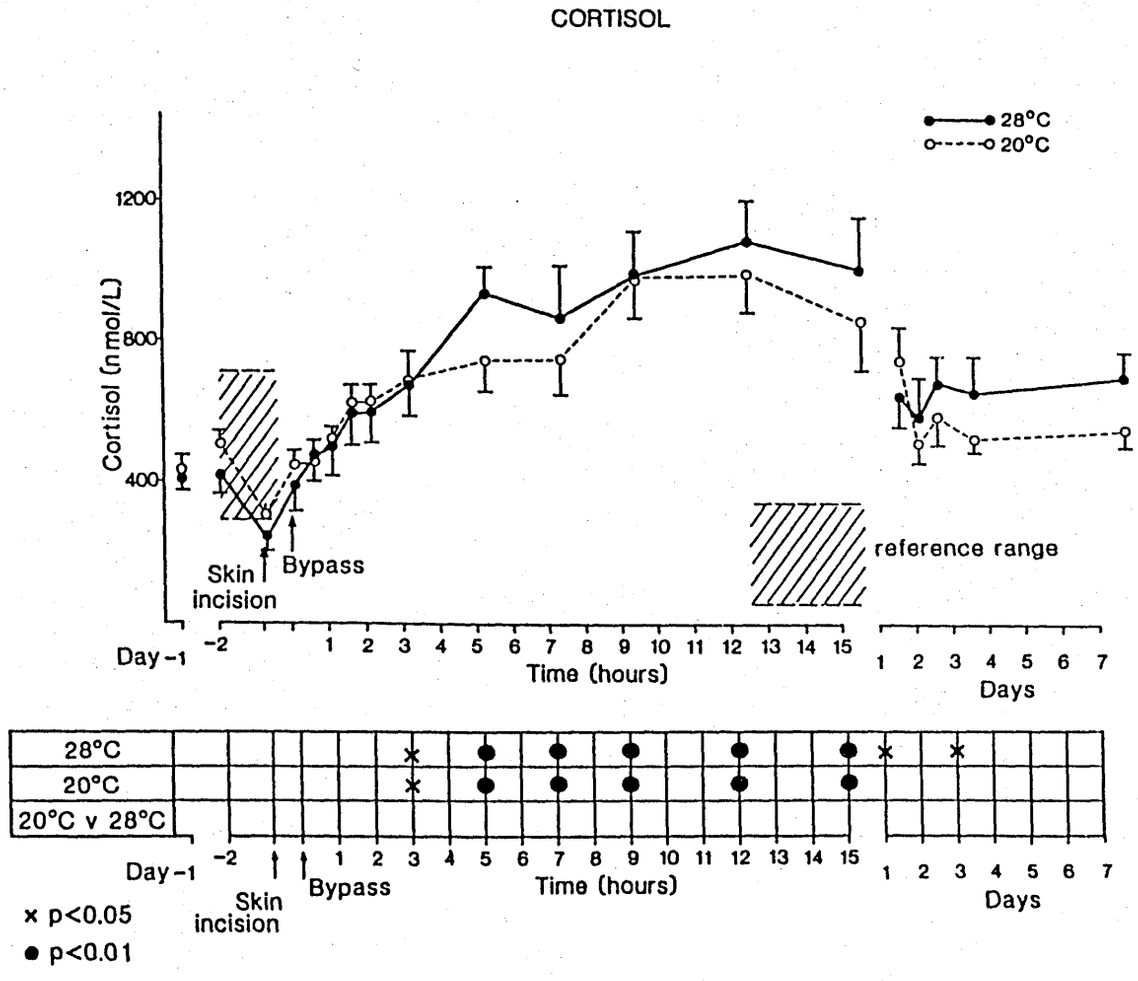


FIG 9.3 Mean (SE) changes in serum cortisol in the two temperature groups.

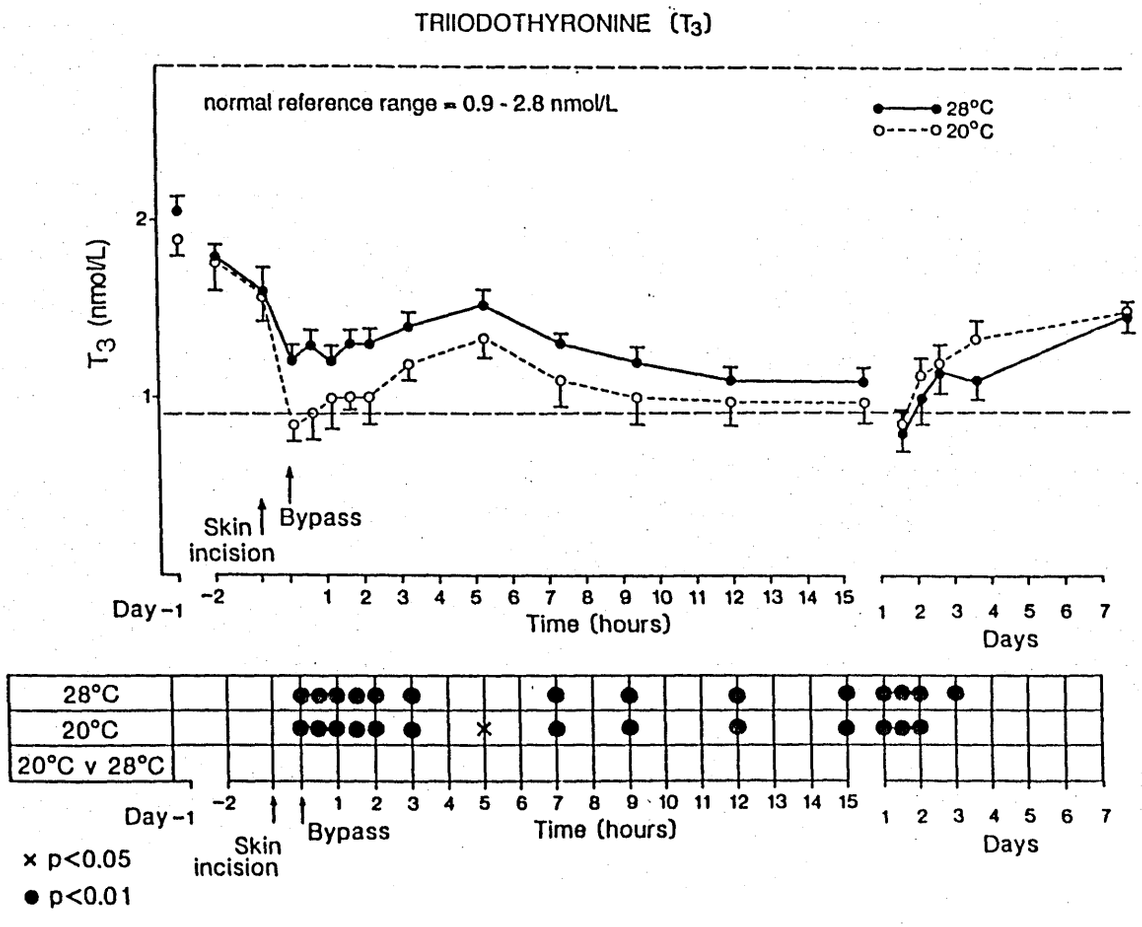


FIG 9.4 Mean (SE) changes in serum T3 in the two temperature groups.

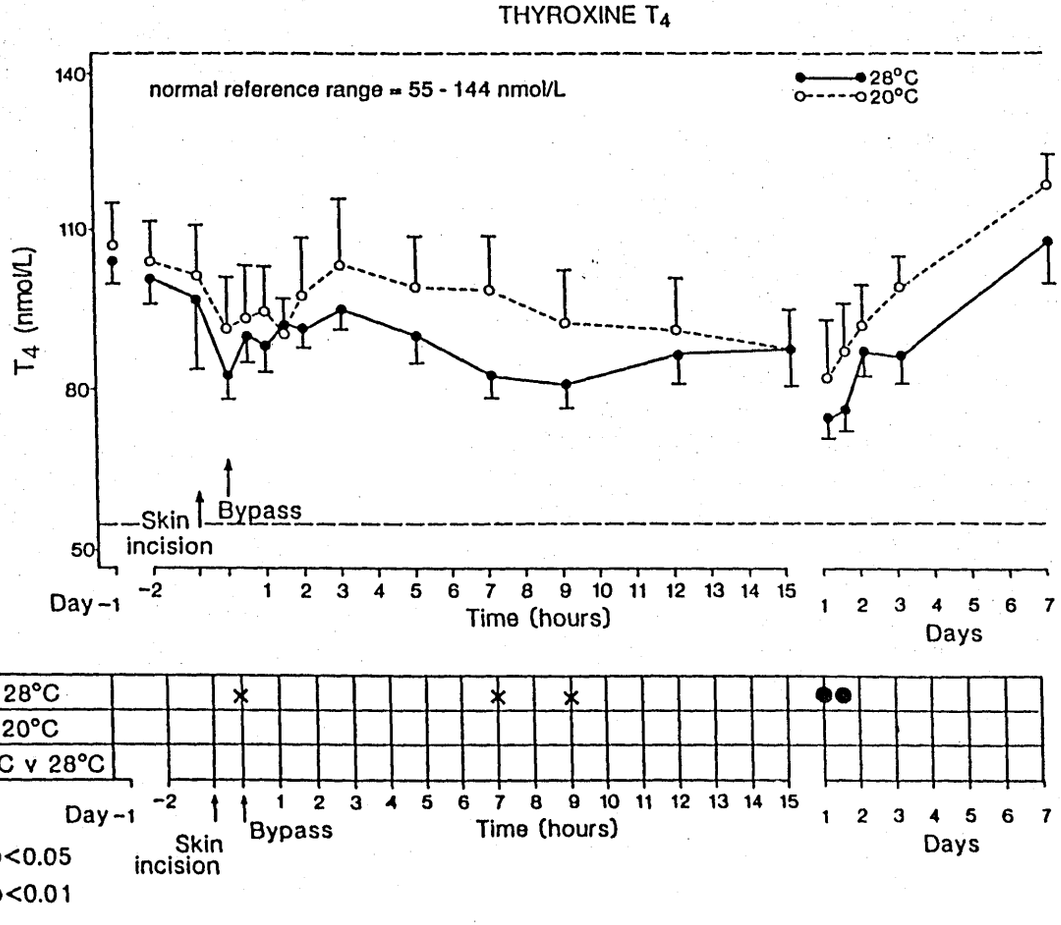


FIG 9.5 Mean (SE) changes in serum T4 in the two temperature groups.

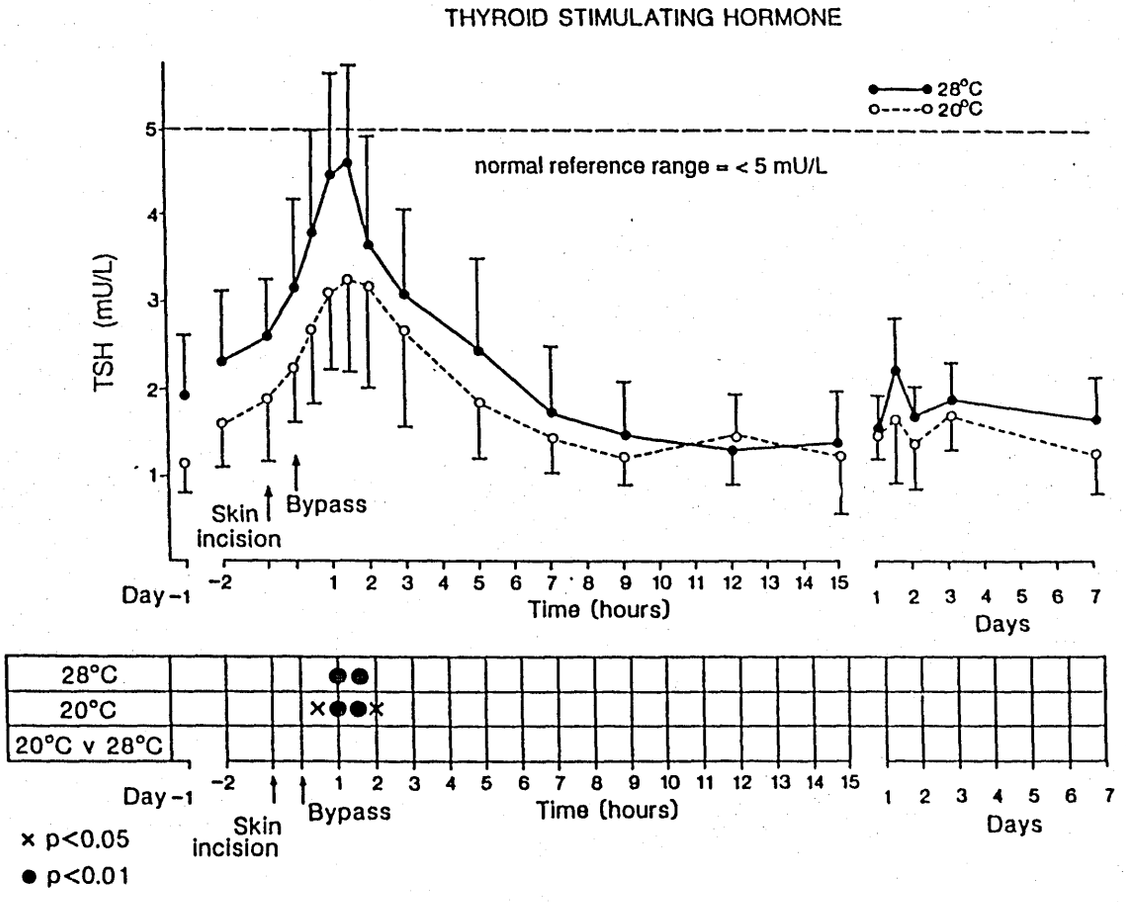


FIG 9.6 Mean (SE) changes in serum TSH in the two temperature groups.

CHAPTER 10

THE ENDOCRINE RESPONSE. PART 2: URINARY EXCRETION OF HORMONES

CHAPTER 10

THE ENDOCRINE RESPONSE. PART 2: URINARY EXCRETION OF HORMONES

"Carry his water to th' wise woman"

William Shakespeare (1564-1616). Twelfth Night. III. iv. 114.

INTRODUCTION

In contrast to numerous studies detailing the plasma endocrine response to CPB, as discussed in Chapter 9, the urinary excretion of hormones and their metabolites after cardiac surgery is less well documented. Measurement of plasma concentrations of hormones is necessary to detect rapid and/or subtle changes in the endocrine response to surgery but provides little information on quantitative changes. Measurement of urinary excretion of hormones, over fixed time periods, provides quantitative information on the magnitude of the endocrine response.

As indicated in Chapter 9, no previous study has prospectively addressed the effects of two levels of intraoperative hypothermia on the endocrine response to CPB. The urinary excretion of adrenaline, noradrenaline, dopamine and cortisol was determined in 20 male patients preoperatively and on the first, second, third and seventh days following coronary artery surgery. The role of the counter-regulatory hormones adrenaline, noradrenaline and cortisol, in mediating the metabolic response to trauma is discussed in Chapter 12.

PATIENTS and METHODS

The 20 male patients have been described in Chapter 7 and the operative details in Chapter 3.

The twenty-four hour urine collections were obtained 24-48 hours prior to surgery and on the first, second, third and seventh postoperative days.

Adrenaline, noradrenaline and dopamine were measured by reversed phase high performance liquid chromatography with electrochemical detection in a modification of the method described by Davidson and Fitzpatrick (352). The measurements were performed by Mr William Borland in the Department of Biochemistry, Royal Infirmary, Glasgow. The between batch precision was: adrenaline 6.9%, noradrenaline 7.0% and dopamine 6.1% and the mean recoveries of catecholamines added to urine were: adrenaline 99.3%, noradrenaline 98.8% and dopamine 102.6%.

Urinary free cortisol was measured by an in-house radioimmunoassay (Glasgow Royal Infirmary) using antisera obtained from the Scottish Antibody Production Unit, Carluke ML8 5ES. Separation of the assay is performed using a solid phase technique (302). The detection limit of the assay was 40 nmol/L and the between batch coefficient of variation <10% across the working range of the assay.

