AN INVESTIGATION
OF THE ANABOLIC ACTIONS OF
BIOSYNTHETIC HUMAN GROWTH HORMONE
AFTER INJURY BY BURNING

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To my wife, Georgina

ABSTRACT

Previous clinical trials in normal subjects and post-operative patients have shown that biosynthetic growth hormone preparations increase nitrogen retention. It has been suggested that their administration to injured patients may be beneficial.

A clinical trial is presented of twelve adult burned patients of whom six were allocated to receive biosynthetic human growth hormone (somatropin) and six to form a control group. Injury by burning is followed by increases in resting energy expenditure and urinary nitrogen excretion, accompanied by insulin resistance and glucose intolerance. There is a generalised fall in plasma protein concentrations, including the somatomedin, insulin-like growth factor-I. Somatropin administration causes no change in the rate of protein oxidation, the positivity of nitrogen balance or, either serum somatomedin or plasma protein concentrations. It causes an increase in the insulin-resistance already present in burned patients.

Two further studies are presented in which somatropin is compared with a placebo in both unburned and burned rats. Injury by burning causes weight-loss, an increase in urinary nitrogen excretion, a fall in the serum albumin and somatomedin concentrations, and a reduction in the strength of healing laparotomy wounds. Somatropin administration to unburned rats causes a small rise in the serum somatomedin concentration and a transient increase in wound-strength. It causes no increase in the positivity of nitrogen balance or weight-gain. Its administration to the burned rat causes no effect on the serum somatomedin concentration, nitrogen balance, weight-gain or wound-healing.

These three studies show that somatropin has no anabolic effect soon after injury by burning. I postulate that this failure reflects the changes observed after injury in somatomedin concentrations and the responsiveness of somatomedins to somatropin. I conclude that somatropin and related compounds are not suitable for use as anabolic agents soon after injury by burning.

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STATEMENT OF ORIGINALITY

None of the work in this thesis has been performed before. All the experiments described were designed and carried out by myself.

Since the development of biosynthetic human growth hormone (GH) preparations, a number of trials have been conducted which have investigated their anabolic activity in both normal subjects and post-operative patients. A description of the first clinical trial using a biosynthetic GH preparation in burned patients is contained in chapter 8.. Similarly, previous animal studies have investigated the anabolic actions of GH and its effect on wound-healing. Descriptions of the first studies of the actions of a biosynthetic GH preparation in animals injured by burning are contained in chapters 9. and 10..

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ADDITIONAL MATERIAL

Abbreviations

ACTH	Adrenocorticotrophic hormone
AEE	Activity energy expenditure
AUC	Area under the curve
BA	Burn area
BCAA	Branch-chain amino-acid
BEE	Basal energy expenditure (Harris-Benedict formula)
BSA	Body surface area
C	Control
CEE	Calculated energy expenditure (Curreri formula)
DNA	Deoxyribonucleic acid
ECW	Extracellular water
F	Fraction
FFA	Free fatty acid
GFR	Glomerular filtration rate
GH	Growth hormone
GHRH	Growth hormone releasing hormone
GRIH	Growth hormone release inhibiting hormone
ICW	Intracellular water
IGF	Insulin-like growth factor
IgG	Immunoglobulin-G
IL-1	Interleukin-1
iu	International unit
IV	Intravenous
IVGTT	Intravenous glucose tolerance test
N	Nitrogen
NM	Nitrogen metabolism
NPE:N	Non-protein energy:nitrogen (ratio)
p	Partial pressure
PBD	Post-burn day
PCV	Packed cell volume
PEC	Protein energy contribution
PEM	Protein-energy malnutrition
PPF	Plasma protein fraction
RBP	Retinol-binding protein
REE	Resting energy expenditure
RPF	Renal plasma flow
RQ	Respiratory quotient
SPD	Static plasma deficit
STPD	Standard temperature, pressure and dry
T	Time in minutes after the start of a test (eg. T_5)
TBW	Total body water
TBPA	Thyroxine-binding pre-albumin
TBSA	Total burn surface area
TER	Transcapillary escape rate
TPN	Total parenteral nutrition
V	Volume
VE	Ventilation equivalent

(See appendix 7. for statistical abbreviations)

Growth hormone preparations

Pit-GH	Pituitary-derived GH
Somatrem	Biosynthetic methionyl-human GH
Somatropin	Biosynthetic human GH

Symbols

§	Section
#	Number
0-5'	Zero to five minutes

Units and powers

C	Centigrade	M	Mega	10^{6}
cal	Calorie (4.186J)	k	Kilo	10^{3}
D	Dalton	d	Deci	10^{-1}
hr	Hour	c	Centi	10^{-2}
g	Gram	m	Milli	10^{-3}
J	Joule	u	Micro	10^{-6}
l	Litre	n	Nano	10^{-9}
m	Metre	p	Pico	10^{-12}
min	Minute			
N	Newton			
Osm	Osmole			
yr	Year			

PART-I

INTRODUCTION

PREAMBLE

In the introductory part to follow, I will review the literature which has prompted me to study the anabolic activity of biosynthetic human growth hormone after injury by burning. In chapters 1. and 2., I will outline the metabolic changes that occur after injury and discuss why protein depletion may occur rapidly in burned patients. In chapter 3., I will consider the consequences of malnutrition, with particular reference to wound-healing, and examine the evidence that nutrition can prevent or reverse these consequences. In chapter 4., I will review the recent advances that have been made in the nutrition of burned patients. In chapter 5., I will examine the effectiveness of anabolic agents in improving nutrition and discuss the development of biosynthetic human growth hormone. In chapter 6., I will review the physiology of growth hormone and discuss the role of the somatomedins in mediating its growth-promoting and anabolic actions. I will also examine its other actions, particularly those on carbohydrate metabolism and fluid balance. In chapter 7., I will examine in detail all the previous studies of its anabolic activity in man and its effects on wound-healing in animals. I will then discuss its potential for benefit in burned patients.

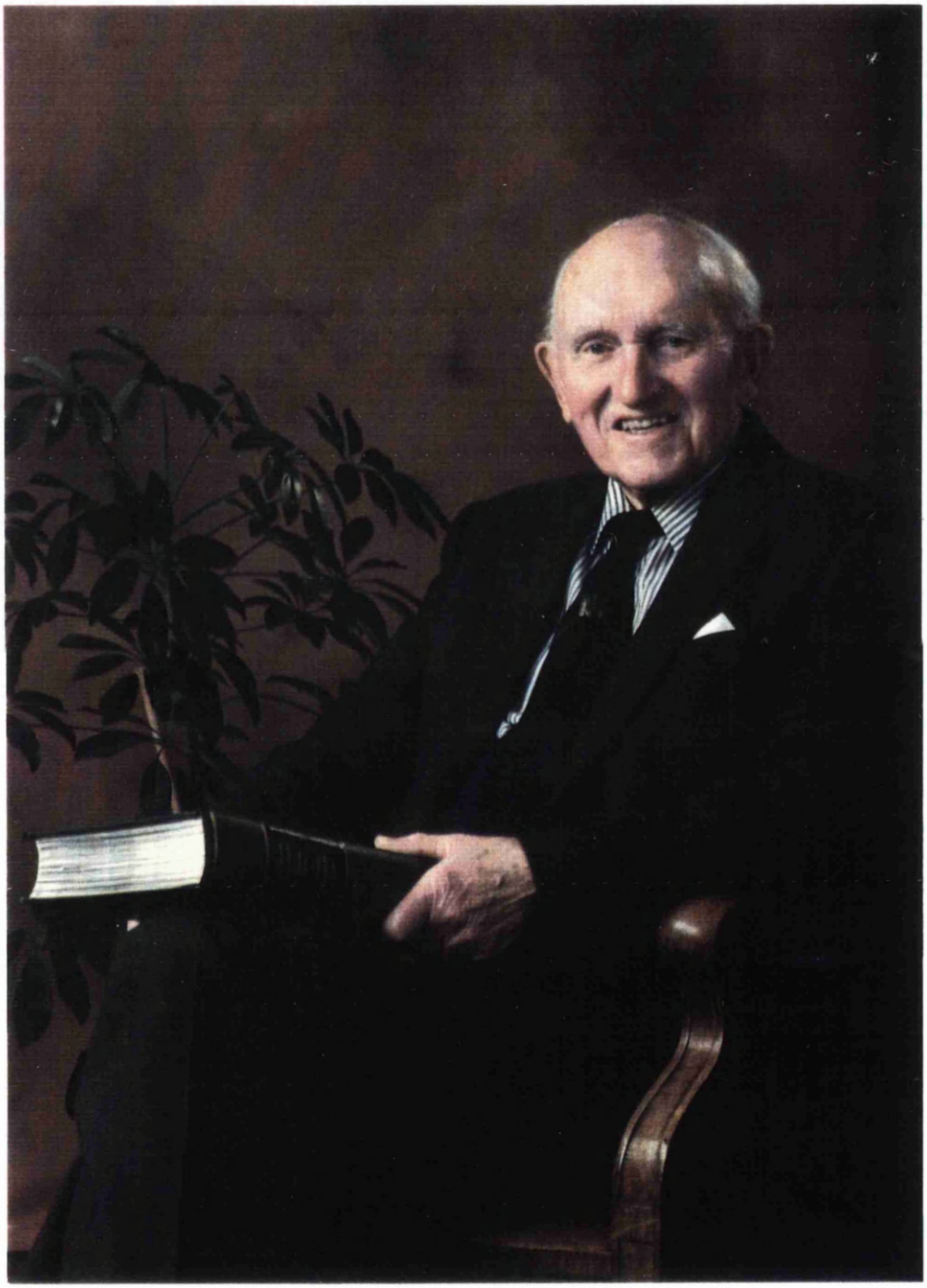
1.1 Ebb and flow

The metabolic response to trauma was first described in detail by Sir David Cuthbertson (Figure 1.1) in a series of studies published between 1930 and 1942 in which he detailed the principal changes which occur following injury [Cuthbertson 1930, 1931, 1932, 1936, Cuthbertson et al. 1939, Cuthbertson 1942].

It has been recognised for many years that there is a depression of metabolism following injury, characterised by a lowering of core temperature and metabolic rate [Meltzer 1908, Henderson 1917]. Although haemorrhage, exposure to cold, pain and anxiety probably contribute to these changes, early studies in experimental animals showed that the fall in metabolic rate was related to the severity of injury and could not consistently be reproduced by haemorrhage alone [Aub 1920]. This response was termed the "ebb phase" by Cuthbertson [1942].

The ebb phase is now recognised to result from a central inhibition of thermoregulation [Stoner 1972] and is well documented in animals after trauma and burns [Lieberman and Lansche 1956, Miksche and Caldwell 1968, Stoner 1969, Herndon et al. 1978, Arturson and Hjelm 1984]. Serum from burned rats given to normal animals induces a fall in oxygen consumption, indicating the presence of a circulating depressant factor [Caldwell et al. 1971]. Cuthbertson [1932] stated that heat production was reduced immediately after injury in patients with fractures but this has not been unequivocally demonstrated in man, although both the core temperature and critical temperature for heat production are lowered early after injury and are related to the severity of trauma [Little and Stoner 1981].

Figure 1.1 Sir David Cuthbertson.



The phase of depressed metabolism persists until resuscitation and is followed by the "flow phase" [Cuthbertson 1942] which is characterised by a raised core temperature and metabolic rate with increased protein breakdown. The rise in the rate of nitrogen excretion after trauma was first noted by Wertheimer et al. [1919] but Cuthbertson [1930] was the first to recognise that this increase was an integral part of the metabolic response to trauma and resulted from increased muscle catabolism. In his subsequent studies, he showed that the rise in core temperature, metabolic rate and nitrogen excretion paralleled each other and were related to the severity of injury [Cuthbertson 1932, 1936]. These observations were soon corroborated in burned patients and it was commented that "the nitrogen excretion (in a burned patient) was so high that it was impossible to bring the patient into nitrogen-balance even though a high caloric, high protein diet was ingested" [Lucido 1940].

Cope et al. [1953] showed that the metabolic rate or resting energy expenditure (REE) rises to a maximum level at about ten days after burning when it is commonly between 30 and 60% above that in normal subjects, following which it declines as healing occurs. Duke et al. [1970] subsequently showed that the REE is increased by approximately 10% after elective surgery, between 15% to 30% after major fractures, between 20 and 50% during major sepsis and by up to 100% after major burns. The early observations of Cope et al. have been repeatedly confirmed [Harrison et al. 1964, Birke et al. 1972, Wilmore et al. 1974b, 1975a, Arturson et al. 1978, Aulick et al. 1979, Caldwell et al. 1981, Saffle et al. 1985, Turner et al. 1985, Matsuda et al. 1987, Allard et al. 1988]. The magnitude of response has been shown to be related in a curvilinear manner to the size of burn

with maximal increases in the REE occurring in patients with total burn surface areas (TBSA) of 50 to 60% or greater [Wilmore et al. 1974b, 1975a, Aulick et al. 1979] (Figure 1.2). Such is the magnitude of this response, it is frequently referred to as "burn hypermetabolism".