Statistical analysis was based on a paired t-test to compare serial differences within the same group and an unpaired t-test to compare differences between groups. Differences were considered statistically significant when the probability of their arising by random sampling error was less than 1 in 20 ($p<0.05$) and highly significant if this probability was less than 1 in 100 ($p<0.01$).

RESULTS

Patient details and operative procedure are described in Table 7.1. There was no significant difference between the two temperature

groups with respect to age, bypass or cross clamp times but a highly significant difference in the lowest intraoperative blood temperature.

URINARY ADRENALINE (Table 10.1,Fig 10.1). Urinary adrenaline excretion increased from preoperative levels to maximal excretion, in both groups, on the first postoperative day with a 514% increase in adrenaline excretion in the 28°C group ($p<0.001$) and a 269% increase in the 20°C group ($p<0.01$). By the second postoperative day adrenaline excretion was still more than double preoperative values in the 28°C group ($p<0.01$) but had almost returned to the baseline value in the colder temperature group. In the 28°C group adrenaline excretion returned to the baseline level by day 3.

URINARY NORADRENALINE (Table 10.1, Fig 10.2). Urinary noradrenaline excretion increased to maximum levels on the first and second postoperative days in both groups. The increase was almost treble in the 28°C group ($p<0.01$) and was by 165% in the 20°C group ($p=0.01$) on the first postoperative day; the same magnitude of increase persisted in each group on the second postoperative day. Noradrenaline excretion remained elevated on the third postoperative day, by 77% in the 28°C group ($p=0.01$) and 32% in the 20°C group ($p=0.57$). In both groups noradrenaline excretion returned to preoperative values by the seventh postoperative day.

URINARY DOPAMINE (Table 10.1, Fig 10.3). Urinary dopamine excretion is based on seven patients in the 28°C group and nine patients in the 20°C group, as the remaining patients received inotropic dopamine in the early postoperative period producing very high dopamine concentrations in the urinary collections. There was a 200% increase in urinary dopamine excretion over the first and second postoperative days in the 28°C group ($p=0.03$, $p=0.02$ respectively) compared to a 50% increase in the 20°C group over the same period

($p=0.03$, $p=0.01$ respectively). The statistical significance of the increases in the 28°C group was not greater than that of the 20°C group, presumably because of the smaller group size. In both groups dopamine excretion was still elevated, but not significantly so, on the third postoperative day. A 20% increase in dopamine excretion persisted in the 28°C group, but not the 20°C group, until the 7th postoperative day.

URINARY CORTISOL (Table 10.1, Fig 10.4). In both groups there was a highly significant increase in cortisol excretion on the first postoperative day representing a 25-fold increase in the 28°C group and a 19-fold increase in the 20°C group. On the second postoperative day there was an eleven-fold increase in cortisol excretion in both groups. By day three cortisol excretion was still five times greater than the preoperative value in the 28°C group ($p<0.01$) and three times greater in the 20°C group ($p<0.05$). By day seven cortisol excretion remained elevated in both groups but only significantly so in the 28°C group, being three times greater than the preoperative value ($p<0.05$).

DISCUSSION

Since 1970 there have been more than 200 publications concerning the plasma endocrine response to surgery of which approximately 30 have pertained to CPB. In contrast there have been relatively few reports concerning postoperative changes in the urinary excretion of the same hormones or their metabolites. In particular, there has been no study of the effects of two levels of intraoperative hypothermia on the subsequent urinary excretion of catecholamines and cortisol.

Detection of rapid changes in plasma hormone concentrations is not possible with urinary collections but requires frequent blood sampling. In contrast, changes in the urinary excretion of hormones

and their metabolites following trauma are more prolonged than alterations in the plasma concentrations of the same hormones and provide a more composite picture of the magnitude and duration of the endocrine response (318,353).

Wilmore has incriminated catecholamines as major mediators of the metabolic response to injury, particularly burns, and estimated that noradrenaline may quantitatively account for more than 80% of the adrenergic response (89). Post-traumatic increases in the plasma concentration of catecholamines are proportional to the severity of tissue injury (89,304). Whereas the plasma concentration of catecholamines returns to normal within 12 hours of tissue injury, urinary excretion of adrenaline, noradrenaline and their metabolites remains elevated for a number of days (305,318,354-356). The role of of the counter-regulatory hormones in mediating various components of the post-traumatic metabolic response is discussed further in Chapter 12.

There have been relatively few reports of urinary catecholamine excretion after CPB and none have examined the effects of different levels of intraoperative hypothermia on the catecholamine response. One recent report attempted to quantify the urinary adrenergic response to CPB but interpretation of the results was hampered as 12 of the 13 patients studied required catecholamine infusions (357).

In the current study there were smaller postoperative increments in urinary adrenaline and noradrenaline excretion in the 20°C group. Furthermore, in the 20°C group urinary catecholamine excretion returned to preoperative values earlier than in the 28°C group. These findings are consistent with the hypothesis, proposed in Chapter 9, that a more profound level of intraoperative hypothermia may attenuate the catecholamine response to CPB and that the effect can persist

until the third postoperative day.

Dopamine is the immediate precursor of noradrenaline and is metabolised in a similar fashion to the other catecholamines. Plasma dopamine concentration increases exponentially with the severity of tissue injury and this increase is believed to be secondary to a rise in noradrenaline (358). There has been no previous report regarding the effects of surgery on urinary dopamine excretion. Although it has been suggested that the kidney may itself be responsible for some of the dopamine it excretes (359), recent work has confirmed that urinary dopamine is largely derived from plasma dopamine (360). In the current study four patients (three from the 28°C group and one from the 20°C) were excluded from urinary dopamine analysis after receiving inotropic dopamine infusions (dopamine in a concentration > 5ug Kg^{-1} $minute^{-1}$). Postoperative increases in dopamine excretion were greater in the 28°C group and remained elevated for longer than in the 20°C group.

Cortisol is present in plasma in both a free and bound form. The free, physiologically active, component is water soluble and excreted in urine as urinary free cortisol, as in the current study. Urinary excretion of the plasma bound cortisol components depends on the rate of reduction of cortisol in the liver, subsequent conjugation of the relatively insoluble free compounds, and on renal function.

Following trauma or major surgery, plasma concentrations of cortisol usually peak within 5- 6 hours and return to within a normal range by 10 hours, whereas urinary excretion of cortisol and its metabolites remains elevated for up to a week depending on the severity of tissue injury (45,179,305,318). Furthermore plasma concentrations of cortisol do not bear a simple relationship to the magnitude of trauma; maximum plasma cortisol concentrations are

reached with sub-maximum injuries so that there is no further increase in the plasma cortisol concentration with increasing severity of injury (319). In contrast the duration of elevated urinary cortisol excretion does correlate with the severity of injury (361).

In the current study, the magnitude of increases in urinary cortisol excretion was similar in the two temperature groups until the third postoperative day. This is consistent with the plasma cortisol response, which was similar in the two temperature groups, and the premise that timed urinary collections can accurately reflect the situation in plasma. By day seven, however, urinary cortisol excretion remained significantly elevated only in the 28°C group, similar to the findings of Swan and colleagues who reported elevations in urinary cortisol excretion until the seventh postoperative day in 21 patients undergoing cardiac surgery using topical hypothermia (45).

An attenuation of the endocrine response with a more profound level of intraoperative hypothermia and the persistence of the effect until at least the the third postoperative day for catecholamines, and the seventh postoperative day for cortisol is of major theoretical interest. It suggests that the final mediators of the endocrine response are elaborated at the time of surgical insult as the operative and subsequent clinical course of the two temperature groups was otherwise identical.

Whereas the acute phase response is controlled by cytokines, catecholamine and cortisol secretion are controlled by the neuroendocrine response via the hypothalamo-pituitary-adrenal axis and sympathetic systems. As discussed in Chapter 12, cytokines may also have some synergistic effects on these systems. Further work is required to identify if the various control systems are modified by intraoperative hypothermia.

SUMMARY and CONCLUSIONS

The endocrine response to CPB and the effects of two levels of intraoperative hypothermia (28°C and 20°C) on this response were assessed by measuring the urinary excretion of adrenaline, noradrenaline, dopamine and cortisol in 24 hour urine collections obtained preoperatively and on the first, second, third and seventh postoperative days. This is the first study to report the effect of surgery on urinary dopamine excretion.

Over the last two decades estimation of the endocrine response to surgery by measurement of urinary excretion of hormones has largely been abandoned in favour of measurement of plasma hormone concentrations, although the former provides a more integrated assessment of the endocrine response. The urinary excretion of adrenaline, noradrenaline, dopamine and cortisol was found to reflect the situation in plasma.

The current study demonstrated that a more profound level of intraoperative hypothermia attenuates, but does not abolish, the endocrine response to surgery with smaller increases in the postoperative excretion of adrenaline, noradrenaline and dopamine which also return to baseline excretion rates more quickly. An attenuation of the cortisol response is not seen until the third postoperative day but is still evident on the seventh postoperative day and is consistent with the response observed in plasma.

The results imply that controlling mechanisms of the endocrine response are elaborated at the time of surgery and are susceptible to manipulation at this time.

	28°C					20°C				
	PREOP	DAY 1	DAY 2	DAY 3	DAY 7	PREOP	DAY 1	DAY 2	DAY 3	DAY 7
ADRENALINE	90	465	224	99	106	112	302	139	143	138
(nmol/24 hrs)	(10)	(147)	(55)	(17)	(19)	(36)	(73)	(16)	(29)	(43)
p vlaue		0.003	0.01	0.80	0.70		0.01	0.52	0.55	0.57
NORADRENALINE	410	1153	1192	726	465	489	806	785	645	460
(nmol/24 hrs)	(50)	(183)	(165)	(91)	(94)	(63)	(92)	(64)	(78)	(55)
p value		0.009	0.01	0.01	0.80		0.01	0.01	0.12	0.55
DOPAMINE	515	2304	2173	1305	1382	1119	1700	1830	1320	1151
(nmol/24 hrs)	(195)	(427)	(199)	(232)	(458)	(153)	(189)	(201)	(203)	(138)
p value		0.03	0.02	0.64	0.64		0.03	0.01	0.43	0.88
CORTISOL	195	4877	2138	935	587	243	4519	2662	783	436
(nmol/24 hrs)	(21)	(506)	(426)	(268)	(182)	(59)	(704)	(1079)	(117)	(114)
p value		0.006	0.006	0.008	0.014		0.009	0.009	0.02	0.24

TABLE 10.1 Mean daily (SE) urinary excretion of hormones pre and postoperatively in the two temperature groups (with p value against preoperative excretion).

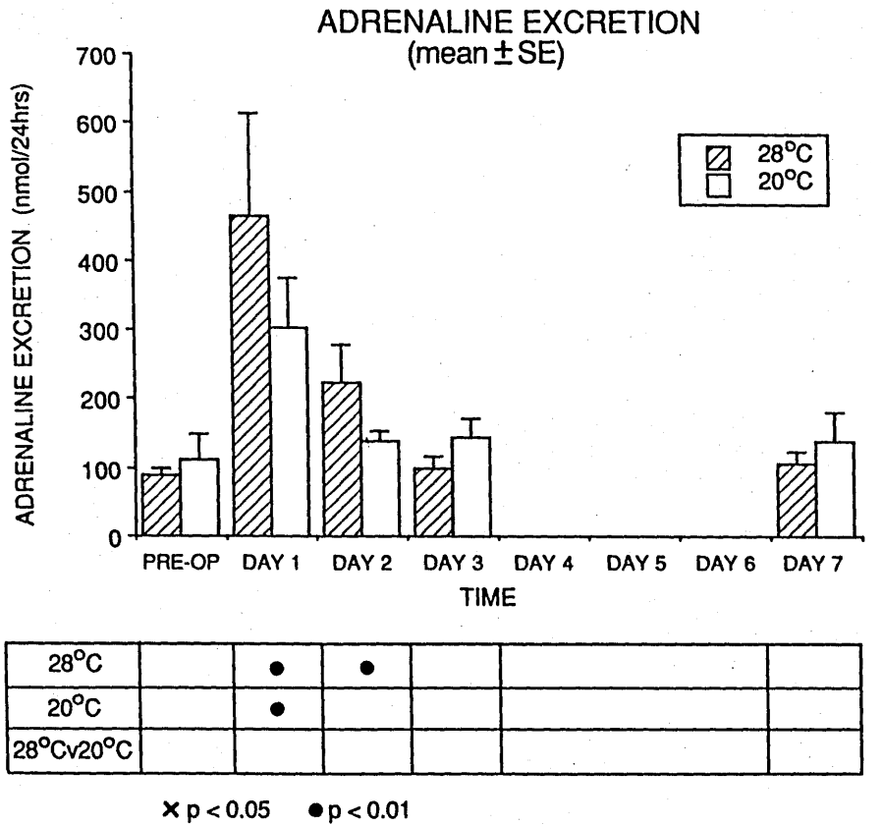


FIG 10.1 Mean (SE) urinary adrenaline excretion in the two temperature groups.

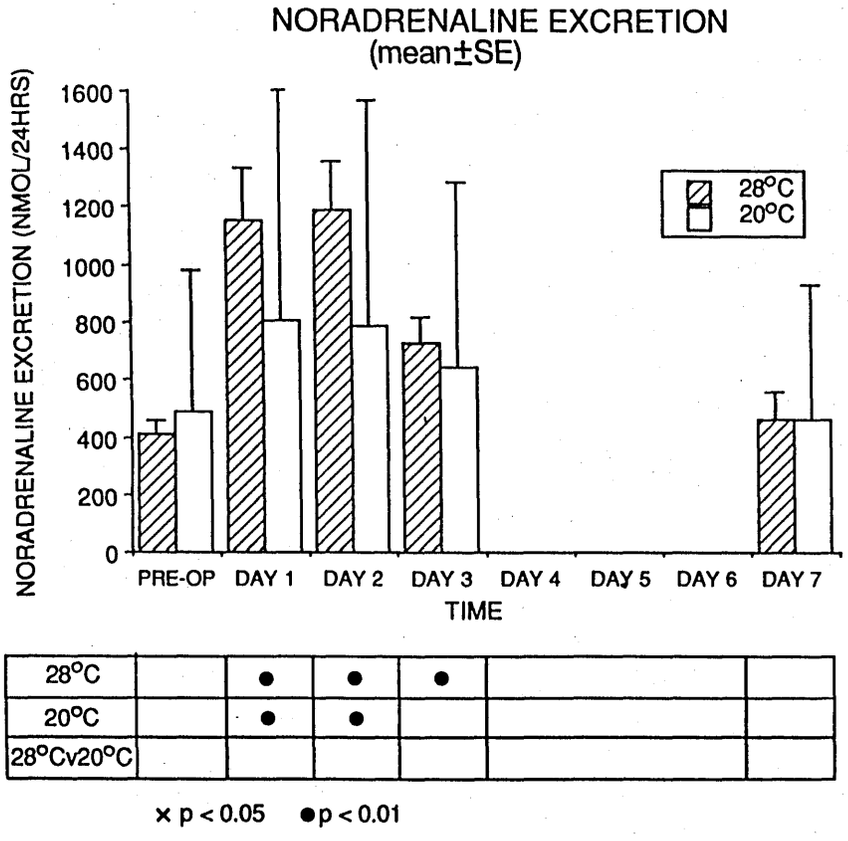


FIG 10.2 Mean (SE) urinary noradrenaline excretion in the two temperature groups.