1.2 The genesis of hypermetabolism

A number of authors have suggested that hypermetabolism occurs in response to increased heat loss from the burn. This view is supported by the reduction in REE that follows the application of impermeable dressings to burns [Lieberman and Lansche 1956, Harrison et al. 1964, Neely et al. 1974, Caldwell 1976, Caldwell et al. 1981] and the management of patients in a warm environment [Arturson et al. 1978, Caldwell et al. 1981]. Nevertheless, patients remain hypermetabolic despite the use of these techniques (Figure 1.2) and these findings fail to explain the rise in REE that occurs after other forms of trauma. Other authors have suggested that hypermetabolism results from protein catabolism and the rise in core temperature that occurs after injury [Miksche and Caldwell 1967]. This fails to explain why these changes occur. Douglas Wilmore (Figure 1.3) has therefore suggested that the principal event is a resetting of hypothalamic thermoregulatory control [Wilmore et al. 1974b, 1976]. This is supported by Arturson et al. [1978] who showed that burned patients managed in a warm environment had a normal REE if corrected for their raised core temperature.

Figure 1.2 The relationship between REE and TBSA at three environmental temperatures. (i) Both normal men and burned patients had an increased REE at the lower temperature. (ii) At each temperature there was a curvilinear relationship between REE and TBSA with the maximum REE being reached in patients with a TBSA of between 50 and 60%. (iii) The REE in burned patients did not return to a normal value at a temperature of 33^0C (Reproduced from Wilmore et al. 1974b, with permission).

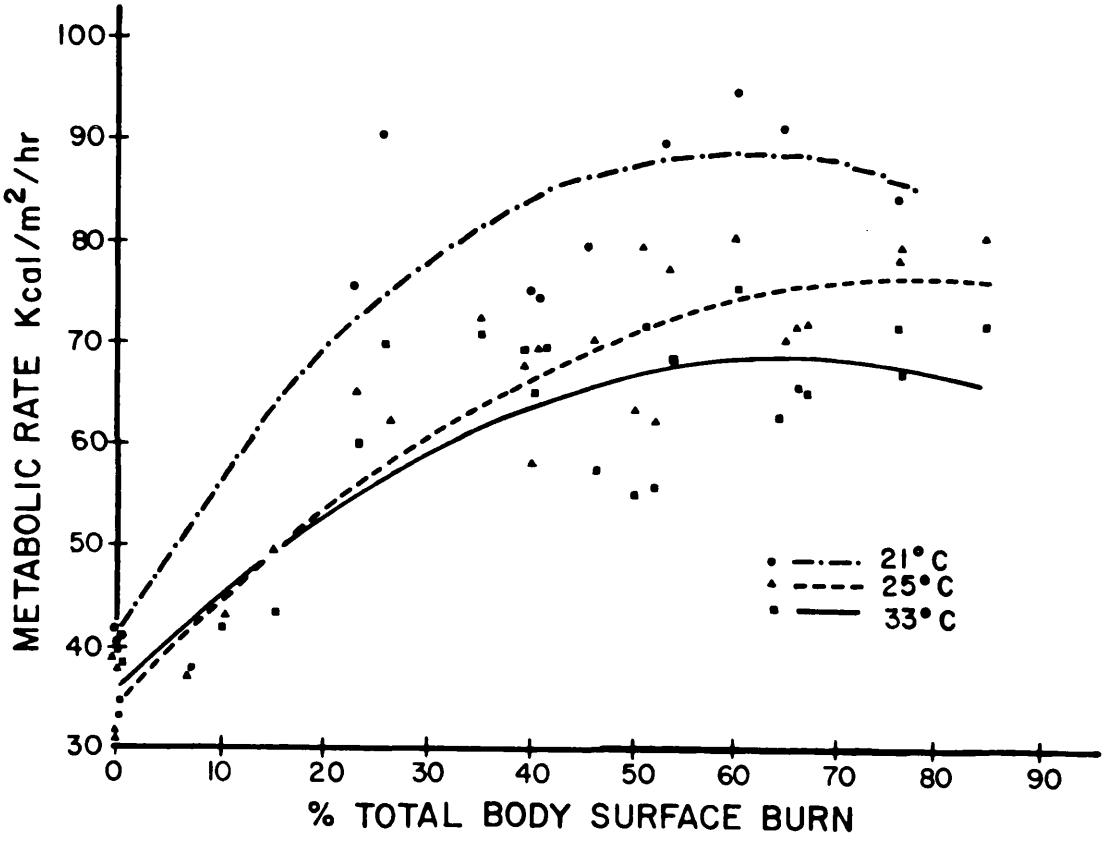


Figure 1.3 Douglas W. Wilmore.

Wolfe et al. [1987] have recently suggested that the rise in REE is secondary to substrate cycling. This is consistent with studies which have indicated that the burn-wound is an important factor contributing to the hypermetabolism that occurs in burned patients. The blood flow to full-thickness burns is near that in unburned limbs for two to three days after injury because of vascular thrombosis but flow rises with the formation of a vascular wound-bed [Gump et al. 1970, Wilmore et al. 1977]. Plethysmographic studies have shown that limb blood flow is best correlated with the size of limb-burn rather than TBSA and that blood flow is normal in unburned extremities. The cardiac index and oxygen consumption rise in proportion to the TBSA. Limbs with large burns have greatly increased rates of glucose uptake and lactate production whereas glucose uptake in uninjured limbs is normal. In contrast, oxygen consumption is comparable in uninjured and burned limbs [Aulick et al. 1977, Wilmore et al. 1977]. These studies indicate that much of the increase in cardiac output in burned patients is directed to the burn and that much of the peripheral utilization of glucose occurs there. These findings are consistent with the glycolytic metabolism of fibroblasts, leucocytes and epithelial cells within a healing wound [Im and Hoopes 1970]. It has been proposed that the rise in REE and therefore core temperature during the flow phase is the result of substrate mobilisation and cycling, and that in burned patients much of this occurs in response to the increased metabolic demands of the burn [Aulick et al. 1977, Wilmore et al. 1977, 1980].

1.3 Mediation of hypermetabolism

There is evidence that the hypermetabolic response is mediated by the counter-regulatory hormones; adrenalin, cortisol, glucagon and possibly growth hormone (GH). There is an immediate increase in the secretion of catecholamines and glucocorticoids following all forms of trauma. The magnitude and duration of the increases are proportional to the severity of trauma [Batstone et al. 1976, Chansouria et al. 1979, Coombes and Batstone 1982, Vaughan et al. 1982, Davies et al. 1984, Vaughan et al. 1985]. Plasma glucagon levels rise after severe burns, despite the prevailing hyperglycaemia but peak levels are seen only after three to four days after injury [Wilmore et al. 1974a, Batstone et al. 1976, Wachtel et al. 1978]. Growth hormone release is increased by stressful stimuli but its release early after burns is not documented and studies performed later after injury are contradictory (see §6.2).

The role of the counter-regulatory hormones during the flow phase has been clarified by a series of studies in which these hormones have been infused into normal subjects [Wilmore et al. 1974b, Rizza et al. 1979, Shamoon et al. 1981, Bessey et al. 1984, Hendler et al. 1984, Gelfand et al. 1984, Waldhausl et al. 1987]. The infusion of adrenalin is followed by increases in REE, alanine and free fatty acid (FFA) release, and lactate production but there is no significant increase in nitrogen excretion. Although glucose production is transiently increased, even in subjects who have been fasted for four days, there are sustained rises in the plasma insulin and glucose concentrations due to a persistent decrease in peripheral glucose uptake. The combined infusion of all three hormones produces an increase in both the REE and nitrogen excretion. The increase

in glucose production becomes sustained, glucose clearance is further lowered and there is an increase in plasma glucose which exceeds the sum of increments resulting from the hormones when given alone. This occurs despite a two to threefold increase in plasma insulin levels. The role of GH is unknown but its infusion, in addition to the other three hormones, causes a further rise in plasma insulin, FFA and ketone levels [Sherwin et al. 1983].

Further studies have indicated that inflammatory mediators, such as interleukin-1 (IL-1), also contribute to the hypermetabolic response. Although the combined infusion of the three counter-regulatory hormones results in many of the changes seen in hypermetabolic patients, other features such as fever and leucocytosis are absent. A proteolytic peptide has been isolated in the plasma of septic patients which may be a breakdown product of IL-1 [Clowes et al. 1983]. Etiocholanolone is a naturally occurring steroid metabolite which is known to induce IL-1 synthesis. Its administration to normal volunteers results in fever and leucocytosis [Watters et al. 1985] and in conjunction with hormonal infusion, its administration results in the full spectrum of changes seen in patients with sepsis or after severe injury [Watters et al. 1986].

1.4 Limitation of hypermetabolism

Although the precise cause of hypermetabolism has yet to be established unequivocally, it appears to consist of an obligatory, febrile component, which provides the burn with substrate and a non-obligatory, non-febrile component. There is agreement that the latter component can be safely and usefully limited by the use of occlusive dressings and by

managing patients in a warm environment. However, the merits of manipulating the former, which constitutes the normal physiological response to injury, is debatable. Wilmore et al. [1974b] showed that the administration of ß-adrenergic blockers significantly reduces the REE of burned patients but Wolfe et al. [1987] have not confirmed this finding. The administration of anti-inflammatory drugs does not result in a significant reduction in REE or muscle catabolism in burned rodents [Clark et al. 1984, Alexander et al. 1986] or protein catabolism in surgical patients receiving TPN [Shaw and Wolfe 1988], although the administration of acetaminophen (paracetemol) to burned children lowers core temperature [Childs and Little 1988]. There is some evidence that the blockade of histamine type-2 receptors reduces protein catabolism [Shaw and Wolfe 1988].

The stimuli that promote hypermetabolism can be limited by clinical measures such as the control of sepsis and pain which exacerbate the effects of injury. The incidence of sepsis can be reduced by the selective use of central venous catheters, isolating patients in laminar flow units, barrier nursing, frequent dressings and the use of topical antiseptics [Demling 1985]. Experimental studies in rats have shown that the application of antiseptic agents to the burn limits the rise in REE [Aulick et al. 1986]. Anxiety and pain cause further rises in the REE of burned patients [Arturson et al. 1978]. The use of epidural blockade during surgical procedures results in a reduction in nitrogen excretion [Shaw et al. 1987]. It is clinically evident that the humane use of anxiolytics and analgesics in burned patients reduces the stress associated with

procedures such as dressing changes and it is reasonable to assume that this also limits any rises in REE.

2 - MOBILISATION OF SUBSTRATE

2.1 Gluconeogenesis

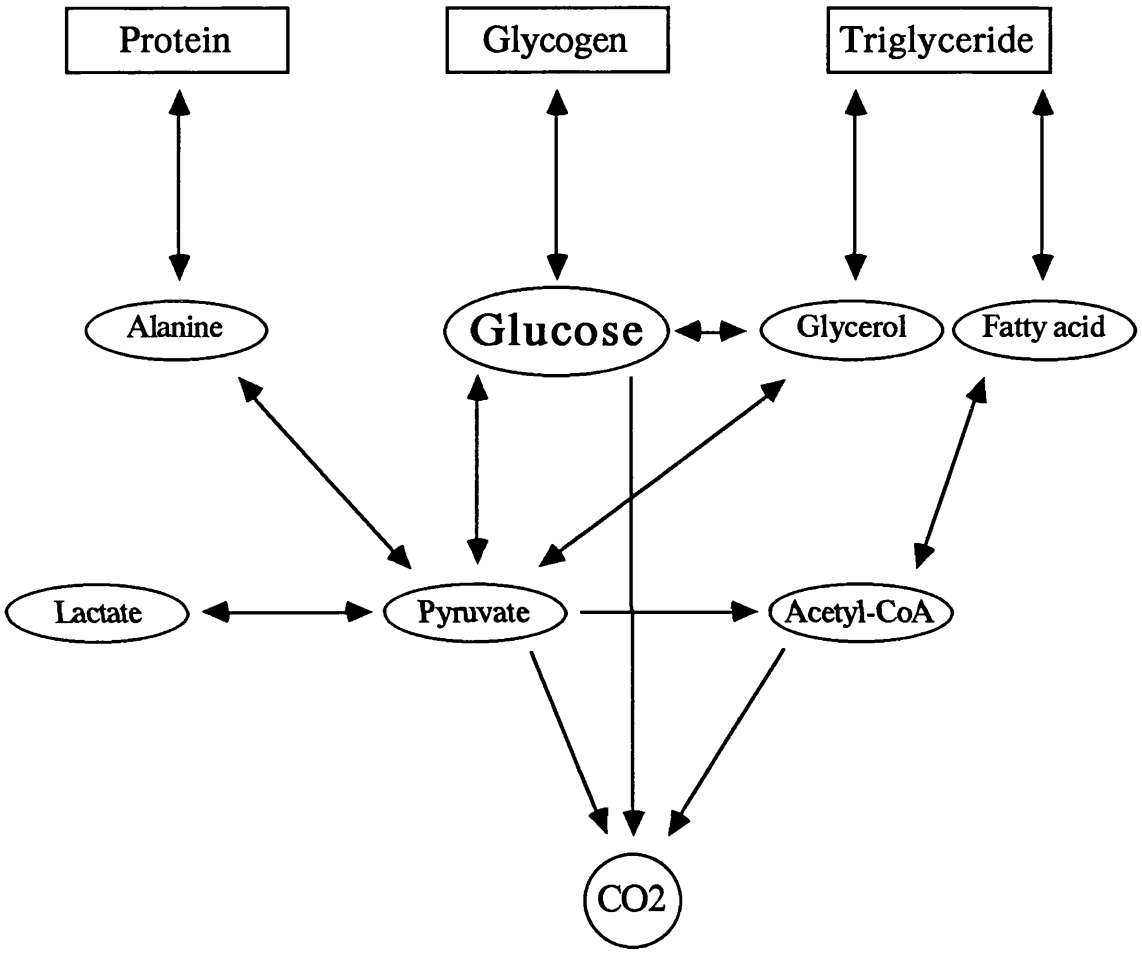
Patients with major burns have a 50% increase in the rate of glucose production which is paralleled by increased splanchnic blood flow, oxygen consumption and uptake of lactate, pyruvate and alanine [Wolfe et al. 1979b, Wilmore et al. 1980, Jahoor et al. 1986]. Glycogen stores are quickly depleted [Nilsson and Hultman 1973] and therefore the majority of this increased glucose production is due to gluconeogenesis [Wilmore et al. 1980]. This glucose is derived from a number of sources including lactic acid, amino-acids [Wilmore et al. 1980] and glycerol [Shaw and Wolfe 1989] (Figure 2.1). Gluconeogenesis is normally inhibited by an increase in blood glucose levels but during sepsis or after burns, glucose infusions fail to suppress hepatic glucose output, unlike normal subjects in whom there is a reversal of hepatic glucose output to a net uptake [Gump et al. 1974, Long et al. 1976, Allsop et al. 1978]. This loss of suppressibility may be caused by the increased availability of substrates occurring in a favourable hormonal environment.

2.2 Glucose metabolism

It has been recognised for many years that hyperglycaemia is common in burned patients [Davidson 1925] and that it is a characteristic feature of the hypermetabolic response to injury. Its occurrence reflects abnormalities in insulin metabolism.

Within the first six to twelve hours following a burn, basal plasma insulin levels may be low and the insulin response to an exogenous glucose load is markedly impaired [Allison et al. 1968, Batstone et al. 1976,

Figure 2.1 A simplified scheme of glucose metabolism.



Turinsky et al. 1977, Chansouria et al. 1979], as seen, perioperatively, in general surgical patients [Wright et al. 1974, Aarimaa et al. 1978]. This initial impairment of insulin release is due to the transient inhibitory effect of catecholamines due to alpha-adrenergic activity [Porte and Robertson 1973] and to the increase in peripheral insulin degradation that occurs in the first 24 hours after injury [Hoare et al. 1980]

With the establishment of the flow phase, basal insulin levels recover and may rise above normal due, in part, to the later, ß-adrenergic, stimulatory effect of catecholamines on insulin release [Porte and Robertson 1973]. However, patients continue to have impaired glucose disposal despite the presence of an exaggerated insulin response [Allison et al. 1968, Turinsky et al. 1977]. There are reductions in both the maximum glucose disposal rate and the rate of glucose clearance at all insulin concentrations compared with normal subjects [Thomas et al. 1979, Black et al. 1982]. This decrease in insulin-mediated glucose uptake occurs primarily in muscle and fat.

Peripheral glucose uptake, clearance and oxidation are increased in burned patients, despite the presence of insulin resistance and hyperglycaemia [Wolfe et al. 1979b]. It appears, therefore, that hyperglycaemia in burned patients is primarily due the increased glucose production which exceeds the concomitant increase in glucose clearance. Under basal conditions, most glucose uptake takes place in insulin-independent tissues at a rate which is dependent on the prevailing blood glucose. Significantly, glucose uptake in most of the cellular components of a wound is insulin-independent and it has been postulated by Black et al. [1982] that "the body's response following injury appears to selectively

direct glucose toward injured tissue to insure an ongoing nutrient source for wound repair". This suggestion is consistent with studies which have indicated that the burn is the major site of glucose utilization (see §1.2).

2.3 Fat breakdown

Although carbohydrate and protein oxidation are increased after injury, fat remains the major source of energy. Plasma free fatty-acid (FFA) and glycerol levels are elevated according to the severity of injury indicating increased lipolysis [Birke et al. 1972, Batstone et al. 1976, Stoner et al. 1979, Harris et al. 1982].

Severely injured patients or those with sepsis have, even when fasted, low plasma ketone levels [Birkhahn et al. 1981, Harris et al. 1982, Grecos et al. 1984] which indicates that much of the mobilised free-fatty acids are re-esterified rather than being oxidised. Recent studies have confirmed that increased triglyceride-fatty acid cycling occurs after injury [Shaw and Wolfe 1989] and that it contributes to the rise in REE in burned patients [Wolfe et al. 1987]. Despite this, there is an absolute increase in energy production from fat and Shaw and Wolfe [1989] have shown that glycerol is a major substrate for gluconeogenesis.

2.4 Protein breakdown

Skeletal muscle constitutes about 40% of total body mass and contains 80% of the free amino-acid pool. Alanine and glutamine are the major routes of amino-group release by skeletal muscle, alanine being the major transport compound to the liver. *In vitro* studies in muscles obtained

from fasted rats, two to three days after a 50% burn, have shown that the rate of muscle protein breakdown is increased whilst protein synthesis is either unchanged or decreased [Clark et al. 1984, Newman et al. 1984]. Studies in burned children using ^{15}N-labelled glycine have shown increased rates of both protein breakdown and synthesis which were significantly correlated with the TBSA. In patients with burns greater than 60%, the rates of both were approximately twice that seen in unburned children [Kien et al. 1978]. The higher synthetic rates in burned children may be explained by their large energy and protein intakes.

Although the absolute rate of protein utilization for energy production is increased in burned patients there is no change in the proportion of energy that is derived from protein. In both normal subjects and burned patients the protein energy contribution (PEC) is 15 to 16%, although in young, fit patients, with soft tissue trauma, it may rise to 20%. This implies that the increase in energy derived from protein parallels that from carbohydrate and fat [Duke et al. 1970].

2.5 Amino-acid release

As a result of increased muscle protein breakdown, there is an increased rate of amino-acid release from the periphery. It has been shown that total amino-acid release from the lower limbs is increased by between two and five times in patients following trauma, with sepsis or after burns. The rate of alanine release is correlated with the TBSA and oxygen consumption of the patient but is unrelated to limb blood flow or the extent of limb-burn [Aulick and Wilmore 1979, Clowes et al. 1980].

The relative molar proportions of the majority of amino-acids released are identical to those in muscle, indicating that their release results from simple proteolysis. The exceptions are alanine, which is released in excess of three to four times its molar percentage in muscle, and the branch chain amino-acids (valine, leucine, isoleucine), asparagine and aspartic acid which are released in reduced amounts. The latter five amino-acids, along with glutamic acid, can be transaminated in muscle to form alanine and glutamine [Goldberg and Chang 1978] and indicates that there is a net increase in the rate of muscle transamination [Clowes et al. 1980].

Despite the increase in peripheral amino-acid release, the plasma concentrations of the majority of amino-acids are reduced in burned patients compared with control subjects [Aulick and Wilmore 1979, Stinnett et al. 1982]. This is consistent with the increased rate of splanchnic uptake of amino-acids [Wilmore et al. 1980].

2.6 Nitrogen losses in burned patients

Wilmore et al. [1980] showed that 22.2% of glucose produced by gluconeogenesis in burned patients without sepsis was derived from amino-acids. These are deaminated, prior to their utilization, with the release of ammonia which is predominantly converted to urea via the urea cycle and excreted in the urine. The increase in amino-acid breakdown in injured and infected patients is therefore reflected by an increase in urinary nitrogen excretion. The rate of nitrogen excretion parallels the severity of injury [Duke et al. 1970]. In adult burned patients, who were receiving their calculated dietary requirements, the daily urinary nitrogen losses were

shown to be 11.1, 18.9 and 25.3g in those with a TBSA of 1 to 10%, 11 to 30% and 31 to 60% respectively [Kagan et al. 1982].

The increase in urinary nitrogen losses is exacerbated by protein losses from the burn-wound which have been estimated to be 5.9g/m^2 burn/hr from partial thickness burns and 9.8g/m^2 burn/hr from full thickness burns in the first three days after injury, decreasing to 2.9 and 5.1g/m^2 burn/hr respectively between four and seven days after injury [Waxman et al. 1987]. The mean protein losses in the first week and second weeks are 5.0 and 2.3 g/m^2 burn/hr respectively and it can be calculated that the total protein loss from a 30% burn in an adult, with a body surface area of 1.8m^2, is 65g/day in the first week after injury and 30g/day in the second. Detailed nitrogen balance studies, in adult patients with major burns who were receiving 3500kcal/m^2/day (non-protein), showed that the nitrogen requirements for equilibrium were 20.7 to 25.5g/m^2/day between 7 and 17 days after injury. These declined with time as the burns were grafted and from 90 days onwards were not significantly different from the normal recommended nitrogen intake of 6.5g/m^2/day [Soroff et al. 1961].

2.7 Protein depletion

Body protein stores may be rapidly eroded by the increased utilization of amino-acids for gluconeogenesis and losses from the burn wound unless replaced by dietary protein. Weight-loss was an inevitable consequence of a major burn before the advent of modern feeding techniques. Newsome et al. [1973] emphasised this in a retrospective analysis of burned patients treated prior to the availability of parenteral feeding. Patients with a

TBSA of less than 19% had a mean maximum mean weight loss of 6%, those with burns between 20 and 39% had a maximum weight loss of 12% whilst those with burns over 40% had a maximum weight loss of 22%.

Unlike fat or glycogen, each protein molecule is functional, being a contractile protein, a structural protein or an enzyme and consequently, the loss of 25 to 30% of total body protein is invariably fatal. Lesser amounts of protein depletion result in disordered cell function which is clinically manifested as weakness, immunosuppression or impaired wound healing.

3 - CONSEQUENCES OF MALNUTRITION

3.1 Injury and starvation

Normal subjects when starved undergo an adaptive process whereby they use progressively less protein and more fat as an energy source. This process is associated with decreased insulin levels, increased fat metabolism, fat oxidation with the production of ketone bodies and a fall in urinary nitrogen excretion [Cahill 1970]. In contrast, burned patients maintain high plasma levels of insulin, have a markedly blunted ketonaemic response and there is little or no reduction in nitrogen excretion in response to starvation [Harris et al. 1982, Grecos et al. 1984]. The metabolic adaptation to starvation, which allows protein conservation, is therefore overridden after injury. This occurs at a time when energy utilization is high and therefore the onset of protein energy malnutrition (PEM) may be rapid.

3.2 Consequences of malnutrition

Studley [1936] was the first to correlate weight loss with an increase in morbidity and mortality in surgical patients. Rhoads and Alexander [1955] subsequently demonstrated a significantly higher rate of post-operative infective complications in patients who were hypoproteinaemic compared with those who had a normal plasma protein content. Recently developed predictive nutritional indices, based on plasma protein levels, anthropomorphic data and immunological function, are well correlated with the incidence of postoperative complications and mortality in general surgical patients [Mullen et al. 1980] and a more recent review of 5000

surgical admissions showed that weight-loss of ten pounds was associated with a 19-fold increase in mortality [Seltzer et al. 1982].

Although loss of muscle mass and peripheral oedema are readily apparent clinically and may complicate the recovery of burned patients by hindering expectoration of phlegm and early mobilisation, the major cause of morbidity and mortality is sepsis [Sevitt 1979]. Burned patients, have a profound, global, loss of immunocompetence which is contributed to by impaired nutrition [Winkelstein 1984]. Uncomplicated malnutrition is associated with abnormalities of all components of the immune system. Although T-lymphocyte abnormalities are the best documented and provide a useful marker of nutritional status [Leite et al. 1987], complement, fibronectin, neutrophil, macrophage and reticulo-endothelial function are also impaired in malnourished subjects [Law et al. 1974, Saba et al. 1983].

Normal healing is dependent, to a great extent, on the non-specific immune system. Immunosuppression also contributes to delayed healing which is a further source of morbidity resulting from malnutrition. This is usually manifested in a burned patient by a skin-graft donor site that fails to epithelialize promptly, thereby delaying subsequent grafting procedures.

3.3 The healing of incised wounds

Tissue healing can be divided into three, overlapping, phases; namely inflammation, granulation tissue formation and matrix formation [Ordman and Gillman 1966, Forrest 1983, Hunt 1984, Clark 1985]. Following the extravasation of blood into the wound, platelets adhere to injured tissue,

causing the release of substances which trigger further platelet aggregation, blood coagulation, activate the complement and kinin cascades and attract inflammatory cells.

During the early inflammatory phase, neutrophils and macrophages enter the wound in response to chemotaxins. The major function of the neutrophil infiltration is to eliminate micro-organisms and it usually ceases within three days, unless gross bacterial contamination has occurred. Macrophages continue to enter the wound phagocytose micro-organisms, tissue debris and effete neutrophils. Macrophages are central to the repair process and initiate the formation of granulation tissue by the release of factors such as fibroblast growth factor and angiogenic factor.

Granulation tissue consists of new blood vessels, macrophages and fibroblasts surrounded by a loose matrix containing collagen, fibronectin and hyaluronic acid which are secreted by fibroblasts. All these components enter the wound as a single interdependent unit from about the second day onwards. During this time, fibroblasts, as they proliferate, undergo a phenotypic change to become *myofibroblasts* which have contractile and motile abilities but which retain their secretory capabilities.

Re-epithelialization of the wound begins within hours of injury. Epithelial cells, also undergo a phenotypic change that allows them to become detached from the basement membrane and move from the free edge across the defect whilst the cells remaining at the wound edge proliferate. Once epithelialization is complete, the epithelial cells revert to their original phenotype and become firmly re-attached to the basement membrane.

Matrix formation and remodelling starts with the formation of granulation tissue. The matrix initially contains considerable amounts of fibronectin but, as "fibroplasia" continues, this is replaced initially by type-III collagen and later by type-I. Similarly, hyaluronic acid is replaced by proteoglycans such as chondroitin sulphate as the wound matures.