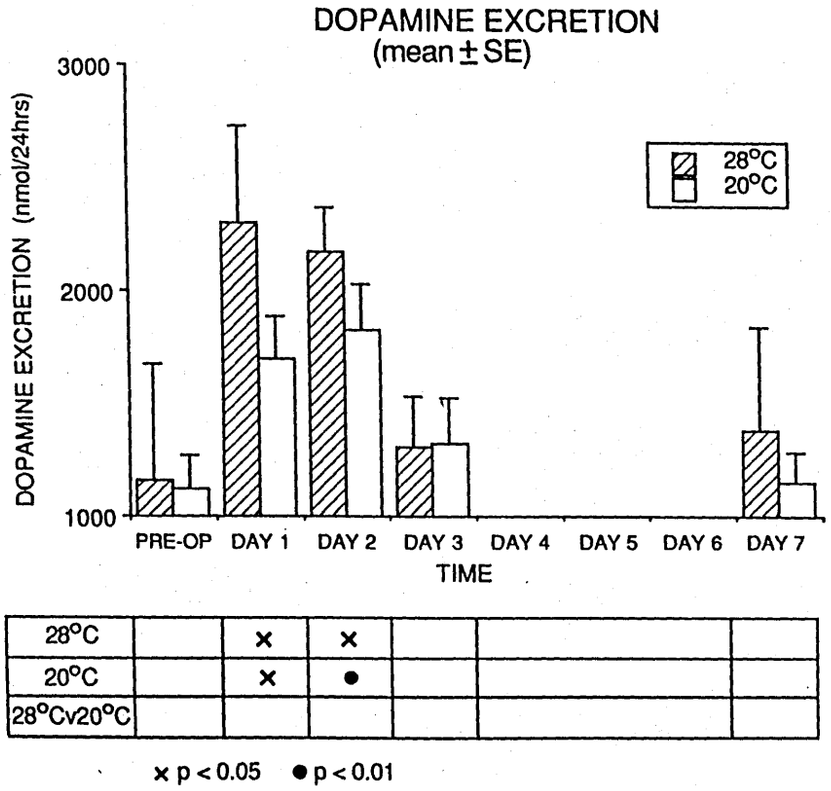


FIG 10.3 Mean (SE) urinary dopamine excretion in the two temperature groups.

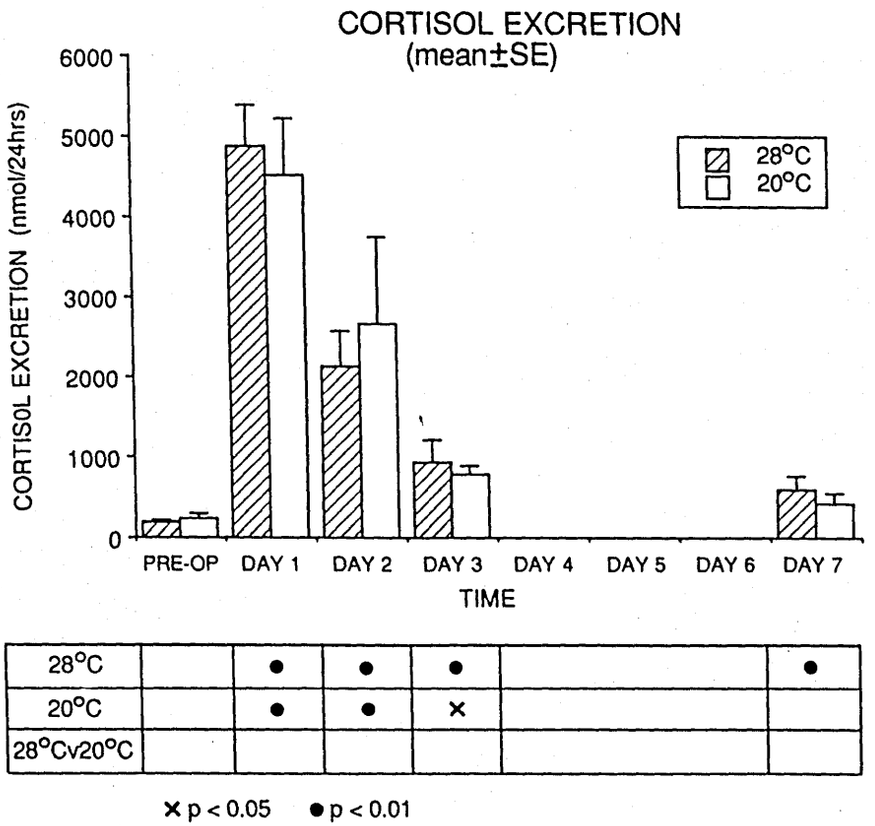


FIG 10.4 Mean (SE) urinary cortisol excretion in the two temperature groups.

CHAPTER 11

NITROGEN BALANCE AND WHOLE BODY PROTEIN TURNOVER

CHAPTER 11

NITROGEN BALANCE AND WHOLE BODY PROTEIN TURNOVER

I balanced all, brought all to mind,
The years to come seemed waste of breath,
A waste of breath the years behind,
In balance with this life, this death.

William Butler Yeats (1865-1939). An Irish Airman Foresees His Death.

INTRODUCTION

Injury elicits a complex metabolic response in surgical patients. Prominent amongst the physiological and biochemical changes is an increase in nitrogen excretion (60,61) due to an alteration in whole body protein metabolism. Studies of nitrogen balance provide important information on body protein metabolism in the clinical setting but give no information on the rate of protein synthesis or breakdown. Hence, methods using isotopically labelled amino acids have been developed to allow direct measurement of rates of whole body protein turnover (WBPT), synthesis (WBPS) and breakdown (WBPB). Methods involving stable isotopes of nitrogen and carbon (^{15}N and ^{13}C) have been most widely used and protein metabolism after elective surgery (100), trauma (104), and sepsis (106) has been studied.

Such techniques involve making basic assumptions about protein metabolism and the distribution of isotopic label throughout the body. ^{15}N tracer techniques commonly use the non-essential amino acid glycine and analyse the ^{15}N enrichment of a urinary nitrogenous end product. In this method it is assumed that the tracer mixes freely throughout a single, homogeneous, metabolic pool. ^{13}C tracer methods

involve labelling of the precursor pool and amino-acid flux is then calculated from the plateau enrichment of an amino acid in plasma, usually leucine. Rates of protein synthesis and breakdown are calculated from the proportion of the tracer irreversibly lost to breath CO_2 and therefore not used for protein synthesis. In both these methods, short protocols using primed constant infusions of labelled amino acids have been developed to reduce problems associated with re-entry of tracer from protein breakdown.

Amino-acid disposal by pathways other than protein synthesis or breakdown can introduce errors in the measurement of protein turnover when using labelled amino-acids. The use of ^{13}C leucine to estimate protein turnover is subject to error since the flux of leucine through alternative pathways is increased in certain circumstances, eg. when leucine becomes a substrate for energy production in skeletal muscle. Glycine is used in several metabolic pathways in the body, but since the ^{15}N label is rapidly distributed throughout the alpha amino groups of most amino-acids, the potential error associated with glycine metabolism is minimised. Indeed, results using ^{15}N glycine compare well with results obtained from uniformly labelled protein tracers (362).

The greatest potential source of error in the use of labelled amino-acids for the measurement of WBPT is the assumption of the existence of a single metabolic pool through which the label is rapidly distributed. Measurement of WBPT using the stable isotope ^{15}N is based on the excretion of isotopically enriched urea and/or ammonium in urine (the "end-product" method). The major assumption of this stochastic method, widely popularised by Picou and Taylor-Roberts (95), is of a single homogeneous nitrogen pool which derives nitrogen from food intake and the breakdown of body protein and loses nitrogen

by synthesis into new structural proteins or by excretion as waste products. Calculation of rates of whole body protein turnover, in the steady state and at isotopic equilibrium, assume equal distribution of the isotope, like all other metabolic nitrogen from this homogeneous pool, between the synthesis of new proteins and excretory waste products.

Fern and Garlick studied this at length and concluded that the concept of a single pool was an oversimplification and that under certain circumstances compartmentation of the label could be demonstrated (362,363). They argued that since the major nitrogenous end products are ammonium and urea, and since urea is synthesised in the liver and ammonium is largely synthesised from glutamine derived from skeletal muscle, figures obtained for WBPT would be biased towards the end product used in the calculation. These workers suggested that since skeletal muscle protein turnover contributes approximately 50% of the whole body figure, an average of results using ammonium and urea end products- the End Product Average (EPA)- should yield a more accurate picture of whole body protein kinetics.

This use of an EPA to calculate WBPT in the non-stressed individual seems valid (362,363). However, it is well accepted that stress results in an overall increase in hepatic protein synthesis and an increase in muscle protein breakdown. This alteration in body homeostasis might be expected to produce an alteration in the metabolism of urea and ammonium which could, in certain circumstances, cause inaccuracy in either end product method as well as the EPA. Thus, elevation in hepatic protein synthesis might lead to an alteration in urea synthesis, while an increase in muscle protein breakdown could alter the rate of ammmonium production from glutamine. A figure for WBPT based only on the EPA could therefore obscure

independent alterations in the two major protein compartments.

WBPS, WBPB and the relevance of the EPA have been assessed in circumstances in which alterations of protein metabolism in the two major pools of the body, visceral and skeletal muscle, might be expected to diverge. Indeed recently Wilmore's group reported a reduction in "post-traumatic proteolysis" in patients undergoing open-heart surgery (10). These workers used only moderate hypothermia (28°C) and large doses of anaesthetic agents which themselves could modify the metabolic response to injury (10).

In the current study the effects of two conventionally used levels of intraoperative hypothermia (28°C and 20°C) on the protein turnover response to coronary artery surgery were assessed by nitrogen balance, WBPS and WBPB calculated from isotopic enrichment of urinary urea and ammonium, and the EPA.

PATIENTS and METHODS

Nitrogen balance and WBPT studies were performed pre and postoperatively in 20 adult male patients undergoing elective coronary artery surgery. The theoretical considerations and practical details of these studies are described in detail in Chapter 3.

In addition to routine hospital investigations all patients underwent anthropometric assessment.

Operative techniques were standardised as detailed in Chapter 3.

Urinary nitrogen was measured by a standard microkjeldahl method (218). ^{15}N enrichment of urinary urea and ammonium was measured by automated continuous flow isotope ratio mass spectrometry (220) as explained in Chapter 3. WBPS and WBPB rates were calculated by the stochastic method of Picou and Taylor-Roberts (95) detailed in Chapter 3.

BACKGROUND TRACER ENRICHMENT

The timing of postoperative protein turnover measurement period was determined by the need to measure whole body protein dynamics at the time of the maximum inflammatory response. Commencing the second WBPT measurement only 24 hours after surgery resulted in low levels of tracer remaining in the samples used to assay postoperative "baseline". All postoperative baseline samples were analysed for residual ^{15}N in urea and ammonium and results were compared with baseline samples taken at the start of the first infusion. The mean ^{15}N enrichment in urea above the preoperative baseline was 0.00505 +/- 0.00318 (n=20; 1 SD) atom percent excess (APE) and in ammonium 0.00486+0.00189 APE. Tracer enrichment in the rapidly cycling ammonium pool at 48 hours after the end of the first infusion would largely be due to protein degradation while the greater residual tracer enrichment due to the slower turnover of the urea pool was apparent. As these values do not correspond to the "true" postoperative baseline, the residual enrichment "under" the midpoint of the second tracer plateau at 66 hours after operation was calculated assuming first order kinetics. This showed that the "true" postoperative urea baseline was 0.00257 APE ^{15}N and that of ammonium 0.0241 APE ^{15}N. These figures were both within 95% confidence limits of their respective measured preoperative baseline enrichment (2 SD equal to +/- 0.00306 APE ^{15}N for urea and 0.00310 APE for ammonium) and, for the purposes of this comparative study, validates the use of the preoperative baseline to calculate both pre and postoperative tracer plateau enrichments.

STATISTICAL ANALYSIS

Statistical analysis was performed using Wilcoxon's signed rank

test for paired data in the same patient and the Mann-Whitney U test to compare differences between groups. Differences were considered to be significant when the probability of their arising by random sampling error was less than 1 in 20 ($p<0.05$) and highly significant if this probability was less than 1 in 100 ($p<0.01$).

RESULTS

In both groups preoperative anti-anginal therapy, premedication therapy, anaesthetic techniques, operative procedure and early postoperative recovery were similar.

Anthropometric and surgical data for both patient groups are presented in Table 11.1; there was no significant difference between the two groups in age or nutritional status as determined by percentage predicted body weight, arm muscle circumference and triceps skin fold thickness.

Bypass times (ie period utilising extracorporeal circuit) and cross clamp times (ie period of exclusion of heart and lungs from the circulation) were similar in both groups.

The pre and postoperative rates of nitrogen excretion are summarised in Table 11.2 and illustrated in Figure 11.1. Individual values for nitrogen excretion in the two groups are given in Tables 12.1 and 12.2. The rates of WBPS, WBPB and the EPA, calculated from the ^{15}N enrichment of urinary urea and ammonium, are shown in Table 11.3 expressed as median values and ranges. The pre and postoperative values of WBPS and WBPB for individual patients are illustrated in Fig 11.2 to Fig 11.9. Accidental discard of a urine sample led to loss of ammonium data in one patient in the 28°C group. One patient from the 28°C group is not represented in the figures because of outlying values.

NITROGEN BALANCE (Tables 11.2, 12.1, 12.2, Figure 11.1). There was a 35% increase in postoperative nitrogen excretion in the 28°C group ($p=0.02$) and a 20% increase in the 20°C group ($p=0.08$).

WHOLE BODY PROTEIN SYNTHESIS (Table 11.3). The preoperative rate of WBPS was similar for each group calculated from the isotopic enrichment of urinary urea, ammonium and the EPA. Postoperatively there was a 23% fall in WBPS in the 28°C group ($p<0.01$) and a 17% fall in the 20°C group ($p<0.05$) measured by ^{15}N enrichment of urinary urea. In contrast, when WBPS was measured from isotopic enrichment of urinary ammonium there was an increase in the postoperative rates of WBPS in both groups, by 25% in the 28°C group ($p=0.12$) and 22% in the 20°C group ($p=0.04$). The EPA showed little change in the postoperative rate of WBPS in both groups with a 3% decrease in the 28°C group and a 2% decrease in the 20°C group.

WHOLE BODY PROTEIN BREAKDOWN (Table 11.3). The preoperative rate of WBPB was similar for each group when calculated from the isotopic enrichment of either urinary end product and the EPA. Postoperatively there was a decrease in WBPB in both groups measured from the isotopic enrichment of urinary urea (by 12% in the 28°C group and 5% in the 20°C group) but in neither group was this statistically significant. In contrast postoperative WBPB measured from the ammonium end product showed a 30% increase in the 28°C group ($p=0.10$) and a 21% increase in the 20°C group ($p=0.01$). The EPA showed a small increase in postoperative WBPB in both groups, by 5% in the 28°C group and 8% in the 20°C group.

DISCUSSION

Estimation of protein turnover using isotope tracers has been used to investigate nitrogen balance following elective surgery in a

number of studies, with conflicting results (101-106). The widely held premise that a negative nitrogen balance after surgery resulted from increased skeletal muscle protein breakdown was questioned by the protein turnover studies of O'Keefe and colleagues which suggested that this resulted predominantly from a fall in protein synthesis with little alteration in protein breakdown (100). These findings have been confirmed following surgery of moderate severity (101) while it has been reported that after major surgery (102), trauma (103), burns (104) and sepsis (105), a negative nitrogen balance results from an increase in WBPB exceeding a simultaneous increase in WBPS.

The current study is the largest to date to examine the effects of major elective surgery on WBPT and had two aims:
i) to investigate the potential of a brief period of intraoperative hypothermia to modify alterations in protein metabolism after trauma.
ii) to investigate the phenomenon of precursor compartmentation. This was achieved by measuring the isotopic enrichment of urinary urea and ammonium, pre and postoperatively in the same patient, and by calculating an EPA.

^{15}N glycine is one of the most widely used stable isotope tracers in metabolic studies of protein turnover and its value and limitations have been extensively reviewed (364-366). ^{15}N glycine fulfills most of the criteria required for an isotopic tracer and is particularly useful for comparative studies in view of the large body of literature which already exists.

Rates of WBPS and WBPB are affected by preceding nutritional status (365), caloric intake (96,97) and activity levels (98,99). To obviate these variables as far as possible in this study all patients were nutritionally replete, had normal dietary intake prior to hospital admission, remained fasted for both phases of the study and

were allowed to perform only minimal ward activities during the periods of study.