3.4 Collagen and tensile strength

Collagens are a family of closely related triple-chain glycoproteins. Each chain contains a repeating X-Y-Glycine primary sequence, often with proline and hydroxyproline at the X and Y positions respectively. Eight varieties of collagen have been identified. Type-I is the predominant type found in skin and tendinous tissues and consists of two identical alpha-chains designated as {alpha-1, type I} and a third distinct chain designated {alpha-2} [Forrest 1983].

In cutaneous wounds in rats, there is a lag period of about five days, before the development of tensile strength, coincident with a matrix rich in fibronectin and hyaluronic acid. The onset of tensile strength accompanies collagen deposition. Wound hydroxyproline measurements show that there is a rapid increase in collagen content from the fifth to eleventh day which is well correlated with the increase in wound strength. The collagen content subsequently plateaus whilst strength continues to increase for up to a year after injury due to collagen remodelling, at which time it is about 80% as strong as unwounded skin [Sandberg and Zederfeldt 1963, Levenson et al. 1965].

3.5 Nutrition and collagen deposition

Early studies indicated, but without statistical analysis, that the accumulation of hydroxyproline in wounds is impaired in rats and post-operative patients receiving protein-depleted diets [Udupa et al. 1956, Bozzetti et al. 1975]. In a more recent study, Haydock and Hill [1986] assigned surgical patients on the basis of dietary history and anthropomorphic measurements to either normal nutrition, mild PEM or moderate to severe PEM groups and assessed wound-healing by the accumulation of hydroxyproline in subcutaneously placed polytetrafluoroethylene (Goretex) tubes. Hydroxyproline accumulation was significantly impaired in both PEM groups compared with normally nourished patients but unfortunately, the groups were ill-matched for age, incidence of malignancy and surgical procedure. In a further study, using the same technique, the same authors found hydroxyproline accumulation to be impaired in patients who had been referred for TPN compared with normally nourished controls [Haydock and Hill 1987]. Within one week of initiating TPN in the malnourished group, there was a significant improvement in hydroxyproline accumulation.

3.6 Nutritional-depletion and wound-strength

The early observation that malnutrition is associated with weak wounds in experimental animals [Howes et al. 1933] have been repeatedly confirmed [Kobak et al. 1947, Localio et al. 1948, Williamson et al. 1951, Peacock 1960, Daly et al. 1972, Irvin 1978, Devereux et al. 1979, Ward et al. 1982, Greenhalgh and Gamelli 1987, Yue et al. 1987] and this effect correlated with reduced wound collagen deposition [Temple et al. 1975, Devereux et al.

1979]. Many of these studies examined tensile strength after prolonged periods of protein depletion. Irving [1978] measured breaking-strength in the skin and abdominal wall and bursting-strength in colons one week after performing a colonic anastomosis in rats which had been given a protein-depleted diet for between one and eight weeks prior to operation. There was a significant fall in the breaking strength in skin and abdominal wall only after seven weeks of protein-depletion at which time the animals had lost 37% of their original weight. He demonstrated no significant impairment in colonic healing at any time and postulated that visceral protein was better preserved than parietal.

These findings are at variance with other studies which, using similar protocols, have demonstrated impaired skin and colonic healing in rats which had received a protein-depleted diet for only two weeks and which had lost around 15% of their original weight [Daly and Dudrick 1972, Devereux et al. 1979, Ward et al. 1982]. Furthermore, Peacock [1960] showed that the rate of increase of tensile strength in skin wounds was significantly slowed in rats that had received a protein-depleted diet six days before and after operation. More recently, Greenhalgh and Gemelli [1987] have demonstrated a reduction in skin tensile-strength after only seven days of post-operative nutritional depletion.

3.7 Nutritional-repletion and wound-strength

Steiger et al. [1973] performed colonic anastomoses in rats following a six week period of protein-depletion and then provided the rats with intravenous nutrition consisting of either 30% dextrose with 5% amino-acids, 30% dextrose alone or 5% dextrose alone. After one week, those

which had received both 30% dextrose and amino-acids gained significantly more weight and had significantly increased colonic bursting strengths compared with the other two groups. Irving [1978] provided a group of rats receiving a protein-depleted diet (see §3.6) with additional amino-acids during the last week of depletion and the week post-operatively, before wound-healing assessment. The addition of amino-acids resulted in a positive nitrogen balance, the prevention of further weight-loss, increased abdominal wound collagen content and an increase in breaking strength.

These clinical and animal studies indicate that wound-healing can be impaired by malnutrition. This effect can, however, be reversed quickly by feeding. Similarly, Alexander et al. [1980] showed that the immunological abnormalities seen in burned patients can be partly reversed by aggressive nutritional therapy (see §4.3). In a large prospective series of general surgical patients, in whom malnutrition was common, Mullen et al. [1980] showed that the administration of TPN for at least seven days pre-operatively caused a two-fold reduction in the incidence of all postoperative complications and a seven-fold decrease in mortality. The most dramatic decrease was noted in septic complications.

3.8 Clinical implications

These studies show that the onset of malnutrition in surgical patients is associated with an increase in complications and mortality. These consequences, however, can be reversed by feeding and therefore it is apparent that nutrition can contribute significantly to the clinical outcome after surgery. The majority of burned patients are normally

nourished at the time of injury and therefore failure to maintain this situation poses a serious threat to their well-being and represents a failure in clinical care.

4 - FEEDING THE BURNED PATIENT

4.1 Improvements in the care of patients with burns

In the last two decades, the prognosis of burned patients has improved. In the period between 1976 and 1979 there was an overall survival rate of 90% in patients admitted to American burn centres, compared with 81% between 1965 and 1971. More specifically, patients aged between 35 and 49 years with a TBSA of between 30 and 39% had a mean survival rate of 91%, compared with 78% [Feller et al. 1980].

A number of factors can be identified that have contributed to this improvement [Demling 1985]. A better understanding of fluid resuscitation combined with the availability of intravascular pressure and cardiac output monitors has reduced the incidence of renal and respiratory complications. The investigation and management of smoke inhalation injuries has been facilitated by the use of fibre-optic bronchoscopy and early mechanical ventilation. Multiple organ failure, due to sepsis, remains the most common cause of death with the lungs and burn being the most frequent sources of infection. Improved control of sepsis has been achieved with the routine use of topical antiseptic agents and the early excision of burns. Hypermetabolism is limited by routinely managing patients in a warm environment and the rapid cover of burns by grafting. There has been an increased emphasis on nutrition which has had a major impact on the management of burns. Recent studies have demonstrated substantial clinical benefits to accrue from changes in timing, route of administration and formulation of feeding solutions.

4.2 Energy requirements

The increased dietary requirements of burned patients were appreciated many years ago and early dietary recommendations aimed to provide them with energy and nitrogen intakes well in excess of anticipated requirements [Davies 1982]. Most of these regimens were based on body weight alone and failed to take account of the TBSA which had been shown to be the major factor influencing the REE of patients. Two formulae for calculating energy requirements subsequently evolved which included the burn-size in order to provide a more realistic estimation of requirements [Curreri et al. 1974, Sutherland 1976]. Although both have been widely used because of their simplicity, the Sutherland formula has now largely been abandoned because it significantly overestimates the requirements. A number of studies have shown that the Curreri formula (see appendix 3.) also overestimates the REE by between 35 and 60% in patients with large burns, although it is more accurate for those with a TBSA between 11 and 30% [Turner et al. 1985, Saffle et al. 1985, Matsuda et al. 1987, Allard et al. 1988]. For this reason it is usual to limit the burn-size to 50% when estimating the calculated energy expenditure (CEE) using this formula.

There is increasing evidence that over-nutrition is as undesirable as under-nutrition. Furthermore, there are considerable logistic problems in providing large intakes, often necessitating the use of the parenteral route with its attendant risks (see §4.5). Although the administration of increasing quantities of energy in the form of carbohydrate can improve nitrogen balance in patients receiving a fixed intake of nitrogen (see §4.3), this fails to occur once the energy intake exceeds the REE [Long et al. 1977]. Studies in patients receiving TPN have shown that glucose

infused in excess of 7mg/kg/min (40kcal/kg/day) is not oxidised but is stored as fat [Burke et al. 1979, Askanazi et al. 1980, Wolfe et al. 1980]. This results in hepatomegaly due to fatty infiltration of the liver, an increase in REE and an increase in CO_2 production. The latter is undesirable in patients who have any respiratory impairment or who are being ventilated. Furthermore, persistently high insulin levels may contribute to the adult respiratory distress syndrome by inhibiting pulmonary surfactant synthesis [Wolfe et al. 1979c]. Similarly, large protein loads induce calciuresis and may contribute to the increased incidence of renal stones in burned patients [November-Dusansky et al. 1980, Waymack et al. 1987].

Although nutritional formulae have been updated, to improve their accuracy, they fail to allow for either individual variations in response to injury or additional factors such as sepsis. The increased availability of portable indirect calorimeters has made it possible to assess the energy requirements of patients on an individual basis and thereby avoid both under and over-nutrition. Calorimetry in a burned patient therefore allows the measurement of the actual REE and respiratory quotient which together can indicate the correct energy requirements [Saffle et al. 1985]. It is usual to provide a further 25% of energy, in addition to the measured REE, to allow for daily activities.

4.3 Energy and protein inter-relationships

Nitrogen balance is dependent on both protein and energy intake. In the presence of an adequate energy intake, an increase in nitrogen intake results in a more positive nitrogen balance. Similarly, increasing energy

intake, in the presence of an adequate nitrogen intake, will increase the positivity of nitrogen balance [Munro 1951, Calloway and Spector 1954]. The actual nitrogen balance achieved at any given level of intake is subject to a number of variables but this relationship is maintained even in septic and burned patients although the actual energy and protein intakes required to achieve any given nitrogen balance are greater than in normal subjects [Troell and Wretlind 1961, Long et al. 1976, McDougal et al. 1977, Rowlands et al. 1977, Hill and Church 1984].

There is evidence that the administration of protein-rich diets is beneficial to burned patients. The relative amounts of energy and nitrogen are most conveniently expressed as the non-protein energy:nitrogen ratio (NPE:N ratio). Matsuda et al. [1983] compared burned patients fed according to the Curreri formula who were receiving a diet with a NPE:N ratio of 150:1 with those receiving a diet with a ratio of 100:1. The mean nitrogen balances were positive, regardless of burn-size, in those receiving the 100:1 diet whereas patients with burns larger than 31%, who were receiving the lower ratio diet, had negative balances. Similarly, Dominioni et al. [1985] administered enteral diets containing either 175 or 200kcal/kg/day with between 5% and 40% of energy derived from protein to guinea-pigs with 30% burns. The best nutritional and metabolic results were obtained when the energy intake was similar to the measured REE (175kcal/kg/day) and when 20 to 30% of energy was derived from protein (NPE:N ratios of 100:1 to 58:1).

Alexander et al. [1980] have shown that the use of a high protein diet and resultant improvement in nitrogen balance is clinically beneficial. Two groups of burned children received a diet containing either 15% or 25%

of energy as protein (NPE:N ratios of 142:1 and 75:1). Those who received the high-protein diet, despite receiving less energy in total, had an improved opsonic index and higher plasma levels of complement factors, immunoglobulin-G (IgG), transferrin and total protein. These changes were accompanied by a lesser incidence of bacteraemia and a lower mortality.

4.4 Energy source

The nitrogen balance achieved at a given energy intake is also dependent on the non-protein energy source. Some studies have indicated that lipid is less efficient in retaining nitrogen than carbohydrate [Munro 1951, Long et al. 1977, Shizgal and Forse 1980]. Serog et al. [1983] administered four isocaloric diets in random order to burned patients, designated as normal (protein 13%, carbohydrate 50%, lipid 37%), protein-rich (24%, 44% and 32%), carbohydrate-rich (13%, 87% and 0%) or lipid-rich (13%, 27% and 60%). Nitrogen balance was significantly worse when patients were receiving the lipid-rich diet compared with the other diets and it was best during the administration of the protein-rich diet.

Other studies, in patients receiving TPN, have indicated that lipid is comparable to carbohydrate as an energy source in promoting nitrogen retention and that fat-carbohydrate mixtures are advantageous [Jeejeebhoy et al. 1976, Elwyn et al. 1980]. Lipid is a useful energy source because of its high energy content, low osmolality and supply of essential fatty acids. The substitution of fat-derived energy for carbohydrate can reverse the metabolic changes associated with excessive carbohydrate loads [Elwyn et al. 1980, MacFie et al. 1983, Baker et al. 1984]. Mochizuki et al. [1984] administered diets containing 175kcal/kg/day, of which 20% were

protein-derived, with either 0, 5, 15, 30 or 50% of non-protein energy as lipid to guinea-pigs with 30% burns. The best nitrogen balances, serum transferrin levels and the least incidence of fatty infiltration of the liver were seen when between 5% and 15% of non-protein energy was derived from lipid.

4.5 Route of administration

Nutritional support is usually necessary in burned patients with TBSA in excess of 20% in the early weeks after injury, in order to achieve their calculated nutritional requirements. The gut, if functional, is the preferred route for administration of nutrients, being both cheaper and safer than the parenteral route. The majority of burned patients can be satisfactorily fed using the gut thus avoiding the potential complications of the parenteral route, particularly catheter-related sepsis which is both common [Popp et al. 1974] and difficult to detect in burned patients.

Recent clinical studies have shown no significant difference in efficacy between the routes of administration [Burt et al. 1982, Bennegard et al. 1984, Muggia-Sullam et al. 1985]. In contrast, animal studies have shown that use of the enteral route results in improved nitrogen balance, weight-gain and a reduced incidence of fatty infiltration of the liver compared with the parenteral route [Johnson et al. 1975, Lickley et al. 1978, King et al. 1983, Kudsk et al. 1983]. Saito et al. [1987] administered identical diets either enterally or parenterally to guinea-pigs with 30% burns. The enterally-fed animals had significantly better nitrogen balances, less weight-loss, lower catecholamine excretion rates and lower plasma glucagon and cortisol levels. This and other studies

[Johnson et al. 1975, Ford et al. 1984] have shown that the intestinal structure is better preserved in enterally-fed animals and it has been postulated that gastro-intestinal hormones may interact with the counter-regulatory hormones, particularly glucagon, to modify the hypermetabolic response.

4.6 Formulation

There are a large number of products designed for enteral feeding which, in addition to varying in energy content and distribution of energy source, also vary in type of protein or lipid. A number contain protein as oligopeptides or amino-acids and are intended for use in patients with a short-bowel syndrome. Clinical trials have shown no advantage in their use in patients with normal gastro-intestinal function [Jones et al. 1983]. Trocki et al. [1986] provided burned guinea-pigs with identical diets in which the nitrogen-source was either intact or as free amino-acids. Those fed intact protein had a better nitrogen balance, less weight-loss and improved preservation of jejunal mucosal mass. After 14 days they had significantly higher serum albumin, transferrin and complement levels.

There has been recent interest in supplementation of feeds with branch-chain amino-acids (BCAA) but there is little evidence that this improves clinical outcome [Brennan et al. 1986]. Mochizuki et al. [1986] compared whey protein diets with isonitrogenous BCAA-enriched diets in burned guinea pigs and showed no benefit accruing from BCAA administration. There appears to be consensus that the use of solutions

containing no more than 20 to 25% of protein as BCAA is ideal and avoids significant shortfalls in other essential amino-acids.

There is evidence that manipulation of lipid type also affects the efficiency of a feed. Alexander et al. [1986] gave burned guinea-pigs identical diets containing lipid that was rich in the precursors of either trienoic (linoleic acid, safflower oil) or dienoic prostaglandins (eicosapentaenoic acid, fish-oil). Those given fish-oil had a smaller rise in REE, less weight loss and better immune function. The dienoic prostaglandins, principally PGE_2, are known to be immunosuppressive unlike the trienoic prostaglandins, which although structurally similar, are not immunosuppressive.

4.7 Timing of feeding

The timing of feeding may also be important. Mochizuki et al. [1984] compared burned guinea-pigs that were either fed enterally two hours after injury or underwent a conventional 72 hour adaptation period prior to full enteral feeding. Approximately 15% of body weight was lost during the adaptation period compared with less than 5% in animals that were fed early and this difference was still apparent after 14 days. There was a significantly greater decline in intestinal mucosal thickness and weight in the adaptation group and these animals also had significantly higher plasma cortisol and glucagon levels. The REE rose by 40% in the adapted group compared to 10% in the animals fed early.

The use of "starter regimens" for enteral feeding is probably unnecessary in recently injured patients and serves only to delay the

implementation of full feeding. There is little evidence that they reduce the incidence of side-effects. The continuous infusion of feed via a volumetric pump to burned patients results in better tolerance of and permits the earlier achievement of nutritional targets compared with intermittent administration [Hiebert et al. 1981]. The occurrence of diarrhoea is more closely related to the concurrent administration of antibiotics rather than the tonicity of feed and its incidence in burned patients is reduced by early feeding [Keohane et al. 1984, Gottschlich et al. 1988].

4.8 Clinical implications

Although many of these recent studies have been performed in animals, they indicate that nutrition can influence outcome after injury by burning. In summary, they suggest that feeding should be started early after injury and be administered enterally if possible. The total energy administered should reflect, as accurately as possible, an individual patient's requirements. Feeds should be protein-rich and both lipid and carbohydrate should be used as the non-protein energy source. It is unnecessary to use elemental feeds or feeds with high concentrations of BCAA but there is some indication that diets that are rich in lipids derived from fish-oils are advantageous.

5 - ANABOLIC AGENTS

5.1 The need for anabolic agents

The advent of modern feeding methods, combined with environmental control and improved clinical management have resulted in a better prognosis for burned patients. Despite these advances, there still may be difficulties in achieving positive energy and nitrogen balances, particularly in the first fortnight after injury. This may be due to a number of clinical factors such as the need for fasting periods prior to surgery or dressings, limits on feed-rates because of fluid retention, hyperglycaemia or intestinal ileus and shortage of sites suitable for central intravenous feeding-lines. For many years there have been attempts to improve the utilization of administered nutrients by the use of anabolic agents, in order either to delay the onset of PEM or to improve the rate of nutritional repletion once feeding is established.

5.2 Anabolic steroids

Shortly after the identification of testosterone as the principal androgenic steroid produced by the testis, its administration was shown to decrease nitrogen excretion in eunuchoid subjects [Kenyon et al. 1938] and burned patients [Abbott et al. 1946]. A number of anecdotal studies suggested that the administration of testosterone and related androgens to injured or post-operative patients could improve nitrogen balance and this impression was later reinforced by two prospective studies [Johnston and Chenneour 1963, Tweedle et al. 1973]. However, subsequent studies using long-acting anabolic steroids are contradictory. Michelsen et al. [1982] showed that the administration of nandrolone to patients after hip arthroplasty caused a significant improvement in nitrogen balance but

three other studies, performed in patients receiving TPN or after abdominal surgery, failed to show any significant improvement in nitrogen retention in treated patients compared with controls [Yule et al. 1981, Lewis et al. 1981, Young et al. 1983]. More recently, however, two studies showed that the administration of stanozolol prior to abdominal surgery caused a significant improvement in post-operative nitrogen balance [Blamey et al. 1984, Hansell et al. 1987].

No anabolic steroids have been identified without androgenic activity [Wilson and Griffin 1980]. Paradoxically, some may have feminising activity in men because of their metabolism to oestrogens, for example causing gynaecomastia. As a consequence, their use in women and children is relatively contraindicated although stanozolol, which has a high anabolic to androgenic ratio, has not been associated with any masculinising effect in the women studied [Blamey et al. 1984, Hansell et al. 1987]. Increased sodium retention has been observed during treatment with anabolic steroids [Johnston and Chenneour 1963] and there was a significant increase in the diuretic requirements of treated patients receiving TPN [Yule et al. 1981, Young et al. 1983]. Again, this side-effect has not been associated with stanozolol administration [Blamey et al. 1984, Hansell et al. 1987]. Androgens often cause abnormalities in liver function and may cause obstructive jaundice. More rarely their administration may result in the development of a hepatoma or peliosis hepatitis, in which the liver becomes filled with blood-filled cavities. The widespread use of anabolic steroids is therefore limited by these side-effects until there is some clear indication that their administration is clinically beneficial.

5.3 Insulin

Diabetes is characterised by negative nitrogen balance and muscle wasting. Soon after its discovery, insulin was shown to correct the negative nitrogen balance in diabetics. In vitro studies have shown that insulin can both enhance protein synthesis and inhibit protein breakdown in muscle taken from normal and burned rats [Frayne and Maycock 1979, Odessey and Parr 1982, Clark et al. 1984]. Hinton et al. [1971] first showed that the administration of dextrose, insulin and potassium solutions to burned patients could significantly reduce urinary urea excretion. Subsequent studies in burned patients receiving TPN have confirmed that insulin reduces urea excretion [Long et al. 1977] and have shown a trend towards increased protein synthesis [Burke et al. 1979]. Woolfson et al. [1979] compared injured patients receiving TPN either with or without insulin. Insulin administration resulted in a fall in the urea production rate which was most marked in the most catabolic patients. No significant effect was seen when the initial rate of urea production was less than 15g/day. The findings in post-operative subjects receiving TPN, who have little or no rise in REE, are contradictory. One study showed that insulin administration had no effect on body protein content [MacFie et al. 1981], whilst another showed that it resulted in a significant reduction in nitrogen excretion [Inculet et al. 1986].

These findings indicate that insulin can reduce protein catabolism and that this effect is most apparent in more catabolic patients. Despite this, it is not widely used as a nutritional adjunct in burned patients. This probably reflects the frequent monitoring of blood glucose and potassium that are necessary during its administration.

5.4 Naftidrofuryl (Praxilene)

Burns et al. [1981] showed that the administration of naftidrofuryl to surgical patients caused a significant reduction in nitrogen excretion compared with control patients receiving a comparable energy intake. He postulated that naftidrofuryl increased the efficiency of carbohydrate and fat utilization due perhaps to the stimulation of acetyl-CoA or pyruvate entry into the tricarboxylic acid cycle. Subsequent studies have not confirmed this finding and there is little indication that it is an active anabolic agent [Inglis et al. 1983, Jackson et al. 1984, Hansell et al. 1987].

5.5 Growth hormone

It was appreciated at the beginning of this century that the pituitary gland was a growth-regulating centre [Hutchinson 1900] and that hypophysectomy resulted in impaired growth [Crowe et al. 1910]. Anterior pituitary extract was first shown to promote growth in 1921 when administered to normal rats [Evans and Long 1921] and in 1932 when administered to a child with hypopituitarism [Engelbach 1932]. Its administration in normal animals was subsequently shown to cause increases in nitrogen retention and weight-gain [Cuthbertson et al. 1941]. Growth hormone (GH), which was responsible for this activity, was isolated and characterised in 1944 [Li and Evans 1944] and its anabolic effect was first confirmed in hypopituitary subjects using porcine [Raben et al. 1952] and bovine anterior pituitary extracts (pit-GH) [Conn et al. 1952] and then in humans with normal pituitary function using a bovine pit-GH [Kinsell et al. 1954]. Subsequently, a number of studies using bovine and porcine

preparations failed to demonstrate consistent anabolic activity in primates, despite improvements in the quality of preparations. It was postulated that this failure was due to species differences in GH and this was supported by a study in hypophysectomized rhesus monkeys which showed that monkey pit-GH stimulated growth whereas bovine pit-GH did not [Knobil et al. 1956].

Human pit-GH was isolated in 1956 [Li and Papkoff 1956] and was shown to be active in hypopituitary and normal subjects [Beck et al. 1958, Ikkos et al. 1958, Raben 1958, Anonymous 1959]. It was concluded that bovine and porcine pit-GH were unsuitable for clinical use. It was subsequently confirmed that human pit-GH administration to both normal and hypopituitary subjects had anabolic activity, causing falls in the serum urea concentration and urinary urea excretion [Henneman et al. 1960, Soroff et al. 1967, Felig et al. 1971, Hintz et al. 1982].

Sir David Cuthbertson (Figure 1.1) first investigated the anabolic effect of GH after injury in rats which had undergone femoral fracture, showing that it could abolish the sharp rise in nitrogen excretion present in the untreated animals and result in an improvement in weight-retention [Cuthbertson et al. 1941]. Subsequently, Gump [1960] showed that GH administration improved the rate of weight-gain in burned rats and he suggested that GH could modify the metabolic response to trauma. The majority of human studies of the anabolic effects of pit-GH were performed in burned patients and all, which used human pit-GH, showed that its administration resulted in significant improvements in nitrogen balance (see §7.1 and §7.2).

5.6 Recombinant growth hormone

The use of GH has, until recently, been mostly confined to the correction of growth disorders because of its limited availability. In 1985, human pit-GH was withdrawn from use because of the four reports of deaths in young adults, who had received pit-GH, from Creutzfeldt-Jacob disease which is a rare encephalopathy resulting from infection with a slow virus [Preece 1986].

In 1979, using DNA-recombinant techniques, the gene for human GH production was inserted in Escherischia. coli (E.coli) and expressed for the first time with the production of human GH [Goeddel et al. 1979]. This biosynthetic product is identical to human GH except for the addition of a methionyl residue on the N-terminal end which is the result of the bacterial process of protein synthesis. It was administered to adult volunteers and found to have identical activity and potency as human pit-GH [Hintz et al. 1982, Rosenfeld et al. 1982]. Methionyl-human GH (somatrem) was granted a product licence in 1985. Subsequent trials have confirmed its therapeutic potential for children with GH deficiency [Bierich 1986, Takano and Shizume 1986a, Milner 1986].

Further refinements of recombinant techniques have led to the development of authentic human GH (somatropin) [Gray et al. 1984]. It was shown to be both active and safe in human volunteers [Wilton and Sietnieks 1987] and to increase the rate of growth in children with GH deficiency [Takano and Shizume 1986b]. This product was granted a product licence in 1988.

5.7 Clinical implications

Growth hormone is therefore no longer confined to the treatment of hypopituitarism and it is now available for use in other clinical areas such as nutrition. Both preparations are prepared from E.coli which is advantageous as this bacterium is free of mammalian antigens, oncogenes or viruses. There is no evidence that the presence of the extra methionyl-residue in somatrem alters its activity compared with somatropin but there is some indication that somatropin is less antigenic [Takano and Shizume 1986b]. Both preparations are now available in unlimited quantities and initial clinical trials have all indicated that biosynthetic GH preparations have significant anabolic effects under a wide variety of dietary conditions (see §7.3).