In this study the postoperative increase in nitrogen excretion was greater in the 28°C group being consistent with the hypothesis that a more profound level of intraoperative hypothermia attenuates post-traumatic nitrogen loss (10). Wilmore's group have demonstrated a reduction in nitrogen excretion after open-heart surgery with a combination of moderate hypothermia (28°C) and high-dose fentanyl anaesthesia (10). The latter can itself modify the metabolic response to trauma (189) and, furthermore, these workers did not examine nitrogen balance in terms of alterations in protein kinetics. In the current study only modest doses of fentanyl anaesthesia were used (Chapter 3) and whole body protein metabolism was studied using the amino acid glycine, labelled with ^{15}N.

The preoperative rate of WBPS and WBPB was similar for each group when calculated from the isotopic enrichment of urinary urea, ammonium and the EPA, validating the use of the latter to calculate WBPT in the steady, non-stressed state. In the postoperative period there was a significant decrease in the rates of WBPS which exceeded a simultaneous decrease in the rates of WBPB in both groups when measured from the isotopic enrichment of urinary urea. This pattern of response is consistent with the hypothesis that development of a negative nitrogen balance after elective surgery of moderate severity is predominantly due to a fall in WBPS (101,102,105). The magnitude of the decrease in WBPS was significantly greater in the 28°C group implying that a more profound level of intraoperative hypothermia can modify the postoperative fall in WBPS.

In contrast, when isotopic enrichment of urinary ammonium was used to measure WBPT in the postoperative period, there was a marked

increase in WBPB exceeding a simultaneous increase in WBPS in both groups, although statistically significant only in the 20°C group. An increase in WBPB exceeding a simultaneous increase in WBPS has been reported to explain the negative nitrogen balance after major surgical and accidental trauma (102,103), burns (104) and sepsis (106). In the current study the more profound level of intraoperative hypothermia appeared to make little difference to the rates of WBPB and WBPS when judged by isotopic enrichment of urinary ammonium.

These results are not consistent with the concept of a single metabolic nitrogen pool as postulated in the stochastic model of Picou and Taylor-Roberts (95) and there is already evidence to suggest the existence of more than a single metabolic pool. At least 75% of total urinary nitrogen occurs in the form of urea in the post-absorptive state with the remainder consisting mainly of nitrogen in creatinine and ammonia. Urea is predominantly synthesised in the liver from both hepatic and extra-hepatic sources of nitrogen while the N of urinary ammonium is derived mostly from the amide N of glutamine (367), the main source of which is skeletal muscle (368). The ^{15}N abundance in urinary ammonium may be biased towards the precursor pool of muscle in the same way that urea reflects the precursor pool in the liver.

Fern and Garlick have argued that if the single pool concept was valid in physical terms then administration of the same tracer source by different physical routes should result in the same rate of turnover for any end product derived from the pool, although this is not the case (363). Likewise if the single pool concept was valid in metabolic terms then administration of any tracer amino acid by the same physical route would give a similar rate of turnover for the pool. Again this is known not to be the case (363). Based on differences between the rates of WBPT derived from urinary urea and

ammonium after administration of ^{15}N glycine, Fern and Garlick have proposed a two-pool model for compartmentation of metabolic nitrogen where urinary urea and ammonium are generated from two functionally distinct precursor pools of nitrogen (363). Urinary ammonium predominantly reflects activity in the skeletal muscle pool and urinary urea activity in the hepatic precursor pool. To resolve some difficulties in accounting for the activities of both precursor pools Fern and Garlick proposed the use of an EPA, based on isotopic enrichment of urinary urea and ammonium, to calculate WBPT (362,363).

In a two-pool model these results imply that in the visceral protein precursor pool, largely represented by liver, a decrease in protein synthesis exceeds a simultaneous fall in protein breakdown leading to a net fall in protein synthesis. In contrast, in the peripheral protein precursor pool, largely represented by skeletal muscle, an increase in protein breakdown exceeds a simultaneous increase in protein synthesis leading to a net increase in protein breakdown.

These results are in accordance with the concept of "hepatic reprioritization" following trauma, when there is an overall increase in skeletal muscle breakdown (369) to provide amino acids both as a source of fuel and for hepatic synthesis of the acute phase reactants (Chapter 12). Although it is generally assumed that there is an overall increase in hepatic protein synthesis after trauma, there is little direct evidence to support this concept in man, and the results of the current study imply a net decrease in hepatic protein synthesis after surgery. Although the liver increases production of some acute phase proteins such as CRP after surgery, these increases are quantitatively small compared to the fall in synthesis of proteins such as albumin ("negative acute phase proteins") as discussed in

Chapter 7.

It appears that while a more profound level of intraoperative hypothermia may attenuate the postoperative fall in protein synthesis, it does not modify skeletal muscle breakdown. This impies that the latter is one of the most basic and fundamental responses to tissue injury and is consequently less susceptible to modulation. This observation is discussed further in Chapter 12.

SUMMARY and CONCLUSIONS

Nitrogen balance and whole body protein kinetics were measured by standard techniques pre and postoperatively in 20 male patients undergoing elective coronary artery surgery and randomised to an intraoperative blood temperature of 28°C or 20°C. The aims of the study were to assess the ability of intraoperative hypothermia to modify changes in post-traumatic protein metabolism and to investigate the phenomenon of precursor compartmentation by measuring isotopic enrichment of urinary urea and ammonium and by calculating an EPA.

There was a significant postoperative increase in nitrogen excretion only in the 28°C group.

In the preoperative and therefore non-stressed state the EPA produced similar results to the values for WBPT when measured by either urinary end product. In the postoperative state the EPA suggested little overall change in WBPT but obscured large changes in synthesis and breakdown within the visceral and peripheral precursor pools. Isotopic enrichment of urinary urea suggested the development of a negative nitrogen balance was due predominantly to a fall in WBPS while isotopic enrichment of urinary ammonium suggested that a postoperative negative nitrogen balance was due to an increase in WBPB exceeding a concomitant rise in WBPS. It is possible that the failure

by many groups to observe or comment on the activities of both precursor pools has led to conflicting conclusions on the aetiology of a post-traumatic negative nitrogen balance. Measurement of the isotopic enrichment of urinary urea implies that a negative nitrogen balance is due to a fall in protein synthesis while isotopic enrichment of urinary ammonium implies it is due to an increase in protein breakdown. Indeed using only one of these end product methods would have led to similar erroneous conclusions in the current study and it is therefore essential to examine both end products in a post-traumatic state.

It appears that a more profound level of intraoperative hypothermia can attenuate the postoperative fall in protein synthesis but not the postoperative increase in muscle breakdown, implying that the latter is a more fundamental resonse to trauma.

	28°C	20°C	
NUMBER OF PATIENTS	10	10	
AGE	55(5)	57(7)	NS
% IDEAL BODY WEIGHT	113(14)	113(15)	NS
% PREDICTED ARM MUSCLE CIRCUMFERENCE	105(7)	104(6)	NS
% PREDICTED TRICEPS SKIN FOLD THICKNESS	83(46)	99(22)	NS
BYPASS TIME (mins)	93(26)	97(19)	NS
CROSS CLAMP TIME (mins)	53(19)	55(15)	NS
BLOOD TEMPERATURE (°C)	28(1)	20(1)	<0.001

Table 11.1. Clinical, anthropometric and operative data of the two temperature groups.

NITROGEN EXCRETION (g/day)

	Preop	Postop
28°C	8.8(4.8)	11.9(16.2) : p=0.02
20°C	9.0(7.0)	10.8(13.6) : p=0.08

Table 11.2. Nitrogen excretion (g/day) pre and postoperatively in the two temperature groups expressed as median and range.

		WHOLE BODY PROTEIN SYNTHESIS			WHOLE BODY PROTEIN BREAKDOWN		
		g protein/ Kg body mass/ day					
		PREOP	POSTOP	p	PREOP	POSTOP	p
^{15}N UREA	28°C	2.58(2.49)	1.98(2.01)	0.01	3.34(2.46)	2.93(1.50)	0.10
	20°C	2.47(1.34)	2.05(0.95)	0.04	3.11(1.19)	2.95(1.04)	0.09
^{15}N AMMONIUM	28°C	2.58(1.85)	3.23(3.13)	0.12	3.39(1.88)	4.41(3.48)	0.10
	20°C	2.03(0.48)	2.47(0.73)	0.04	2.78(0.49)	3.36(0.66)	0.01
EPA	28°C	2.77(0.88)	2.68(1.40)	1.00	3.59(0.91)	3.78(1.75)	0.41
	20°C	2.21(0.36)	2.17(0.54)	1.00	2.95(0.33)	3.18(0.36)	0.10

Table 11.3. Rates of whole body protein synthesis and breakdown, in the two temperature groups, measured by the isotopic enrichment of urea, ammonium and the end product average (EPA). Figures are expressed as median and range.

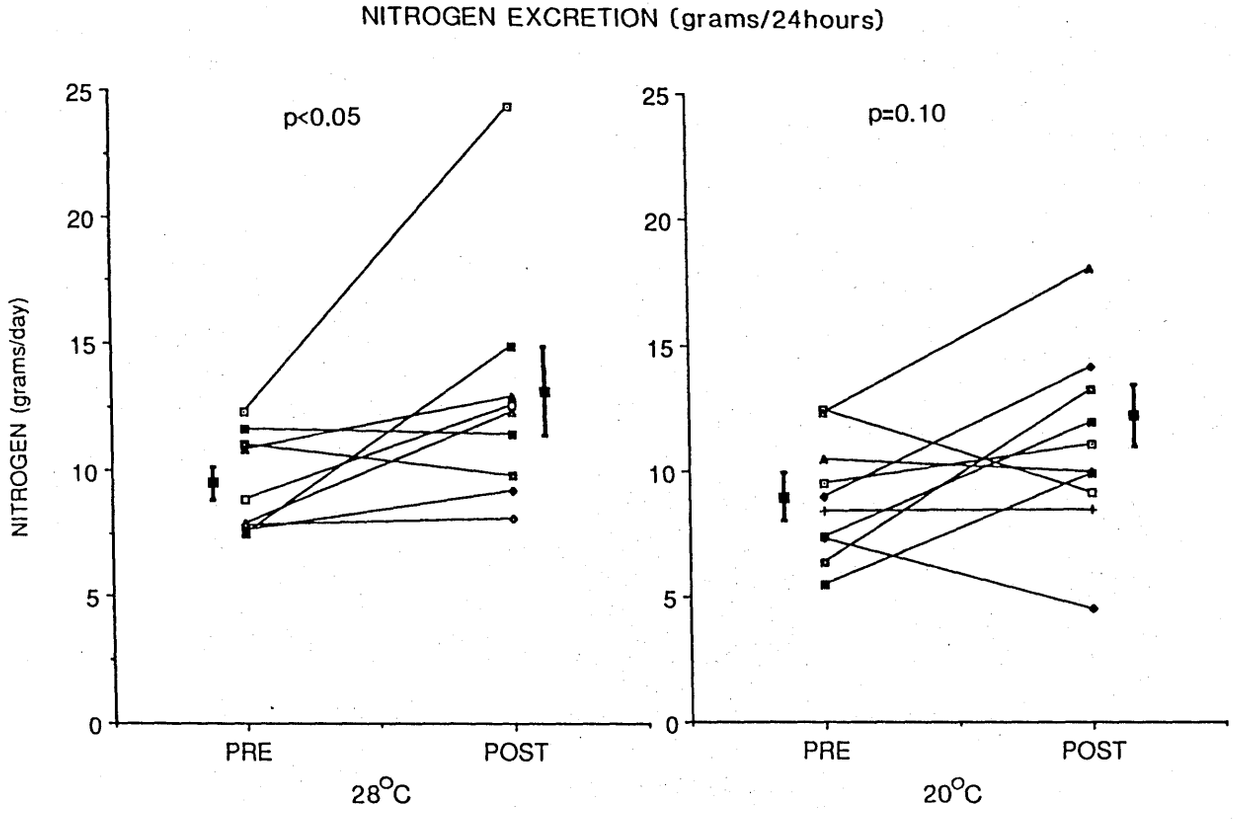
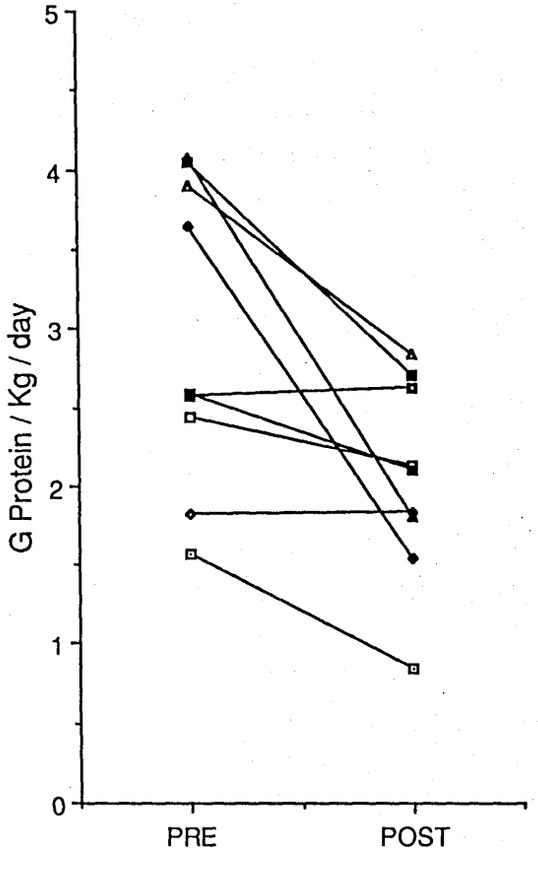


FIG 11.1 Mean (SE) urinary nitrogen excretion in the two temperature groups.

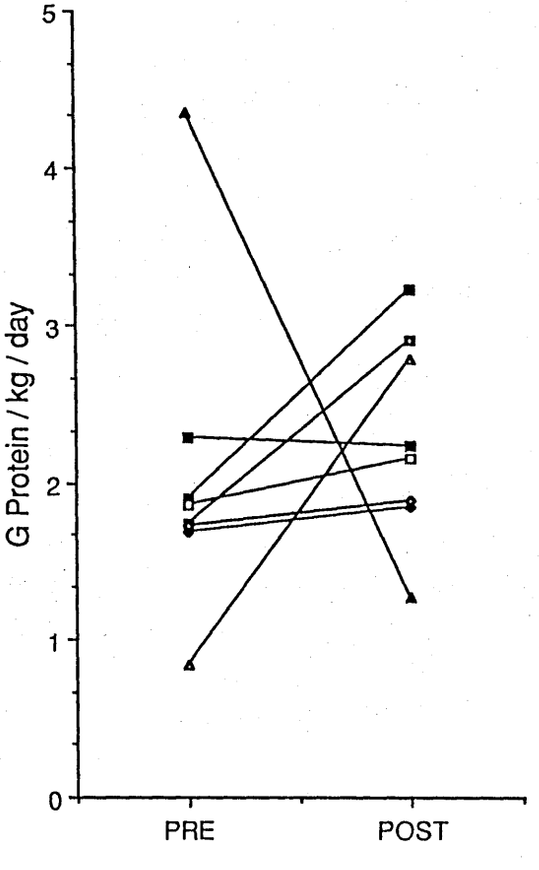
WHOLE BODY PROTEIN SYNTHESIS
(UREA END PRODUCT)



28 DEGREES CENTIGRADE

FIG 11.2

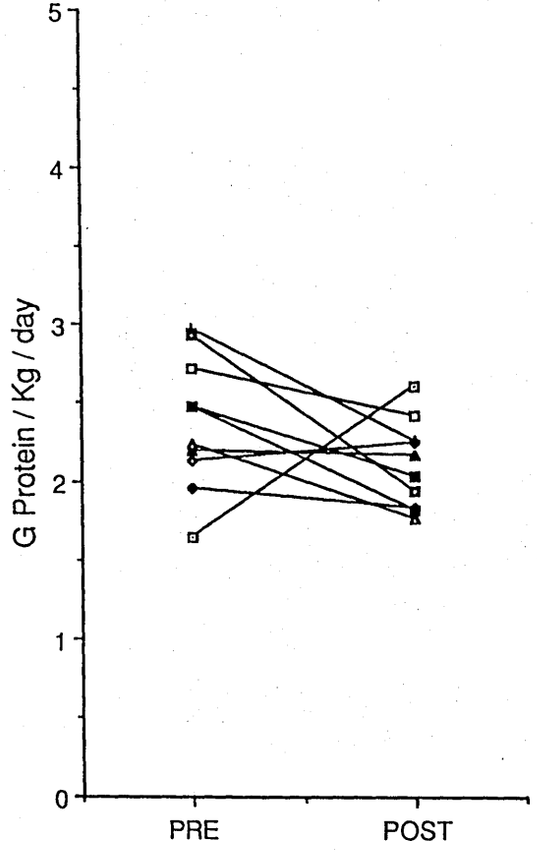
WHOLE BODY PROTEIN SYNTHESIS
(NH3 END PRODUCT)