6 - GROWTH HORMONE PHYSIOLOGY

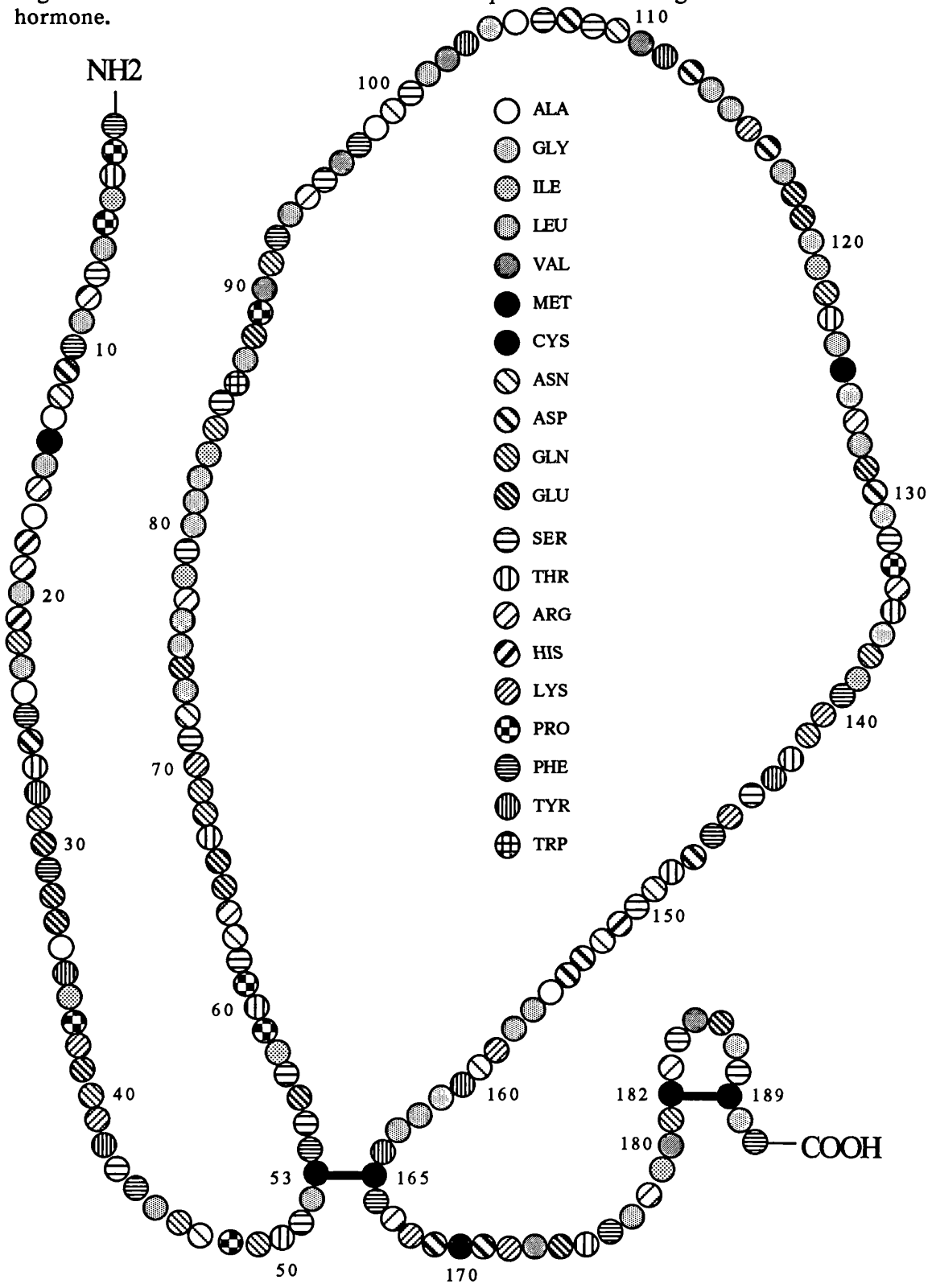
6.1 Structure

Growth hormone is a 191 amino-acid, 22kD, single-chain peptide hormone which is constrained by two disulphide bonds into two loops (Figure 6.1). It is the most abundant hormone in the pituitary which stores approximately 10mg, of which approximately 1mg is released each day [Press 1988]. About 10% of stored GH and 7% of circulating GH is a 20kD variant, which is identical except for the deletion of amino-acids 32 to 46 [Baumann et al. 1983]. About 50% of circulating GH is complexed with a binding-protein [Baumann et al. 1987].

6.2 Secretion

Growth hormone is released from the anterior pituitary gland under the control of two hypothalamic, peptide hormones; GH-releasing hormone (GHRH/somatotrophin/somatocrinin) and GH-release inhibiting hormone (GRIH/somatostatin). GH release is modulated by negative feed-back by GH itself and somatomedins which both trigger the release of somatostatin from the hypothalamus [Muller 1987]. Additional metabolic loops involving glucose, insulin and free fatty acids, probably regulate secretion [Press 1988]. GH secretion is both pulsatile and cyclical. There are four to six peaks each day with maximum levels occurring at night [Popp et al. 1977, Ho et al. 1987]. Secretion varies with age, with the highest levels occurring in pubertal children followed by a decline during adulthood [Zadik et al. 1985, Ho et al. 1987].

Figure 6.1 The structure and amino-acid sequence of human growth hormone.



6 - GROWTH HORMONE PHYSIOLOGY

Growth hormone release is stimulated by conditions of energy deficit, such as hypoglycaemia and fasting as well as raised plasma levels of arginine and glucagon, whereas secretion is suppressed by hyperglycaemia and raised plasma levels of free fatty acids and cortisol [Glick et al. 1965, Schalch 1967]. GH release has been shown to increase in response both to emotional and physical stresses including exercise, electroconvulsive therapy and elective surgery [Glick et al. 1965, Greenwood and Landon 1966, Schalch 1967, Charters et al. 1969, Carey et al. 1971, Newsome and Rose 1971, Wright and Johnston 1975]. In Wright and Johnston's study, performed peri-operatively in general surgical patients, the plasma GH levels were inversely correlated with the glucose disappearance constant. They postulated that GH levels rise in response to diminished cellular glucose uptake.

Growth hormone secretion is markedly elevated immediately after soft-tissue injuries but declines within 24 hours to a low mean level with occasional samples showing high levels [Schalch 1967, Carey et al. 1971, Frayn et al. 1984]. There is contradictory information on the effect of burns on GH secretion and none on secretion in the first 48 hours after injury. The majority of authors have found no significant change in GH levels although high levels have been recorded episodically [Batstone et al. 1976, Popp et al. 1977, Dolecek et al. 1979, Balogh et al. 1984, Vaughan et al. 1985]. Only one study has demonstrated persistently elevated levels but following an overnight fast [Wilmore et al. 1975b]. The factors contributing to these differences may include the pulsatile manner of GH release, variations in sampling time and concurrent dietary input.

6.3 Actions

Growth hormone receptors have been identified on many cell types [Mendelsohn 1988] and as a consequence, it has many actions of which the most important are the stimulation of growth and both an early insulin-like and a later insulin-antagonistic effect. Deficiency of GH secretion, whether idiopathic or secondary to intracranial tumours, results in dwarfism. This has been amenable to treatment with pit-GH for many years [Tanner et al. 1971, Frasier et al. 1977] and more recently with somatrem [Bierich 1986, Milner 1986, Takano and Shizume 1986a, Vicens-Calvet 1986] and somatropin [Takano and Shizume 1986b]. Despite the known growth promoting activity of GH, *in vitro* studies showed that GH itself was unable to promote cartilage uptake of sulphate and this led to the hypothesis that growth was mediated by circulating "sulphation factors" [Salmon and Daughaday 1957] later renamed somatomedins [Daughaday et al. 1972].

GH has a number of actions which are complementary to growth which are also probably mediated by somatomedins. These include increased nitrogen and potassium retention (see chapter 7.), which are accompanied by increased calcium absorption. Its widespread promotion of cellular growth is also reflected by enhancement of erythropoiesis [Fruhman et al. 1954, Golde et al. 1977] and lymphocyte proliferation [Williams and Frohman 1986]. In contrast, the effects of GH on glucose metabolism appear to be independent of somatomedins [Schoenle et al. 1983].

6.4 Somatomedins

It is now recognised that somatomedins are a family of peptides which are mainly synthesised in the liver and which circulate in the plasma bound to large carrier proteins. The factors which have been fully characterized, to date, are insulin-like growth factor-I (IGF-I), which is thought to be identical to somatomedin-C, and IGF-II. Both peptides and structurally related to pro-insulin [Baxter 1986] and consequently the insulin and IGF receptors are similar [Froesch and Zapf 1985]. Two types of plasma membrane receptor have been identified for the IGF. The type-I receptor has a greater affinity for IGF-I than IGF-II and also binds insulin. The type-II receptor has a greater affinity for IGF-II but does not bind insulin and has no known function.

It has been shown in chronically cannulated normal rats that the plasma IGF-I concentration varies in a pulsatile manner that is correlated with secretory bursts of GH [Baxter et al. 1983]. The administration of GH to both normal and hypopituitary subjects causes a gradual rise in plasma IGF-I concentration which reaches a maximum after about five days and which persists for as long as treatment continues [Hintz et al. 1982, Bierich 1986, Takano and Shizume 1986b, Clemmons et al. 1987, Snyder et al. 1988]. In children with hypopituitarism and short children with normal GH secretory function, the somatomedin response to exogenous GH is correlated with their increase in growth-velocity [Vicens-Calvet et al. 1986, Albertson Wikland and Hall 1987].

Under normal conditions, IGF-I, and to a lesser extent IGF-II, are dependent on GH status but IGF-I is also a sensitive index of nutritional status [Clemmons et al. 1985] and insulin activity. Plasma IGF-I levels

fall by 60 to 70% in volunteers fasted for five days and return to normal within eight days of refeeding [Isley et al. 1983, 1984]. Under these circumstances, plasma levels are closely correlated with nitrogen balance. Dogs which have undergone pancreatectomy have very low somatomedin levels but following the administration of insulin, somatomedin levels return to normal [Eigenmann et al. 1977]. The responses to both fasting and insulin-deficiency occur despite the presence of high plasma GH concentrations. Furthermore, the growth-rate of children is more directly related to their insulin activity than their rate of GH secretion [Laron et al. 1972]. These findings emphasise the close relationship between somatomedins and insulin activity.

6.5 Somatomedin actions

Somatomedins elicit rapid insulin-like and slower growth-promoting effects. The administration of recombinant IGF-I to normal subjects causes an immediate fall in in blood glucose and FFA concentrations [Guler et al. 1987]. IGF-I has between 6 to 7.5% of the potency of insulin but it is doubtful if it has a physiologically significant effect on glucose homeostasis as high levels of IGF-I are found in diabetics and the long-term administration of GH results in insulin-resistance, despite raised somatomedin levels.

The major actions of somatomedins are concerned with cellular growth and differentiation [Froesch and Zapf 1985]. Although these actions are shared by insulin, insulin has only 1 to 2% of the potency of IGF-I as a growth-promoting hormone. Schoenle et al. [1985] have shown that the administration of IGF-I to hypophysectomized rats causes increases in

tibial epiphyseal growth, thymidine incorporation by costal cartilage and body weight which are indistinguishable from those due to GH [Schoenle et al. 1985]. IGF-II induces a lesser increase in the two growth indices but no change in body weight. Recent *in vivo* experiments in hypophysectomized rats have shown that GH can induce growth when administered either directly to the tibial epiphyseal plates or via an arterial infusion [Isaksson et al. 1982, Schlechter et al. 1986]. Although this apparently contradicts the somatomedin hypothesis of Salmon and Daughaday [Salmon and Daughaday 1957, Daughaday et al. 1972], a number of cell types, including fibroblasts, are now known to be capable of producing somatomedins [Clemmons et al. 1981, Adams et al. 1983, Clemmons 1984]. It is apparent that somatomedins also have a local autocrine or paracrine mode of action. In support of this, the local effects of GH can be blocked by the addition of IGF-I antiserum [Schlechter et al. 1986].

6.6 Carbohydrate metabolism

GH has a biphasic action on fat and carbohydrate metabolism having both insulin-like and insulin-antagonistic effects [Davidson 1987]. The insulin-like effects include enhanced glucose utilization and reduced fat breakdown. The administration of GH to humans may result in a transient fall in blood glucose and plasma FFA concentrations [Cheng and Kalant 1970, Fineberg and Merimee 1974, Adamson et al. 1977, Adamson and Efendic 1979]. This effect is most prominent in animals that have had little previous exposure to GH, for example after hypophysectomy, and therefore its physiological significance is uncertain.

The anti-insulin effects of GH become apparent within as little as 30 minutes of administration and persist for as long as it continues. Hyperglycaemia, hyperinsulinaemia, insulin-resistance and glucose intolerance are common manifestations of acromegaly [Trimble et al. 1980, Bolinder et al. 1986], whilst GH-deficient subjects and rats that have been rendered GH-deficient by anti-GH antibodies are insulin sensitive [Merimee at al. 1971, Schwartz et al. 1980]. The prolonged administration of GH to normal subjects usually results in either no or sometimes a small increase in the basal or fasting blood glucose concentration. In contrast, both the fasting insulin concentration and insulinogenic index have consistently been shown to rise [Metcalfe et al. 1981, Rosenfeld et al. 1982]. Evidence as to the effect of GH on the insulin responsiveness to glucose is contradictory. Human studies have variously shown increased [Rosenfeld et al. 1982, Sherwin et al. 1983], unaltered [Cerosi et al. 1972] and decreased insulin release [Adamson and Cerasi 1975, Adamson and Efendic 1979] in response to exogenous glucose administration. This lack of accord is contributed to by differences in calculation and in timing between GH and glucose administration perhaps with the persistence of the early insulin-like effect of GH. Hyperglycaemic clamp studies have suggested that the sensitivity of the pancreatic ß-cells to changes in blood glucose concentration are unaltered by GH when corrected for the rise in basal insulin level [Bratusch-Marrain et al. 1982]

The disposal of exogenous glucose is impaired after prolonged GH administration [Rosenfeld et al. 1982, Sherwin et al. 1983]. Euglycaemic clamp studies have shown that GH administration results in insulin-resistance. The insulin dose-response for both stimulation of glucose

utilization and suppression of glucose production is impaired by GH [Rizza et al. 1982]. The major effect of physiological elevations of GH is to inhibit insulin-mediated peripheral glucose uptake and metabolism in muscle and adipose tissue [Bratusch-Marrain et al. 1982, Bolinder et al. 1986]. Insulin-binding studies, using a variety of cell types, have shown either no alteration or a small decrease in cellular insulin-binding at low concentrations [Maloff et al. 1980, Rizza et al. 1982, Bratusch-Marrain et al. 1982, Rosenfeld et al. 1982, Bolinder et al. 1986]. The insulin-antagonistic effects of GH are therefore exerted predominantly at a post-receptor level.