28 DEGREES CENTIGRADE

FIG 11.3

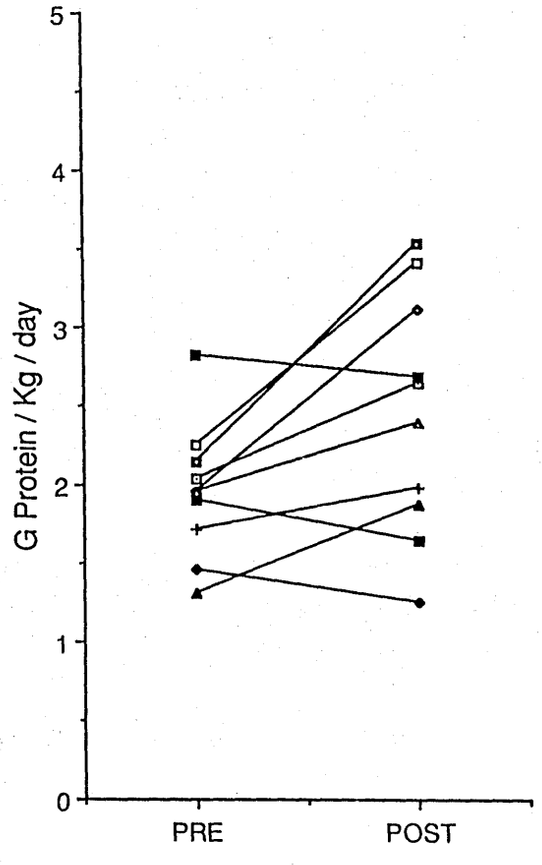
WHOLE BODY PROTEIN SYNTHESIS
(UREA END PRODUCT)



20 DEGREES CENTIGRADE

FIG 11.4

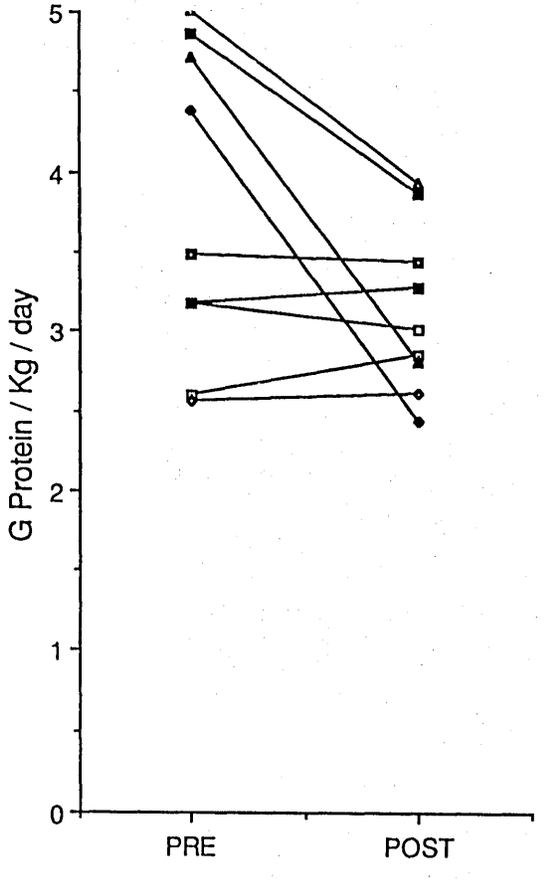
WHOLE BODY PROTEIN SYNTHESIS
(NH3 END PRODUCT)



20 DEGREES CENTIGRADE

FIG 11.5

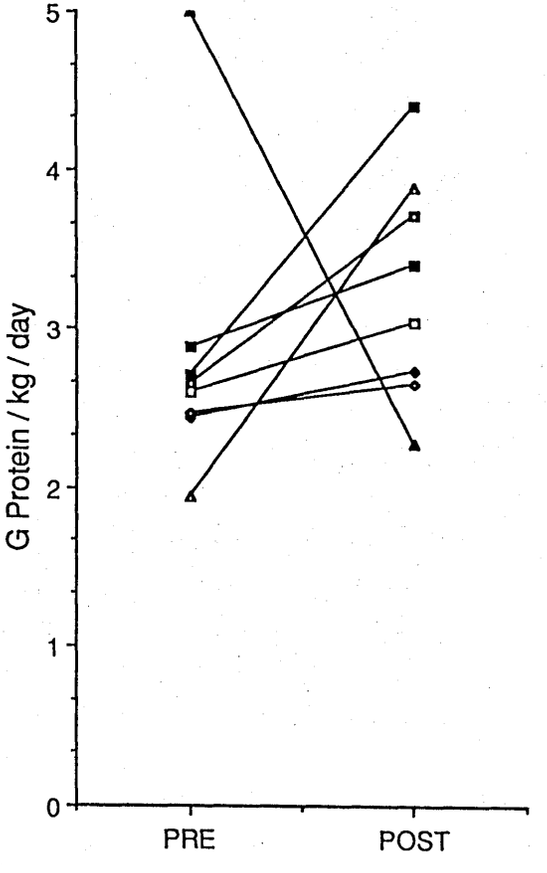
WHOLE BODY PROTEIN BREAKDOWN
(UREA END PRODUCT)



28 DEGREES CENTIGRADE

FIG 11.6

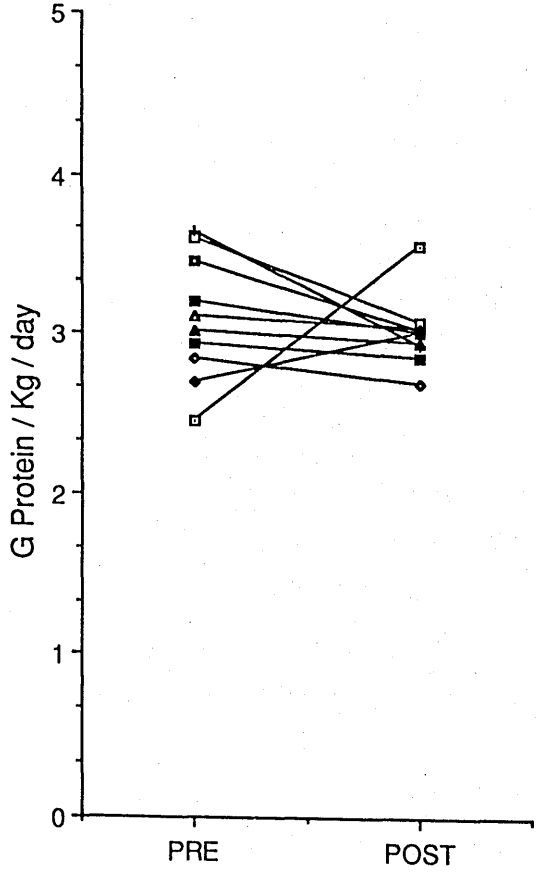
WHOLE BODY PROTEIN BREAKDOWN
(NH3 END PRODUCT)



28 DEGREES CENTIGRADE

FIG 11.7

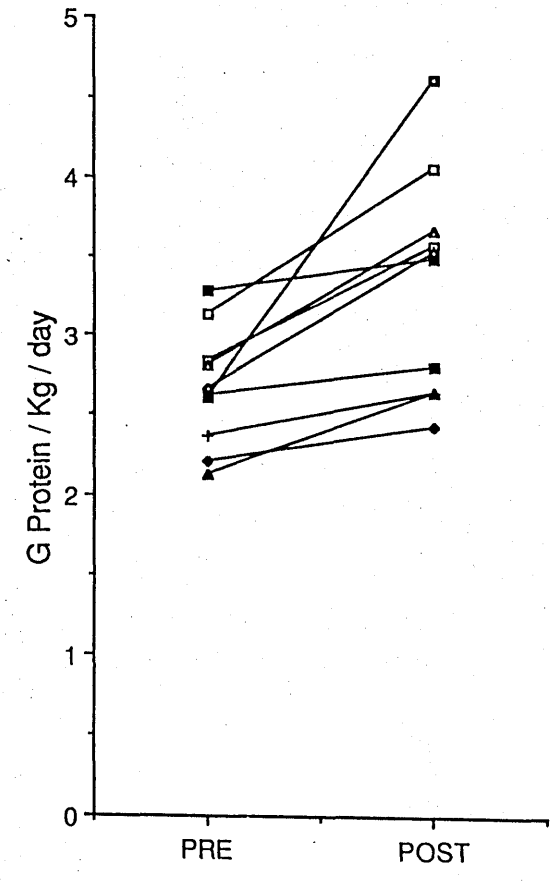
WHOLE BODY PROTEIN BREAKDOWN
(UREA END PRODUCT)



20 DEGREES CENTIGRADE

FIG 11.8

WHOLE BODY PROTEIN BREAKDOWN
(NH3 END PRODUCT)



20 DEGREES CENTIGRADE

FIG 11.9

CHAPTER 12

SKELETAL MUSCLE METABOLISM

CHAPTER 12

SKELETAL MUSCLE METABOLISM

The brain has muscles for thinking as the legs have muscles for walking.

Julien Offroy de La Mettrie (1709-1751). L'homme Machine.

INTRODUCTION

The results of the protein turnover studies discussed in Chapter 11 produced conflicting results regarding the exact aetiology of a postoperative negative nitrogen balance. Isotopic enrichment of urinary urea implied that the postoperative negative nitrogen balance was predominantly due to a fall in WBPS while urinary ammonium enrichment implied that an increase in WBPB was the predominant mechanism. To resolve this conflict the urinary excretion rates of 3 "markers" of skeletal muscle breakdown 3 methylhistidine (3MeH), zinc and creatinine, were measured pre and postoperatively in 20 patients.

PATIENTS and METHODS

The patients have been described in Chapter 11. Measurements of urinary 3MeH, zinc and creatinine were made from the same urinary collections used to determine postoperative changes in nitrogen excretion and isotopic enrichment of urinary urea and ammonium.

3-METHYLHISTIDINE. Urinary 3MeH was analysed on a Hilger (Rank) Chromaspek M amino acid analyser and is based on a cation exchange resin, two buffer gradient dilution, ninhydrin detection method.

ZINC. Urinary zinc was analysed by flame atomic absorption spectrophotometry.

CREATININE. Urinary creatinine was analysed on a Hitachi 704 discrete analyser based on the standard end point Jaffe reaction.

RESULTS

The clinical and operative features of both patient groups are described in Chapter 11 (Table 11.1).

The results of pre and postoperative urinary excretion of 3MeH, zinc and creatinine in the two temperature groups are presented in Table 12.1 and Table 12.2 and respectively illustrated in Figs 12.1, 2 and 3.

There was a 59% increase in the mean postoperative 3MeH excretion in the 28°C group ($p=0.01$) and an 82% increase in the mean postoperative excretion in the 20°C group ($p=0.005$). It should be noted however that the latter result may be biased by the very large increase in postoperative excretion of 3MeH in two patients in the 20°C group (Fig 12.1).

There was a postoperative increase in mean zinc excretion in both groups (Fig 12.2), by 22% in the 28°C group ($p=0.20$) and by 51% in the 20°C group ($p<0.001$).

There was a significant increase in mean creatinine excretion in both groups postoperatively (Fig 12.3), by 31% in the 28°C group ($p=0.05$) and by 35% in the 20°C group ($p<0.001$).

DISCUSSION

The development of a negative nitrogen balance is the hallmark of the catabolic response to tissue trauma resulting from injury (60,61) or surgery (107). As discussed in Chapter 11 the aetiology of a negative nitrogen balance after trauma may depend on the severity of the injury; after surgery of moderate severity a fall in WBPS is

thought to be the predominant factor but after severe injury an increase in WBPB may be of greater importance (106). As indicated by the results in Chapter 11, however, it appears that interpretation of the development of a negative nitrogen balance is also dependent on whether isotopically enriched urea or ammonium is chosen as the end product. It is generally believed that following trauma there is an increase in skeletal muscle breakdown (369) to facilitate "hepatic reprioritization" of proteins. The aim of this chapter was to elucidate any other evidence of a postoperative increase in WBPB by examining postoperative changes in the urinary excretion of "markers" of skeletal muscle trauma.

Skeletal muscle protein breakdown was incriminated as the cause of a post-traumatic negative nitrogen balance by Cuthbertson, based on clinical observations in patients with long bone fractures (60,61) and on the urinary excretion ratios of nitrogen, potassium and sulphur in rats with long bone fractures (370). Using stable isotopes Reiss subsequently demonstrated depletion of the skeletal muscle mass but relative preservation of visceral protein (liver and kidney) in animals with a profound catabolic response (371). From a teleological standpoint, release of amino acids from muscle following injury provides an alternative energy supply and, more importantly, a source of proteins for the liver to produce acute phase proteins involved in a variety of host defence mechanisms. In a recent review of protein turnover in trauma and sepsis, Hasselgren implicated muscle protein breakdown as the most important source of peripheral amino acid production but also suggested that reduced protein synthesis may occur (372).

Unfortunately there is no easily applicable technique to directly measure muscle protein breakdown in the clinical setting.

The release of protein from muscle following trauma can be demonstrated by arteriovenous differences in amino acid concentration (108,373) but such techniques are technically difficult to perform accurately and require a high degree of analytic precision. Interpretation is complicated by re-utilisation of some amino acids and the origin of venous blood not only from muscle but also from bone, skin and adipose tissue (374). Furthermore, this technique provides a qualitative rather than absolute measurement of changes in arteriovenous amino acid concentrations as blood flow cannot be accurately measured.

It was thought that 3MeH might resolve these difficulties as it appeared to be a specific marker of muscle protein breakdown (see Chapter 2). Muscle 3MeH accounts for more than 90% of the total body content of 3MeH, is neither metabolised nor re-utilised for protein synthesis, and is rapidly excreted in urine (109,112). Consequently 3MeH has gained wide popularity as a specific marker of muscle protein breakdown (116). 3MeH is not found exclusively in skeletal muscle as small pools exist in the gastrointestinal tract and skin (111) and Rennie has suggested that fast turnover in these pools may make significant contributions to urinary excretion of 3MeH (122, 375). More recently Long's group have again confirmed the validity of urinary 3MeH as predominantly a marker of skeletal muscle breakdown with little contribution from the gastrointestinal tract (376).

Approximately 60% of body zinc is found in skeletal muscle. The main pathway of excretion is in faeces but a smaller fixed amount of zinc is excreted in the urine each day irrespective of dietary intake or urine volumes (239). An increase in urinary zinc excretion accompanies most catabolic states (377). After surgery increased urinary zinc excretion is largely derived from skeletal muscle (378).

Fell and colleagues, using radioactive ^{65}Zn, measured the urinary excretion of ^{65}Zn and total Zn for a three week period after hip replacement (379). They demonstrated an increased urinary output of zinc after surgery and calculated that most of the postoperative increase in urinary zinc excretion was derived from zinc in skeletal muscle. Other groups of workers have confirmed that urinary zinc excretion reflects post traumatic skeletal muscle catabolism (380,381).

Urinary creatinine excretion has also been used as a marker of skeletal muscle trauma (382) based on the rationale that urinary creatinine is derived from creatine, 98% of which is found in skeletal muscle. Heymsfield has extensively reviewed the literature and confirmed that in non-stressed individuals, on a stable diet and without evidence of renal impairment, urinary creatinine excretion is an indicator of the skeletal muscle mass (383). Changes in urinary creatinine excretion have therefore been used by various groups as an indicator of skeletal muscle breakdown following trauma (382,384) and surgery (380,385).

The results from the current study demonstrated a postoperative increase in the urinary excretion of 3MeH, zinc and creatinine in both temperature groups. The magnitude of the postoperative increase in 3MeH, zinc and creatinine excretion was statistically greater in the $20^{o}C$ temperature group implying a greater degree of skeletal muscle breakdown in this group. This was surprising in view of a smaller postoperative negative nitrogen balance in this group but in accordance with the results of isotopic enrichment of urinary ammonium, discussed in Chapter 11, which implied a greater increase in postoperative WBPB in the $20^{o}C$ group. The discordant demonstrations of a greater degree of postoperative WBPB, without a proportionally

greater increase in nitrogen excretion, in the 20°C temperature group could have a number of possible explanations:

(i) Nitrogen balance is the net result of protein synthesis and breakdown in the visceral and peripheral protein pools. The former is largely accounted for by liver and the latter by skeletal muscle. An increase in skeletal muscle breakdown does not by itself determine nitrogen balance. It was suggested in Chapter 11 that a more profound level of intraoperative hypothermia may attenuate the postoperative fall in protein synthesis and this may modify the increase in negative nitrogen balance which would otherwise be expected on the basis of an increase in skeletal muscle breakdown.

(ii) The counter-regulatory hormones play an integral role in the catabolic response to tissue injury. Wilmore incriminated catecholamines as major mediators of the metabolic response to injury and estimated that noradrenaline accounted for at least 85% of the response (89). Further evidence of hormonal involvement in the catabolic response was the demonstration that infusion of adrenaline, cortisol and glucagon into healthy volunteers produced an elevation in energy expenditure and a negative nitrogen balance through increased protein catabolism (386-388). It had therefore been anticipated that a more profound level of intraoperative hypothermia, accompanied by a smaller postoperative increase in catecholamine secretion (Chapter 10), might lead to a reduction in muscle protein breakdown. Failure to observe such a reduction is in accordance with Hulton's observations that although catecholamine blockade can reduce post-traumatic creatinine excretion and amino-acid efflux, it does not alter overall nitrogen loss (389). Hulton concluded that hormonal blockade inhibits net skeletal muscle protein catabolism without altering whole body nitrogen loss (389). It is apparent that

catecholamines are not therefore the sole determinants of either skeletal muscle breakdown or a post-traumatic negative nitrogen balance. There were no facilities to measure glucagon, another counter-regulatory hormone, in the current studies but it is known to be at least involved in the regulation of skeletal muscle breakdown (386-388).

(iii) Cytokine peptides are known to be involved in post-traumatic muscle proteolysis but the exact mediators are unknown. A major advance was the demonstration that a circulating factor obtained from the serum of postoperative or septic patients induced muscle proteolysis in rat muscle incubated in vitro (390). This substance was isolated as a peptide fragment of 4,200 daltons and called "proteolysis inducing factor". At the same time it was demonstrated that the cytokine interleukin-1 induced prostaglandin E_2 synthesis and consequent proteolysis in incubated rat muscle in vitro at normothermia or even more markedly in a hyperthermic ($39^{o}C$) environment (66). Other workers using recombinant DNA could not reproduce these findings and suggested that the earlier results were due to impurities in the biological samples. This complex area has been extensively reviewed by Hasselgren and colleagues (372) and by Pomposelli and co-workers (391) who suggest that the main stimulant to muscle proteolysis is probably tumour necrosis factor although interleukin-1 has a synergistic action (390) and may also have a further indirect action through stimulation of the endocrine response. High concentrations of catecholamines can inhibit interleukin-1 production in vivo (392) and it is feasible that the higher catecholamine concentrations observed in the $28^{o}C$ group resulted in a relative inhibition of cytokines with a consequent reduction in skeletal muscle proteolysis.

(iv) During perfusion cooling there is a differential cooling of various organs, eg heart, liver and mid-oesophagus cool quickly but not skeletal muscle (28). It is possible that the more profound level of intraoperative hypothermia results in shunting of blood through skeletal muscle without effective cooling of the large skeletal muscle mass and this is currently under investigation.

Whatever the mechanism(s) controlling skeletal muscle breakdown, it appears to be more resistant to modification by profound intraoperarive hypothermia than components of the acute phase, endocrine and protein metabolism responses. This is in keeping with the results reported in Chapter 11, where postoperative isotopic enrichment of urinary ammonium implied an increase in skeletal muscle breakdown. It is possible that the latter represents a more fundamental part of the pathophysiological response to trauma and is not susceptible to modification.

SUMMARY and CONCLUSIONS

The urinary excretion rates of 3 "markers" of skeletal muscle breakdown, 3 methylhistidine (3MeH), zinc and creatinine were measured pre and postoperatively in 20 patients in whom nitrogen balance and protein turnover studies had been performed.

The finding of a greater increase in muscle protein breakdown in the 20^{o}C group correlated with the increase in WBPB determined from isotopic enrichment of urinary ammonium , but was in conflict with a smaller negative nitrogen balance in the same group.

These findings reinforce the importance of both protein synthesis and breakdown in determining nitrogen balance and a number of hypotheses to explain the apparent discrepancy between the endocrine response and muscle breakdown have been postulated.

24 HOUR URINARY EXCRETION RATES

28 DEGREE CENTIGRADE GROUP

	Nitrogen (grams)		3MeH (umol/mmol creat)		Zinc (umol)		Creatinine (mmol)	
	PRE	POST	PRE	POST	PRE	POST	PRE	POST
	12.3	24.3	14.9	21.7	14.7	25.2	11.4	22.6
	7.6	9.2	5.7	12.7	17.8	23.8	13.0	14.2
	11.0	9.8	12.1	22.9	6.5	7.0	9.7	12.1
	7.8	8.1	11.6	16.6	13.8	8.6	10.3	12.7
	7.5	14.9	-	-	9.0	11.2	-	-
	8.8	12.6	14.5	29.6	7.7	13.3	10.8	12.4
	10.8	12.9	20.5	31.8	10.9	15.8	11.1	13.1
	7.9	12.3	28.5	31.6	10.6	14.2	7.8	9.8
	11.6	11.4	16.6	30.8	1.0	7.8	13.7	17.9
MEAN	9.5	12.8	15.5	24.7	11.5	14.0	11.0	14.4
SE	0.6	1.6	2.4	2.6	1.2	2.2	0.7	1.4
p value	<0.05		=0.01		=0.20		=0.05	

Table 12.1 Twenty-four hour urinary excretion rates pre and postoperatively of nitrogen, 3MeH, zinc and creatinine in the 28°C group.

24 HOUR URINARY EXCRETION RATES

20 DEGREE CENTIGRADE GROUP

	Nitrogen (grams)		3MeH (umol/mmol creat)		Zinc (umol)		Creatinine (mmol)	
	PRE	POST	PRE	POST	PRE	POST	PRE	POST
	9.5	11.1	14.5	20.1	8.7	14.0	11.5	14.2
	9.0	14.2	17.2	24.7	5.2	11.2	10.3	13.2
	6.4	13.3	8.6	42.0	10.9	10.8	9.4	13.2
	7.3	4.5	7.9	11.9	8.9	14.3	11.1	11.7
	5.5	9.9	18.6	24.7	17.2	22.3	12.2	16.3
	12.5	9.2	13.1	23.7	1.0	7.8	1.3	11.4
	10.5	10.0	8.6	15.2	10.3	10.1	9.8	13.6
	12.3	18.1	11.4	18.9	9.2	18.1	16.8	20.9
	7.4	12.0	13.3	33.7	9.9	12.6	10.8	12.4
	8.4	8.5	16.1	19.9	4.2	8.5	8.4	10.6
MEAN	8.8	11.8	12.9	23.5	8.6	13.0	10.2	13.8
SE	0.8	1.1	1.2	2.7	1.4	1.4	1.2	0.9
p value	=0.10		=0.005		<0.001		<0.001	

Table 12.2 Twenty-four hour urinary excretion rates pre and postoperatively of nitrogen, 3MeH, zinc and creatinine in the 20°C group.

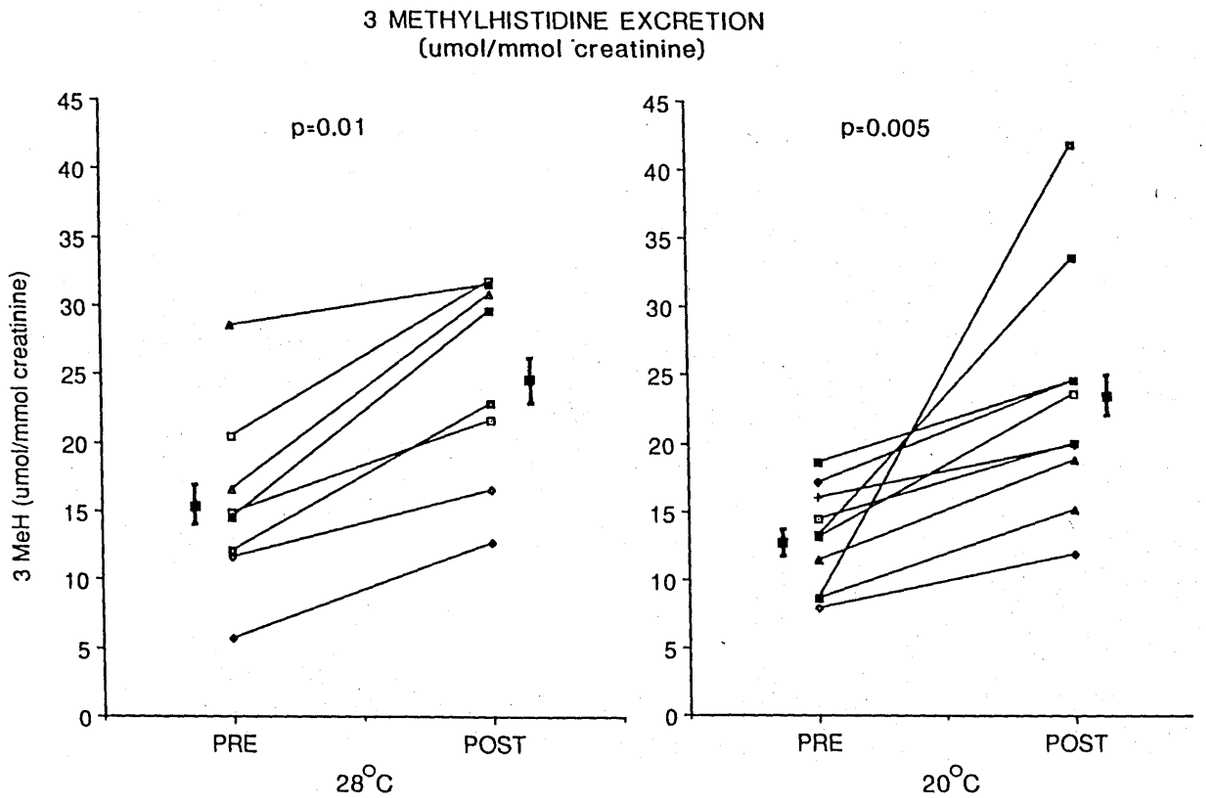


FIG 12.1 Mean (SE) urinary 3 methylhistidine excretion in the two temperature groups.

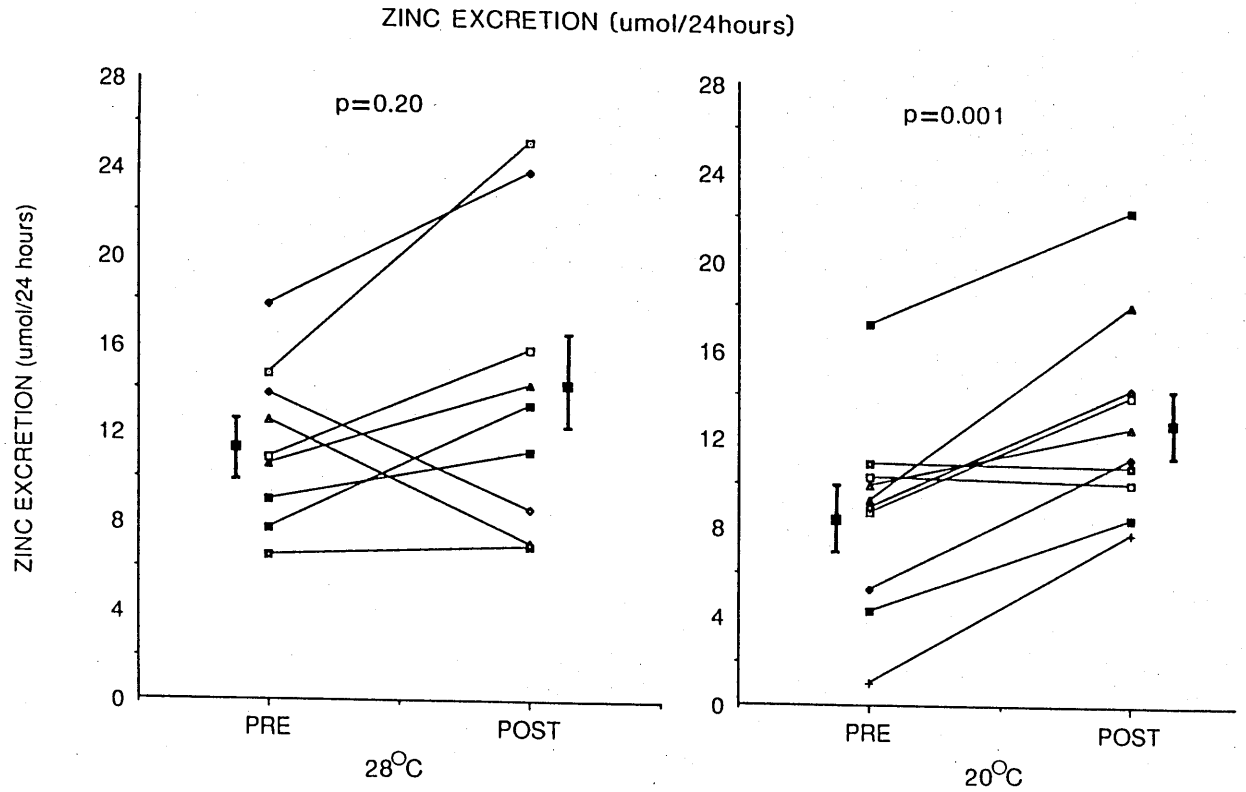


FIG 12.2 Mean (SE) urinary zinc excretion in the two temperature groups.

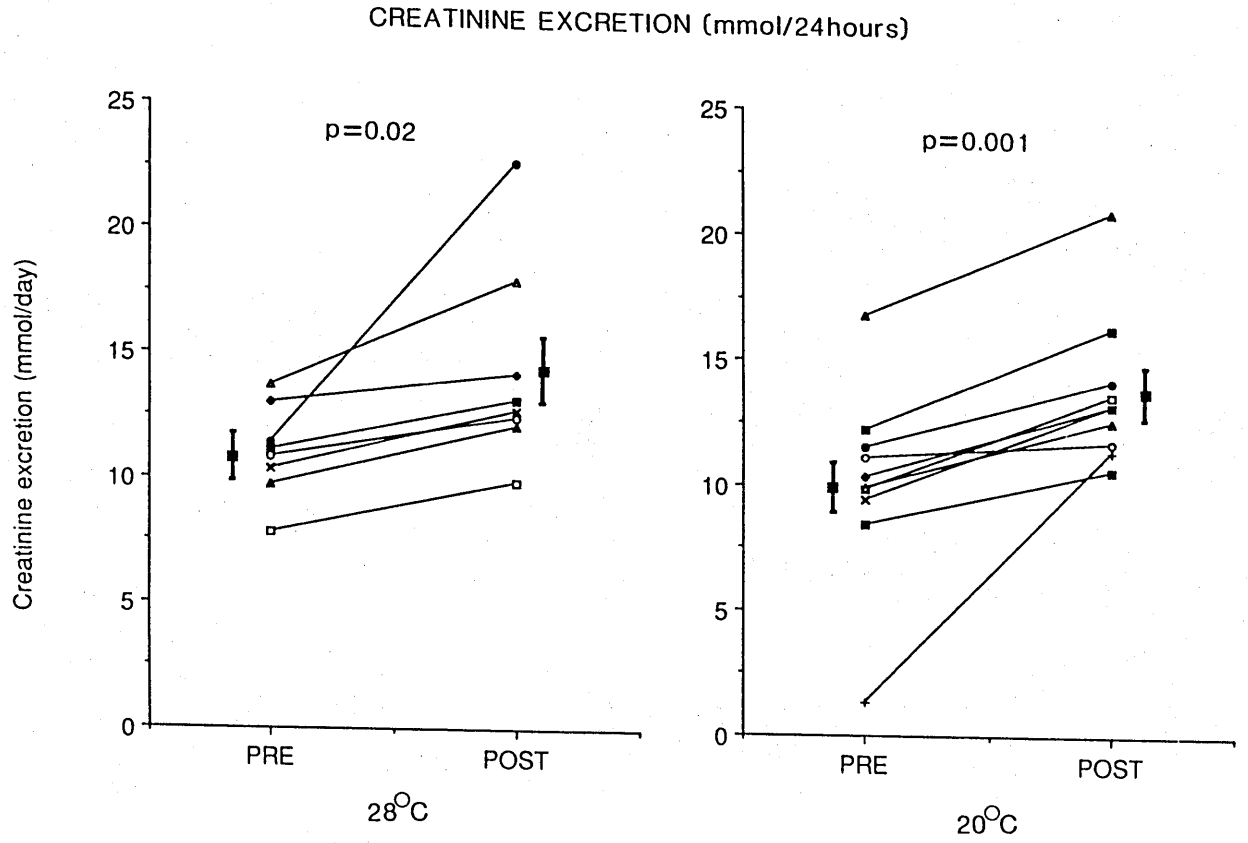


FIG 12.3 Mean (SE) urinary creatinine excretion in the two temperature groups.