6.7 Insulin and GH anabolism

It has been argued that the rise in the plasma insulin concentration during GH treatment is the critical event for protein anabolism to occur and that insulin-resistance serves to antagonise the hypoglycaemic effects of insulin [Press 1988]. There is some evidence to support this view. Insulin, itself, is a potent anabolic agent (see §5.3) and there is close relationship between somatomedin activity and insulin status (see §6.4). As a result, GH administration to diabetic animals fails to cause nitrogen retention unless insulin is also administered [Milman et al. 1951].

6.8 Fat metabolism

Growth hormone is a potent lipolytic agent. The prolonged administration of either human pit-GH or somatrem to normal subjects causes a rise in plasma FFA and glycerol levels accompanied by an increase

in ketone production [Felig et al. 1971, Metcalfe et al. 1981, Hintz et al. 1982, Sherwin et al. 1983, Snyder et al. 1988]. These changes are accompanied by an increased rate of fat oxidation and sometimes by a rise in REE [Rabinowitz et al. 1965, Bray 1969, Manson and Wilmore 1986]. These findings have recently been confirmed in post-operative patients [Ward et al. 1987, Ponting et al. 1988]. Somatrem administration caused a 13 to 19% increase in REE compared with control patients. The rate of carbohydrate oxidation was unaltered but there was a significant increase in fat oxidation and, therefore, a fall in the RQ. As a result, the PEC was significantly lower and nitrogen excretion was significantly decreased in somatrem-treated patients.

These changes in substrate oxidation are similar to the normal response to starvation [Cahill 1970] and suggest that GH administration preserves tissue protein by the increased use of fat stores.

6.9 Sodium and water metabolism

Acromegalics have been shown to have a significantly increased extracellular water (ECW) content compared with normal man, paralleled by increases in the renal plasma flow (RPF) and glomerular filtration rate (GFR) [Falkheden and Sjogren 1964]. All fall following hypophysectomy [Falkheden and Wickbom 1965]. The administration of human pit-GH to GH-deficient dwarfs results in increases in total body water (TBW), intracellular water (ICW) and ECW but these changes are in proportion to the increase in height [Novak et al. 1972, Parra et al. 1979].

A number of early studies in normal subjects indicated that human pit-GH administration caused sodium retention [Ikkos et al. 1958, Biglieri et al. 1961, Corvilain and Abramow 1962, Rudman et al. 1971]. Similarly, human pit-GH administration to burned patients has been shown to cause increased sodium retention by some studies [Liljedahl et al. 1961, Soroff et al. 1967] although not in the most recent [Wilmore et al. 1974c]. These findings may have been the result of contamination of the GH-preparations with other pituitary hormones, such as vasopressin [Biglieri et al. 1961, Baumann et al. 1972, Rabkin et al. 1975].

Recent studies using biosynthetic GH preparations provide evidence that GH causes water retention. In some studies, an increase in weight accompanied by mild oedema was noted in some patients during the first week of administration. This was assumed to be due to fluid retention but fluid balance was not measured [Clemmons et al. 1987, Binnerts et al. 1988, Snyder et al. 1988]. Manson and Wilmore [1986] demonstrated a significant reduction in urine output during somatrem administration in volunteers receiving a fixed intravenous fluid input which amounted to a mean of 200ml/day. They postulated that water retention could have been explained by increased insensible losses secondary to a rise in REE. However, a more recent study by the same group showed that somatrem administration to patients receiving TPN caused significant fluid and sodium retention compared with placebo (means of +259ml/day and +45mmol/day respectively) [Ziegler et al. 1988]. Prolonged somatrem administration to six patients, for between 13 and 25 days, caused ankle oedema in two.

7 - GROWTH HORMONE AS AN ANABOLIC AGENT

7.1 Animal pituitary-derived GH

The first human study of the anabolic effects of GH was performed by Prudden, Pearson and Soroff in four burned adults using a large dose of bovine pit-GH (200mg/day) [Prudden et al. 1956, Pearson et al. 1960]. Its administration improved weight gain and nitrogen balance, particularly in those who were already in positive nitrogen balance but there was some indication that GH administration was detrimental in catabolic patients. As a result of these findings, Gump et al. [1960] studied the effect of 4mg/day bovine pit-GH in burned rats whilst they had free access to food and whilst starved. In the first two weeks after burning, whilst the rats were fed, those receiving GH gained significantly more weight than those receiving saline injections. In contrast, when starved during the subsequent ten days, those rats receiving GH lost weight significantly faster than those receiving saline. It was postulated that there was a "critical level" of nitrogen intake for anabolism to occur following GH administration. In a further study, Soroff et al. [1960] administered 200mg/day of bovine pit-GH to six burned patients receiving a diet containing 2500kcal/m^2/day of energy and either 6.1, 9.9 or 17.3g/m^2/day of nitrogen. GH administration during the "catabolic phase", from 10 days after burning, resulted in a decrease in nitrogen retention at all levels of nitrogen intake and there was no consistent effect when administered during the "anabolic phase", from 59 days after burning.

The failure of these early trials to show a consistent effect are explicable by their use of bovine pit-GH which around this time was shown to have limited activity in man compared with primate or human pit-GH (see §5.5).

7.2 Human pituitary-derived GH

Soon after the isolation of human pit-GH, Liljedahl [1961] administered ten or 20mg/day human pit-GH to five burned patients over a period of seven to nine days from as early as eight days after injury. GH significantly improved nitrogen balance compared with a control period. The findings of this study can be criticised because the treatment periods were always compared with the preceding control periods during which the patients had a lower mean energy intake.

Roe and Kinney [1962] showed that the administration of 10mg/day of human pit-GH to surgical patients for periods up to 45 days altered the pattern of substrate utilization but Johnston and Hadden [1963] failed to show that the administration of between 2.5 and 10mg/day of human pit-GH to patients for five days after inguinal herniorrhaphy resulted in any improvement in nitrogen retention compared with control patients.

Soroff et al. [1967] performed eighteen 16-day cross-over studies in six burned patients receiving a constant dietary intake, starting from between six days and one and a half years after injury. The studies consisted of a four-day control period, followed by two four-day periods of GH administration and finally a further control period. Adults received 8mg/day human pit-GH and children 4mg/day during the GH administration periods. Nitrogen retention was improved by GH administration in twelve studies, significantly so in six. GH seemed to exert its most beneficial effect shortly after grafting, when patients were spontaneously anabolic but nitrogen balance appeared to worsen when GH was administered to previously catabolic patients.

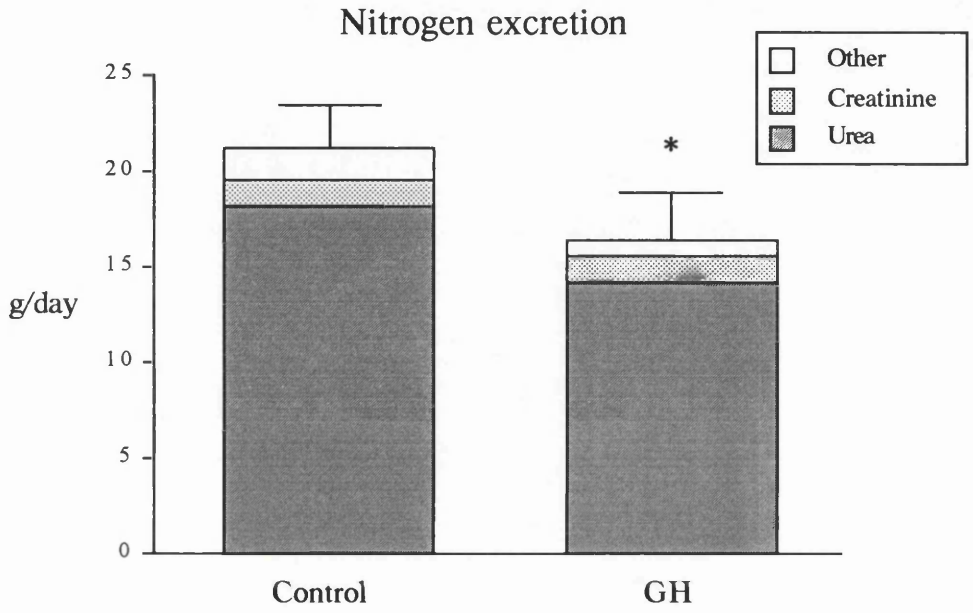
Douglas Wilmore et al. [1974c] (Figure 1.3) studied nine burned patients, whose mean dietary intake was 15.6g/m^2/day (28.3±1.6g/day) nitrogen and 2191kcal/m^2/day, from 14 to 245 days post-injury. Each patient was randomly allocated to receive 10iu of human pit-GH or placebo for seven days and then the alternative preparation for the next seven. GH administration resulted in a significant reduction in the blood urea concentration and nitrogen excretion (Figure 7.1). There was a significant correlation between the reduction in nitrogen excretion and the administered dose when corrected for body weight.

7.3 Biosynthetic GH

No further studies of the anabolic activity of GH were published and there is no evidence that GH was used clinically as an anabolic agent between 1974 and 1986. This almost certainly reflects the scarcity of pit-GH preparations. Following the advent of biosynthetic GH preparations, the effect of GH on nitrogen metabolism has been re-examined by several groups of researchers.

Manson and Wilmore [1986] performed a randomized cross-over study on four normal volunteers who were given either placebo or 10mg/day of either human pit-GH or somatrem (Protropin, Genentech Inc., San Francisco, USA) (0.13-0.16mg/kg/day, 0.26-0.32iu/kg/day, mean=0.28iu/kg/day) during two seven-day periods, separated by at least two weeks. During each period they received TPN consisting of 6g/m^2/day nitrogen with either an "adequate" energy intake, defined as 125% of the calculated BEE, 50% of adequate, or 30% of adequate intake. The administration of both GH

Figure 7.1 The effect of human pit-GH on nitrogen excretion in burned adults. Mean(±SE) urinary total nitrogen and urea excretion during the seven days of human pit-GH or placebo administration (see text). * Urea and total nitrogen excretion was significantly reduced in patients receiving GH compared with those receiving placebo ($p<0.05$) (Drawn from data presented by Wilmore et al. 1974c, with permission).



preparations resulted in significant falls in blood urea-nitrogen, urea excretion and a positive nitrogen balance at all levels of energy intake. Overall, the increase in nitrogen retention amounted to 2.2g/m^2/day. The same group conducted a further cross-over study in nine surgical patients who were receiving TPN, consisting of 60% of their calculated energy requirements and approximately 1.3g/kg/day of protein, during two consecutive seven-day periods [Ziegler et al. 1988]. They were given either placebo or 10mg/day somatrem (Protropin) (0.12-0.21mg/kg/day, 0.25-0.43iu/kg/day, mean=0.32iu/kg/day) during the two periods. Somatrem administration caused a significant fall in their blood urea-nitrogen and an increase in the positivity of their nitrogen balance, amounting to 2.9g/day.

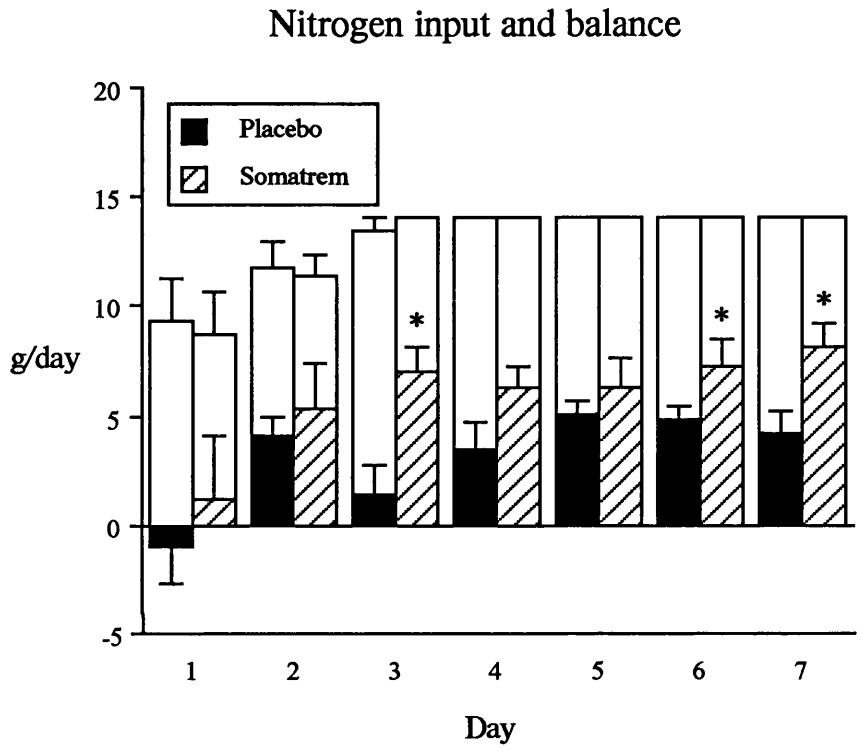
Two studies were performed at the University of North Carolina in obese adults receiving weight-reducing diets of either 24kcal/kg/day with 1g/kg/day protein [Clemmons et al. 1987] or 18kcal/kg/day with 1.2g/kg/day protein [Snyder et al. 1988]. In each, 0.1mg/kg (ideal body weight) of somatrem was administered on alternate days (Protropin, Genentech Inc., San Francisco) (0.05mg/kg/day, 0.1iu/kg/day) and compared with placebo. The first was a cross-over study during which either somatrem or placebo were administered during two three-week periods separated by two weeks. Somatrem administration caused significant nitrogen retention, amounting to 1.9g/day. In the second study, in which the subjects were allocated to receive either somatrem or placebo for 11 weeks, somatrem resulted in significant nitrogen retention, amounting to 0.9g/day. Interestingly, it was noted in the latter study that the nitrogen-sparing effect of somatrem was only apparent during the first 33 days of administration and the

authors postulated that resistance to IGF-I may occur during prolonged treatment.