CHAPTER 13

POSTOPERATIVE CHANGES IN ENERGY EXPENDITURE

CHAPTER 13

POSTOPERATIVE CHANGES IN ENERGY EXPENDITURE

Water, water, everywhere
nor any drop to drink.

Samuel Taylor Coleridge 1772-1834. The Ancient Mariner. ii. 121-122.

INTRODUCTION

The effect of surgery on changes in energy expenditure is controversial. Some groups have reported little alteration in energy expenditure following operation (128,393) while others have reported variable increases up to 25% (130,253,394-396). There has been no report on changes in energy expenditure following cardiac surgery or on the ability of intraoperative hypothermia to modify any such alterations.

The Doubly Labelled Water (DLW) technique permits measurement of total energy expenditure (TEE) by administration of stable isotopes of hydrogen and oxygen (224-230). The technique is based on the observation that oxygen is lost from the body in respired carbon dioxide and in water, while hydrogen is lost only in water (223-226). It follows that any difference in the turnover of isotopic hydrogen and oxygen labelled water is equivalent to carbon dioxide production from which energy expenditure can be calculated. The technique provides an integrated assessment of TEE over specific time periods in contrast to the factorial computations of energy expenditure derived from indirect calorimetry.

This is the first report in the literature of the use of DLW to measure energy expenditure in uncomplicated major surgery.

PATIENTS and METHODS

The DLW study was performed in 16 patients undergoing coronary artery surgery who were subsequently randomised to an intraoperative blood temperature of 28°C or 20°C.

As described in Chapter 3, TEE was measured in 10-day periods before and after surgery.

To examine the relationship of TEE to basal metabolic rate (BMR) two patients underwent indirect calorimetry three times in the preoperative period. Attempts to measure BMR in the same patients in the postoperative period were discontinued as the patients were unable to lie comfortably for the forty minutes required to perform indirect calorimetry, due to discomfort from their sternotomy wounds.

Calculations:

The principles of the DLW method (223-226), including theoretical assumptions and practical considerations, are detailed in Chapter 3.

Lean body mass (LBM) was calculated assuming constant hydration: LBM = Total Body Water (TBW)/0.73 assuming that lean tissue contains 73% water while fat contains negligible amounts (233).

Energy expenditure was measured using Weir's formula (221), after calculating total CO_2 production from the formula of Lifson and Mclintock (224,234).

1. Because the DLW method provides a measure of CO_2 production, rather than O_2 consumption, the calculation of TEE requires some estimation of the average respiratory quotient (RQ) over the study period. Although RQ values may vary widely over short periods, mean values show little variation over longer periods (397,398) except in cases of severe energy imbalance. As in other studies using DLW, a mean RQ 0.85 was assumed over

the study period (398). Even in the unlikely situation of the true RQ having been 0.7 or 1.0 over the study period, this would still have produced a maximum error of +/- 3% in TEE (398) as explained on Page 80.

2. The calculations used to estimate CO_2 production include a 4% correction for deuterium fractionation at epithelial surfaces assuming that 50% of water output is lost as vapour (224,234).

3. There was concern in the preoperative component of the study that although all patients came from the West of Scotland, different sources of fluid intake might vary in background isotope enrichment and therefore produce changes in the isotopic abundance of body water after administration of DLW. This concern was reduced by measuring the baseline enrichments of the patient's urine prior to administration of DLW and also by providing a sufficient dose of DLW so that background fluctuations would have relatively little influence on the final enrichment of the body water pools. In the postoperative period all study patients received fluid from the same source and the natural abundance of isotopes in the urine of other patients receiving fluid from the same source, but not DLW, showed only minor variations.

4. The rate of CO_2 production was derived from differences in the isotope decay constants multiplied by TBW, measured in moles (224). There was no significant difference in the body water pool of the study population over the experimental period. In any case, it has been demonstrated that even a 50% change in TBW would affect calculations of energy expenditure by a maximum of 10% (399).

STATISTICAL ANALYSIS

Statistical analysis was based on a paired t-test to compare serial differences within the same group and an unpaired t-test to compare differences between groups. Differences were considered statistically significant when the probability of their arising by random sampling error was less than 1 in 20 ($p<0.05$) and highly significant if this probability was less than 1 in 100 ($p<0.01$).

RESULTS

Results are presented in Tables 13.1, 13.2, 13.3 and 13.4.

There were nine patients in the 28°C group and seven in the 20°C group. One patient randomised to the 20°C group had only a single vessel suitable for coronary grafting at operation, and it was therefore decided prudent to cool the blood temperature to 28°C rather than 20°C. The 20°C group received significantly more grafts than the 28°C group resulting in a significant increase in the cross-clamp and bypass times in this group (Table 13.1).

The groups were very similar with respect to body weight and LBM, calculated from TBW (Table 13.2).

Table 13.3 gives the calculated first-order rate constants for ^{18}O and ^{2}H decay (k_O and k_H) and Table 13.4 the resulting rates of CO_2 production from which TEE has been calculated (Table 13.2). The mean preoperative TEE was similar in both groups. The mean difference of the product of the oxygen and hydrogen decay rates (k_O - k_H) and body water was greater in the postoperative period than in the preoperative period but not significantly so. This difference is equivalent to the rate of CO_2 production in moles and can be converted into energy expenditure as described in Chapter 3. There was a non-significant increase in the mean 10-day postoperative TEE, calculated in total

calories, in both groups, of 6.7% in the 28°C and 5.1% in the 20°C group. When changes in postoperative TEE were calculated according to LBM the increases were respectively 5.8% and 3.3%.

In two patients the mean BMR, calculated from three daily indirect calorimetry measurements, was 1530 (1510, 1530, 1550 Kcal/day) and 1710 (1700, 1710, 1720 Kcal/day) Kcal/day. The respective figures for mean TEE in these patients over the same period were 2023 and 2230 Kcal/day being respectively 32% and 30% higher. Attempts to repeat BMR measurements in the same patients in the postoperative period were abandoned as neither patient could lie comfortably for the duration of the study (minimum of 40 minutes).

DISCUSSION

Alteration in the metabolic rate in a variety of disease states was first described by Dubois in 1924 (133) and following trauma by Cuthbertson in 1932 (175). There is still, however, no consensus on the effect of major surgery on energy expenditure. Kinney's group reported minimal changes in energy expenditure following elective surgical procedures but a gradual increase in the metabolic rate with increasing severity of injury (128,393). In contrast, other groups have reported modest increases in postoperative energy expenditure of up to 10% (394,395) while other workers have reported increases of up to 25% (130,253,396).

The reason for such discrepancies include differences in the severity of the clinical illness and the type of operative procedure, the pre-existing nutritional status of the patients, and the method of nutritional support employed, as all are known to influence energy expenditure (124). Another explanation for such differences can be found in the methods used to measure energy expenditure. In the

hospital setting energy expenditure is usually calculated from an estimation of resting energy expenditure (REE) as it is often impractical to determine the BMR. The latter requires that the subject has been totally fasted for 12 hours, is studied in the early morning in a darkened, quiet room and is completely at rest in a recumbent position (400). These requirements are often impractical in the clinical situation and it is more common to measure REE which includes the BMR, energy required for the digestion of food (specific dynamic action of food) and minimal physical activity (400). REE is calculated by measurement of indirect calorimetry over a thirty minute period, multiplication of this figure by 48 to give a value over 24 hours, and adjustment of the resulting figure by an "activity factor" to provide an estimate of TEE (128). A fundamental criticism of these techniques is that the true relationship of BMR or REE to TEE is unknown.

A further practical difficulty with indirect calorimetry is the inability of many patients to tolerate nose clips or face masks. This may result in alterations in the respiratory pattern and consequently calculations of energy expenditure (256). Hood or canopy systems ameliorate, but do not eliminate this problem (256, 401).

Another reason for the variability in the effects of surgery on postoperative energy expenditure reported in the literature is, presumably, the practical difficulty of ensuring that the patient is not incapacitated by wound discomfort. In the current study two patients who had completed three indirect calorimetry runs uneventfully in the preoperative period could not tolerate the procedure after surgery, due to discomfort from their sternotomy wound. This is one of the major advantages of the DLW technique which merely requires collection of a small quantity of body fluid - in this

case urine - for calculation of energy expenditure.

Further concerns about the validity of indirect calorimetry measurements include the potentially large errors associated with the current practice of establishing a patient's REE from a single indirect calorimetry measurement which may be considerably higher than second or third measurements (402). This was not evident, however, in either patient in the current study who performed three preoperative indirect calorimetry runs where the values were remarkably similar.

The DLW technique can circumvent many of these problems. DLW is a non-invasive, stable isotope technique for measuring TEE over specific time periods with the advantages of being theoretically simple, safe and of minimal inconvenience to the patient. The method was described in 1955 (224), used for the study of energy expenditure in small animals since then (225), and applied to man by Schoeller and van Santen in 1982 (226). The technique involves the administration of two stable (and, therefore, non-radioactive) isotopes, $^{2}H_2O$ and $H_2{}^{18}O$, as labelled water and is based on the fact that oxygen in body water is in equilibrium with that in bicarbonate (and therefore CO_2) through the action of carbonic anhydrase (223). After mixing with body water ^{2}H is lost from the body only as water, whereas ^{18}O is lost both as water and CO_2, so that the difference in the rate of excretion of the two isotopes in body fluids is due to CO_2 production from which energy expenditure can be calculated. The DLW technique has been demonstrated to have an accuracy to within 2%-5% of CO_2 production rates calculated by whole-body indirect calorimetry (227-230). Furthermore the initial dilution of either isotope in body water reflects the body water pool size and therefore allows estimation of body composition.

Despite the obvious attractions of the DLW technique, clinical

application has been delayed because of the expense of the ^{18}O isotope. While deuterium labelled water is cheap, sufficient ^{18}O isotope for the current study cost approximately 300 pounds per patient (for both phases of the study). With increasing popularity of the technique and expanding facilities for isotope production, costs are likely to fall. Most studies to date in humans have examined healthy volunteers, babies, pregnant women and patients receiving total parenteral nutrition. DLW has been applied to only one surgical population to investigate the effects of operation on energy expenditure but interpretation was complicated by the fact that all seven patients were female with active Crohn's disease and required total parenteral nutrition (396). That study reported a 25% increase in postoperative energy expenditure.

There has been no previous study of alterations in energy expenditure in patients undergoing cardiac surgery or the effects of different levels of intraoperative hypothermia on any such changes. The current report is the largest study of DLW to date and demonstrated a postoperative increase in TEE by 6.7% in the 28°C group and by 5.1% in the 20°C group. When calculated according to LBM the postoperative increases were respectively 5.8% and 3.3%.

It is probable that the modest postoperative increase in TEE in the current study was due predominantly to an increase in BMR as general activity levels were reduced in the postoperative period. Attempts to measure BMR in the postoperative period were abandoned as the patients could not lie comfortably at rest because of discomfort from their sternotomy wound. It is also possible that at least part of the increase in TEE was due to withdrawal of beta-blockade therapy in the postoperative period. As discussed in Chapter 5 beta-blockers may reduce energy expenditure through anti-sympathetic actions.

Although the exact control of changes in the post-traumatic metabolic rate is not fully elucidated, the counter-regulatory hormones are strongly incriminated (386-388). Infusion of counter-regulatory hormones in healthy volunteers can mimic some components of the metabolic response and in particular an elevation in energy expenditure (386-388). The increase in postoperative TEE was slightly less in the 20°C group and could be consistent with the smaller postoperative increases in counter-regulatory hormones observed in this group (Chapter 10).

In two patients studied six times by indirect calorimetry, the daily BMR was remarkably constant and approximately 70% of the TEE measured by DLW. The BMR is generally thought to account for 75%-90% of TEE (124). Although some workers have recommended a postoperative caloric intake 1.76 times the BMR (253), the current results are in accordance with the recommendations of Long and colleagues, who suggested postoperative energy requirements can be adequately met with a caloric intake 1.24 times the BMR (403).

SUMMARY and CONCLUSIONS.

TEE was measured using a novel stable isotope technique over two 10-day periods, pre and postoperatively, in 16 patients randomised to an intraoperative blood temperature of 28°C or 20°C during coronary artery surgery.

The BMR, measured three times preoperatively in two patients, was remarkably constant and accounted for 70% of measured TEE.

There was a modest increase in postoperative TEE in both groups but no significant difference between the groups. The postoperative rise in TEE was assumed to result predominantly from an increase in BMR.

NUMBER OF PATIENTS	9	7	
AGE	53(2)	53(3)	NS
HEIGHT (cm)	175(1)	170(2)	NS
WEIGHT (Kg)	79(3)	76(6)	NS
BYPASS TIME (mins)	78(8)	99(6)	<0.05
CROSS CLAMP TIME (mins)	46(5)	63(3)	<0.05
NUMBER OF GRAFTS	2.7(0.3)	3.6(0.3)	<0.05
LOWEST BLOOD TEMPERATURE (^{o}C)	28(1)	20(1)	<0.001

Table 13.1 Clinical and operative features of the two temperature groups.

	WEIGHT (Kg)	TBW (Kg)	LBM (Kg)	PREOP (Kcal /day)	POSTOP (Kcal /day)	PREOP (Kcal /Kg/ day)	POSTOP (Kcal /Kg/ day)	PREOP (Kcal/ KgLBM /day)	POSTOP (Kcal/ KgLBM/ /day)
					28°C				
	80	39	53	1779	2153	22	27	33	40
	67	49	66	2384	2657	36	40	36	40
	80	40	55	2331	2810	29	35	43	52
	78	36	49	2827	1430	36	18	57	29
	63	32	44	2080	1863	33	30	47	42
	85	45	61	2589	2965	30	35	42	49
	87	49	67	2075	2408	24	28	31	36
	86	40	55	1377	2608	16	30	25	48
	81	51	69	1973	1815	24	22	28	26
MEAN	79	42	58	2157	2301	28	29	38	40
SD	8	7	9	435	517	7	7	10	9
SE	3	2	3	145	172	2	2	3	3
paired t-test				NS		NS		NS	
					20°C				
	63	30	41	1946	1514	31	24	48	37
	100	51	70	2073	2327	21	23	30	33
	78	49	66	2024	2425	26	31	30	36
	64	34	46	2033	1789	32	28	44	39
	89	51	70	3552	3401	40	38	51	49
	82	44	61	2230	2207	27	27	37	36
	59	38	53	1913	2914	32	49	36	55
MEAN	76	42	58	2253	2368	30	31	39	41
SD	15	9	15	582	640	6	9	8	8
SE	6	3	4	220	242	2	3	3	3
paired t-test				NS		NS		NS	

TABLE 13.2 Weight, total body water (TBW), lean body mass (LBM), and changes in energy expenditure expressed as total calories and related to LBM. There was no significant differences between the groups.

	K_{O-1}	K_{D-1}	$[K_{O-1}-K_{D-1}]$	K_{O-2}	K_{D-2}	$[K_{O-2}-K_{D-2}]$
			28°C			
	0.151583	0.133809	0.017774	0.143684	0.123376	0.020308
	0.150733	0.131970	0.018763	0.145453	0.125230	0.020223
	0.120930	0.100340	0.020590	0.091392	0.068223	0.023169
	0.179866	0.130295	0.027472	0.172006	0.155320	0.016686
	0.121835	0.099536	0.022299	0.090244	0.070852	0.019392
	0.091396	0.062133	0.019263	0.093129	0.071066	0.022063
	0.084357	0.069598	0.014759	0.089777	0.072892	0.016885
	0.148266	0.133853	0.014130	0.099883	0.078053	0.021830
	0.140840	0.125304	0.015536	0.117087	0.103171	0.013916
MEAN	0.114550	0.109649	0.018954	0.115850	0.096465	0.019386
SD	0.047279	0.028261	0.004191	0.030640	0.031729	0.003010
SE	0.015759	0.009421	0.001397	0.0.0213	0.010576	0.001003
			20°C			
	0.096312	0.074598	0.022014	0.067871	0.051194	0.016777
	0.101887	0.087055	0.014832	0.083310	0.067602	0.015708
	0.093326	0.078455	0.014871	0.092151	0.074927	0.017224
	0.136878	0.115281	0.021597	0.102124	0.083675	0.018449
	0.109659	0.086213	0.023446	0.097075	0.074867	0.022208
	0.092423	0.075076	0.017347	0.071548	0.054981	0.016567
	0.126303	0.108106	0.018197	0.094211	0.069465	0.024766
MEAN	0.108112	0.089255	0.018900	0.086898	0.068101	0.018814
SD	0.017337	0.016224	0.003496	0.013083	0.011511	0.003378
SE	0.006553	0.006132	0.001321	0.004944	0.004350	0.001277

TABLE 13.3 Oxygen and deuterium decay constants pre and postoperatively in the two groups. There was no significant difference in the constants before and after surgery.

	1-rCO_2 (mole/ day)	2-rCO_2 (mole/ day)	1-rCO_2 (mmole/ Kg/day)	2-rCO_2 (mmole/ Kg/day)	1-rCO_2 (mmole/ KgLBM/day)	2-rCO_2 (mmole/ KgLBM/day
			28°C			
	14.16	17.14	176.99	214.20	265.15	320.91
	18.98	21.14	283.26	315.56	285.56	318.12
	18.56	22.36	231.97	279.55	340.42	410.25
	22.50	11.38	288.44	145.90	456.38	230.86
	16.56	14.82	262.86	235.30	374.24	335.00
	20.60	23.60	242.36	277.63	337.78	386.94
	16.51	19.16	189.78	220.28	245.43	284.88
	10.96	20.76	127.40	241.38	199.60	378.17
	15.70	14.44	193.80	178.30	226.69	208.57
MEAN	17.17	18.31	221.87	234.23	303.47	319.30
SD	3.46	4.11	53.80	52.60	81.22	68.77
SE	1.15	1.37	17.93	17.53	27.07	72.92
			20°C			
	15.49	12.05	245.82	191.21	378.00	294.02
	16.50	18.52	164.96	185.16	236.23	265.16
	16.10	19.30	206.46	247.41	242.22	290.26
	16.18	14.24	252.81	222.44	350.97	308.81
	28.27	27.07	317.63	304.12	404.70	387.48
	17.75	17.57	216.45	214.23	292.57	289.57
	15.23	23.19	258.06	393.07	289.04	440.25
MEAN	17.93	18.84	237.45	251.09	313.39	325.08
SD	4.63	5.09	47.99	74.27	65.79	63.84
SE	1.85	1.92	18.14	28.07	24.87	24.13

TABLE 13.4 Mean daily carbon dioxide production pre and postoperatively in the two groups. There was no significant difference in carbon dioxide production before and after surgery or between groups.

CHAPTER 14

CLINICAL OUTCOME

CHAPTER 14

CLINICAL OUTCOME

Primum non nocere

Latin maxim from Hippocratic school (circa 450 BC).

INTRODUCTION

Improvements in surgical practice and anaesthetic techniques, accompanied by refinements in extracorporeal perfusion technology, have led to a marked fall in the mortality of elective coronary artery surgery over the last decade to approximately 1-2% (404). The use of systemic hypothermia is a standard part of the CPB technique for coronary artery surgery, largely influenced by the need to ensure adequate myocardial protection. After almost three decades of open heart surgery, however, there is no consensus as to the optimal intraoperative temperature and various levels of systemic hypothermia from 30°C to 20°C have been recommended (11-14).

The basic aim of this thesis was to investigate the effects of two levels of intraoperative hypothermia (28°C and 20°C) on the metabolic response to CPB. Although it was not a fundamental objective of these studies to determine the effect of different levels of intraoperative hypothermia on the postoperative clinical course, this chapter examines the effects of an intraoperative blood temperature of 28°C or 20°C on subsequent clinical outcome.

PATIENTS and METHODS

Sixty of the patients studied in this thesis were randomised to an intraoperative blood temperature of 28°C or 20°C.

The postoperative course of each patient was monitored for complications until death or discharge from hospital. In particular the following parameters were recorded (and where necessary defined).

CLINICAL COURSE

VENTILATION TIME: postoperative period of mechanical ventilation in hours.

INOTROPE REQUIREMENTS: the administration of dopamine in inotropic quantities (> 5 ug/Kg/minute) for a systolic blood pressure, measured by radial artery catheter, consistently less than 100 mmHg and accompanied by poor peripheral perfusion and oliguria.

VASODILATOR REQUIREMENTS: the administration of vasodilator agents (sodium nitroprusside or glyceroltrinitrate) for a systolic blood pressure, measured by radial artery catheter, consistently greater than 140 mmHg in the early postoperative period despite adequate sedation and analgesia.

BLOOD TRANSFUSION: The total number of units of blood used during the perioperative period to ensure a haemoglobin value of > 10 g/dl.

COMPLICATIONS

DEATH

RE-OPENING: need for surgical re-exploration due to excessive bleeding, low cardiac output or cardiac arrest.

POSTOPERATIVE ARRHYTHMIA: sustained arrhythmia (usually atrial fibrillation) requiring drug treatment or cardioversion.

RESPIRATORY FAILURE: inability to wean a patient from mechanical ventilation within 24 hours of surgery or inability of patient to maintain self-ventilation after extubation, requiring a further period of mechanical ventilation (usually accompanied by a deterioration in

arterial blood gases and abnormal chest X-ray)

RENAL FAILURE: urine volume of <200 ml/24 hours despite diuretic and/or renal dopamine (ie <5ug/Kg/min) therapy.

PERIOPERATIVE MYOCARDIAL INFARCTION: a postoperative rise in creatine kinase > 2000 accompanied by ECG changes (eg q waves >0.1mm).

SEPTICAEMIA: spiking temperature and/or hypotension in the presence of positive blood cultures.

PNEUMONIA: clinical and radiological signs accompanied by positive sputum cultures and need for antibiotics.

URINARY TRACT INFECTION; quantitative culture of >100,000 organisms/ml.

WOUND DEHISCENCE: complete instability of the sternum requiring re-suturing.

WOUND INFECTION: discharge from wound, associated with positive culture of discharge.

DECUBITUS ULCER: sacral or heel sore.

OBVIOUS CEREBRAL DEFICIT: obvious cerebral insult with localising neurological signs (does not include postoperative confusion).

PULMONARY EMBOLUS: clinical signs confirmed by lung scan (VQ scan).

STATISTICAL ANALYSIS

Statistical analysis was performed using the Mann-Whitney U test to compare differences in age, body weight, number of grafts, cross-clamp and bypass times, ventilation times and blood requirements in the two groups. A chi-square test, with Yates's correction factor, was used to compare the significance of differences in the incidence of various complications between the groups. Differences were considered to be significant when the probability of their arising by random sampling error was less than 1 in 20 ($p<0.05$) and highly

significant if this probability was less than 1 in 100 ($p<0.01$).

RESULTS

A summary of relevant postoperative parameters and complications is given in Table 14.1, 14.2 and 14.3.

CLINICAL and OPERATIVE FEATURES (TABLE 14.1)

The groups were very similar with respect to age and body weight.

The 20°C group received significantly more grafts [3.2(0.7) v 2.7(0.8): $p<0.05$] resulting in a significant increase in the mean cross-clamp time by 11 minutes and the mean bypass time by 19 minutes.

POSTOPERATIVE PARAMETERS (TABLE 14.2)

Despite the longer cross-clamp and bypass times in the 20°C group, the mean postoperative ventilation periods were similar in the two groups and fewer patients in the 20°C group required inotropic support (Table 14.2).

Postoperative blood requirements were less, by approximately one unit of packed red cells (400 ml), in the 20°C group.

POSTOPERATIVE COMPLICATIONS (TABLE 14.3)

There was no significant difference in the incidence of postoperative complications between the groups except for a reduction in the incidence of atrial fibrillation in the 20°C temperature group (1 v 6; $p< 0.05$).

DISCUSSION

Various levels of intraoperative hypothermia have been proposed

for coronary artery surgery (11-14), effective myocardial protection being the prime consideration. There is, however, little objective evidence to demonstrate the overall superiority of any particular level of systemic cooling. The aim of this chapter was to determine any beneficial or prejudicial effect on clinical outcome of an intraoperative blood temperature of 28°C or 20°C.

The temperature groups were well matched with respect to age and body weight. The 20°C temperature group received significantly more grafts than the 28°C group, however, producing a significant increase in the mean cross clamp time (by 11 minutes) and the mean bypass time (by 19 minutes). This was unexpected as the patients were randomised only on the basis that they required at least two coronary grafts. It is a matter of speculation whether the surgeon's prior knowledge of the allocated intraoperative temperature may have influenced his decision on the number of vessels requiring bypass grafts.

Clinical outcome was defined in easily measurable terms of postoperative recovery and occurrence of complications. Length of hospital stay was not used as a parameter of outcome because patients came from all over Scotland and final discharge was frequently determined by the practice and geographical location of the referral centre.

There was no death in either temperature group. The immediate postoperative recovery pattern was similar in the two groups in terms of ventilation periods, the majority of patients being extubated within 12 hours of surgery. Despite a longer bypass and cross clamp time in the 20°C group, only three patients (10%) required inotropic support compared to six patients in the 28°C group (20%) although this difference failed to reach conventional levels of statistical significance.

All forms of arrhythmia occur after cardiac surgery and the most common are supraventricular tachycardias, particularly atrial fibrillation, which occurs in up to 20% of patients (213). The most frequent predisposing factor for arrhythmia is hypokalaemia and therefore in this study only sustained arrhyhmias occurring in the presence of a serum potassium between 4.5-5.5 mmol/L and requiring digitalisation or other drug therapy are included. There was a significantly lower incidence of atrial fibrillation in the 20°C temperature group (1v6: $p<0.05$). The pathophysiology of postoperative atrial arrhythmias remains controversial but inadequate myocardial protection of the right atrium has been incriminated (405). It is feasible that a more profound level of intraoperative hypothermia enhanced myocardial protection by a direct effect and/or a reduction in non-coronary collateral blood.

Hypertension after coronary artery surgery is so frequent that it may be considered the rule rather than the exception. Numerous explanations have been advanced including inadequate sedation and analgesia, increased peripheral vascular resistance (406) and increased catecholamine secretion (147). As discussed in Chapters 9 and 10 there were greater increases in catecholamine secretion in the 28°C group. In the postoperative period 57 of the 60 patients studied required vasodilator therapy. As the choice of vasodilator sodium nitroprusside or glycerol trinitrate was left to the discretion of the anaesthetist, the total requirements of both groups is not directly comparable and it is unknown whether attenuation of the catecholamine response in the colder group reduced the total requirement for vasodilator therapy.

There was a reduction in the mean requirement for blood transfusion, of approximately one unit, in the postoperative period in

the 20°C group although this failed to reach conventional levels of statistical significance. It is known that a more profound level of intraoperative hypothermia significantly impairs blood coagulability, predominantly through an effect on platelets (44), and rewarming to 37°C prior to discontinuation of CPB presumably adequately reverses the tendency to reduced coagulability in the colder group. There was an unusually high incidence of three re-openings in patients in the 28°C group due to excessive bleeding and none in the 20°C group.

There was one case of respiratory failure in a patient whose blood gases deteriorated after the second postoperative day due to the adult respiratory distress syndrome. This patient required a further seven-day period of mechanical ventilation.

The incidence of perioperative myocardial infarction is reported at 0-30% after coronary artery surgery depending on the criteria used for diagnosis (213). The criteria in this centre to diagnose perioperative myocardial infarction, a large rise in creatine kinase accompanied by changes in the electrocardiogram, are equivalent to those required for a "medical diagnosis" of myocardial infarct, and indicate transmural infarction. There was no definite case of perioperative infarct in this study (although on these criteria small non-transmural infarcts are unlikely to be detected).

Two patients in the 28°C group developed an unstable sternum requiring re-suturing. Two additional patients in each group developed minor sternal wound infections from which Staphylococcus epidermidis was isolated. The potential pathogenicity of this coagulase negative organism is being increasingly recognised (407).

Up to 60% of patients undergoing coronary artery surgery may develop neurological or neuropsychological deficits (408) but recognition of such abnormalities requires sophisticated

neuropsychological examination. Only one patient in this study developed an obvious cerebral deficit (409), this being a left-sided hemiparesis. The patient made an excellent recovery with no clinically obvious residual deficit.

SUMMARY and CONCLUSIONS

Clinical outcome was followed in 60 patients randomised to an intraoperative blood temperature of 28°C or 20°C. Despite a longer mean cross-clamp and bypass time in the 20°C group there was a trend towards reduced inotropic and blood requirements in this group. The only significant difference between the groups was a reduction in the incidence of postoperative atrial fibrillation in the 20°C group, presumably reflecting improved myocardial protection.

	28°C	20°C	
NUMBER OF PATIENTS	30	30	
AGE (years)	53.8 [5.1]	53.8 [5.8]	NS
WEIGHT (Kg)	77.5 [9.6]	76.2 [11.3]	NS
NUMBER of GRAFTS	2.7 [0.8]	3.2 [0.7]	$p < 0.05$
CROSS CLAMP TIME (minutes)	45.4 [16.5]	56.9 [18.8]	$p < 0.05$
BYPASS TIME (minutes)	79.8 [23.5]	99.1 [25]	$p < 0.05$

TABLE 14.1 Clinical and operative features of the two temperature groups expressed as mean and SD.

	28°C	20°C	
NUMBER OF PATIENTS	30	30	
VENTILATION TIME (HOURS)	11.1(0.9)	10.6(0.6)	NS
INOTROPIC SUPPORT	6	3	NS
VASODILATOR THERAPY	24	23	NS
BLOOD TRANSFUSION (UNITS)	4.1(0.5)	3.2(0.3)	NS

TABLE 14.2 Clinical features of the two temperature groups with respect to ventilation, requirements for inotropes and/or vasodilators and need for blood transfusion expressed as mean and SD.

	28°C	20°C	
NUMBER OF PATIENTS	30	30	
DEATH	0	0	
RE-OPENING	3	0	
RESPIRATORY FAILURE	0	1	
RENAL FAILURE	0	0	
PERIOPERATIVE MYOCARDIAL INFARCTION	0	0	
ATRIAL FIBRILLATION	6	1	$p < 0.05$
SEPTICAEMIA	0	0	
PNEUMONIA	1	1	
URINARY TRACT INFECTION	0	0	
WOUND DEHISCENCE	2	0	
WOUND INFECTION	2	2	
DECUBITUS ULCER	0	1	
NEUROLOGICAL SIGNS	0	1	
PULMONARY EMBOLUS	0	0	

TABLE 14.3 Incidence of complications in the two temperature groups.

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