The St. Mary's Hospital group performed three studies in which 0.1 mg/kg/day of somatrem (Somatonorm, KabiVitrum AB., Stockholm, Sweden) (0.29iu/kg/day) was compared with placebo in patients who had undergone gastro-intestinal surgery and who were receiving TPN consisting of either 400kcal/day and no nitrogen intake [Ward et al. 1987], 1125kcal/day and 7g/day of nitrogen [Ponting et al. 1988] or 2000kcal/day and 14g/day of nitrogen [G. Ponting et al., unpublished work]. Each was a randomized cross-over study during which the patients were allocated to receive either somatrem or placebo over a six or seven day period. Together, these studies showed that somatrem administration caused a significant reduction in the rate of protein oxidation and an increase in the net rate of protein synthesis. In these studies, somatrem resulted in significant nitrogen retention, compared with placebo, amounting to approximately 1.9, 2.7 and 2.6g/day respectively (Figure 7.2).

To date, only one study of the anabolic effect of somatropin has been published [Binnerts et al. 1988]. Four malnourished patients were studied over five four-day periods when they received 120% of their calculated energy and protein requirements. During the second and fourth periods, they received 0.025 and 0.05mg/kg/day of somatropin respectively (Humatrope, Eli-Lilly Co., Indianapolis, USA) (0.07 and 0.14iu/kg/day). The remaining periods served as controls. Somatropin caused a significant increase in nitrogen retention, regardless of dose, which overall amounted to approximately 1.5g/day.

Figure 7.2 The effect of somatrem on nitrogen balance in post-operative patients receiving full intravenous nutritional support. Mean(±SE) nitrogen input and balance over seven days whilst patients were given somatrem or placebo (see text). * Nitrogen balance was significantly more positive in patients receiving somatrem compared with those receiving placebo ($p<0.05$). (Reproduced from Ponting et al. unpublished, with permission).



These eight studies clearly show that the administration of biosynthetic GH causes nitrogen retention under a wide variety of dietary conditions and refute the "critical level" hypothesis (see §7.1). Indeed, Ziegler et al. [1988] concluded that "positive nitrogen balance can be easily achieved and sustained when hypocaloric parenteral nutrition is combined with recombinant GH administration". These studies indicate that the efficiency of administered nutrients is improved by GH and Ponting et al. [unpublished work] postulated that "in circumstances where a reduced calorie load may be desirable (peripheral feeding) or requirements increased (such as sepsis) administration of biosynthetic human growth hormone may be of benefit". None of these studies was performed in injured patients or in those with sepsis and therefore this comment could only be speculative.

7.4 Dosage

The biological activity of GH has been studied in a variety of experimental animal systems and human trials. It has been shown that its activity is proportional to the logarithm of the dose administered [Frasier et al. 1981, Thorlacius-Ussing et al. 1988]. The administration of between 0.03 and 0.1iu/kg of human pit-GH three times each week is effective in promoting growth in children with GH deficiency [Frasier et al. 1977, 1981]. Initial clinical trials using both somatrem and somatropin in children with GH deficiency have shown that the administration of 0.5iu/kg/week significantly increases growth [Takano and Shizume 1986a, 1986b, Girard and Gourmelen 1986]. It has therefore been recommended that the dose of

somatropin for treatment of GH deficiency should not exceed 0.5iu/kg/week [Humatrope Clinical Investigation Manual, Lilly Research Laboratories 1987].

Clinical trials of the anabolic effect of somatrem and somatropin in adults with normal pituitary function have used doses ranging between 0.07 and a mean of 0.32iu/kg/day (see §7.3). Clinically important hyperglycaemia was reported in one study [Ponting et al. 1988] and significant water retention was observed in a number (see §6.9). Insulin-resistance has been observed during the administration of 0.11 [Rizza et al. 1982] and 0.14iu/kg/day [Sherwin et al. 1983] of human pit-GH and approximately 0.23iu/kg/day of both human pit-GH and somatrem [Rosenfeld et al. 1982]. Both insulin-resistance and sodium retention are common in burned patients and therefore the usefulness of GH as an anabolic agent in them may be limited by these side-effects.

7.5 GH and clinical outcome

There was little or no attempt in any of these studies to examine the effect of GH on biochemical or clinical markers of nutrition or recovery. Wilmore et al. [1974c] noted mood elevation during treatment. Liljedahl [1961] showed a significant improvement in the serum albumin and claimed that appetite was improved during treatment but these findings may reflect the timing of the study periods. Ponting et al. [unpublished work] noted a significant increase in fibronectin levels in treated patients which was interpreted as evidence of improved nutritional repletion.

Immediately after their demonstration of the improved nitrogen retention during pit-GH administration in normal and injured rats (see

§5.5), Cuthbertson et al. [1941] examined its effects on wound-healing. Although, their study did not show any increase in the rate of closure of circular skin wounds, subsequent studies have indicated that the anabolic effect of GH is associated with improved healing.

7.6 GH and wound-healing

The rate of hydroxyproline excretion, which reflects collagen metabolism, is greatest in children and is correlated with their rate of growth [Jasin et al. 1962, Wit and Van den Brande 1984]. Acromegalics have an increased excretion rate [Jasin et al. 1962] and the administration of GH to normal or hypopituitary subjects causes an increase in urinary hydroxyproline excretion and, by implication, collagen turnover [Wit and Van den Brande 1984].

Experimental studies have indicated that GH can increase the collagen content of healing wounds. Kowalewski and Yong [1968] performed skin incisions in rats which were then randomized to receive either 1mg/kg/day bovine pit-GH or no treatment for three weeks. GH administration resulted in an increase in the total hydroxyproline present in both normal and incised skin after one week, although no increase was apparent three weeks after operation. Shaar compared the effect of 0.8mg/kg/day somatrem (2.16iu/kg/day) on collagen deposition with placebo in rats in which polytetrafluoroethylene (Goretex) tubes had been placed subcutaneously [C. Shaar, unpublished work]. After three weeks, the total hydroxyproline and collagen content in these tubes was significantly greater in the treated rats.

Three studies, in which wound-healing was assessed tensiometrically in rats with incised wounds in their skin, have shown that the increase in wound collagen deposition results in an increase in wound-strength. Hollander et al. [1984] allocated rats to a control group and six experimental groups which received one of two doses of somatrem (0.5 or 5mg/kg/day) for three days either preoperatively, perioperatively or postoperatively. After three weeks, the strength of the wounds were significantly increased in those rats treated peri-operatively compared with controls, regardless of the dose administered. This effect was not apparent in those treated either pre- or postoperatively. In a similar study, Pessa et al. [1985] randomly allocated healthy and tumour-bearing rats to receive either 5mg/kg/day of somatrem or placebo for three perioperative days. Somatrem caused a significant increase in the strength of the wounds in both groups of animals after six and 12 days compared with placebo. Jorgensen and Andreassen [1988] allocated rats to a control group or to receive 2mg/kg/day of somatropin starting either seven days preoperatively or at the time of wounding. Wound-healing was assessed after four, seven and ten days. Wound-strength was significantly increased after four days in those rats treated preoperatively but no effect was demonstrated thereafter or in rats treated only from the time of wounding.

Jorgensen and Andreassen conducted two studies in rats in which subcutaneously implanted cellulose sponges, which had been sectioned and then sutured, were used as wound-healing models. Rats were randomly allocated to a control group or to receive 0.5mg/kg/day of somatropin starting either seven days preoperatively or at the time of implantation

[PH. Jorgensen and TT. Andreassen, unpublished work]. The hydroxyproline content and mechanical strength of the sponges were assessed after seven, ten and 16 days. No significant increase in hydroxyproline deposition, due to somatropin, could be demonstrated. After seven days of implantation, the maximum stress applied to the sponges was significantly greater in rats which were treated both pre- and postoperatively with somatropin, compared with control rats. No increase was observed thereafter or in rats which had been treated only from the time of implantation. In a subsequent study, rats were allocated to a control group or to receive either 0.5, 2.0 or 8.0mg/kg/day of somatropin starting either seven days preoperatively or at the time of implantation [Jorgensen and Andreassen 1987]. In the rats which received somatropin both pre- and postoperatively there was a significant, dose-independent, increase in hydroxyproline deposition and maximum stress applied to the sponges, compared with control rats. No improvement in wound-healing was demonstrable in those treated only from the time of implantation. In the latter study, there was a significant, dose-dependent, increase in weight in rats receiving somatropin, compared with controls.

Overall, these animal studies show that the administration of GH increases collagen deposition and thereby wound strength. The majority indicate that this is a transient phenomenon occurring at the time of maximum collagen deposition in an incised wound (see §3.4) and therefore provide evidence that the enhancement of protein synthesis due to GH demonstrated in clinical studies can be clinically beneficial.

7.7 Clinical potential of biosynthetic human GH

Periods of negative nitrogen balance are common in burned patients despite the advances made in nutritional techniques. These periods occur particularly in the early stages after injury, before nutrition is fully established (see §5.1). If prolonged, they may lead quickly to malnutrition because, unlike normal subjects, injured patients fail to conserve protein during periods of partial or absolute starvation (see §3.1).

The majority of studies using human pit-GH and all using biosynthetic GH preparations have shown that GH improves nitrogen balance. Whilst there is little evidence that GH administration improves clinical outcome, the consistent enhancement in wound-healing seen in animal studies suggests that the effects of GH on nitrogen metabolism may be clinically advantageous. It has been postulated that biosynthetic GH preparations may be useful anabolic agents in injured or patients with sepsis [Manson and Wilmore 1986, Ponting et al. 1988, G. Ponting et al., unpublished work] and this is supported by previous studies using human pit-GH in burned patients but no studies have examined this suggestion.

There is evidence that the protein-sparing effects of GH result from an increased utilization of fat as energy substrate, which resembles the changes seen in normal starvation. This suggests that the administration of GH early after injury may be appropriate and effective in reducing the erosion of protein stores that can occur at this time. However, because of the effects of GH on carbohydrate metabolism and fluid balance, it is evident that the dose of somatropin used in preliminary studies in hypermetabolic patients should be modest and start at a level that is at

most comparable to that used as physiological replacement in children with GH deficiency.

SUMMARY

In the introductory part, I have reviewed the literature which has prompted me to study the anabolic activity of biosynthetic human growth hormone after injury by burning. In chapters 1. and 2., I have outlined the metabolic changes that occur after injury and discussed why protein depletion may occur rapidly in burned patients. In chapter 3., I have considered the consequences of malnutrition, with particular reference to wound-healing, and examined the evidence that nutrition can prevent or reverse these consequences. In chapter 4., I have reviewed the recent advances that have been made in the nutrition of burned patients. In chapter 5., I have examined the effectiveness of anabolic agents in improving nutrition and discussed the development of biosynthetic human growth hormone. In chapter 6., I have reviewed the physiology of growth hormone and discussed the role of the somatomedins in mediating its growth-promoting and anabolic actions. I have also examined growth hormone's other actions, particularly those on carbohydrate metabolism and fluid balance. In chapter 7., I have examined in detail all the previous studies of its anabolic activity in man and its effects on wound-healing in animals. I have concluded that there is evidence that biosynthetic human growth hormone may cause nitrogen retention in burned patients and that its administration soon after injury may limit or prevent protein-depletion at this time. I have also noted that growth hormone administration has caused glucose intolerance and sodium retention in normal adults and have concluded that modest doses should be used in burned patients.

PART II

CLINICAL STUDY

8.1 - OBJECTIVES

The clinical study to be described in this part was designed to investigate the anabolic effects of biosynthetic human growth hormone (somatropin) in adult burned patients. It also was intended to investigate its effects on glucose metabolism and fluid balance.

8.2 - MATERIAL AND METHODS

8.2.1 Introduction

The clinical study described below was performed on adult burned patients admitted to the the McIndoe Burns Centre. The patients were studied for 15 days and were randomly allocated to receive either somatropin or to form a control group.

In the material and methods section below, I will confirm that the requirements of the district ethical committee were satisfied by the present study (§8.2.2). I will describe the selection of patients for the study (§8.2.3), their randomization into the two study groups (§8.2.4) and the regimen of somatropin administration (§8.2.5). I will list the criteria for withdrawal of patients from the study (§8.2.6) and for completion of the study (§8.2.7). I will summarise the composition of patients in the two groups of the study (§8.2.8), and will detail their clinical management (§8.2.9 and §8.2.10) and their nutritional regimen (§8.2.11). I will list the clinical data recorded during the study (§8.2.12) then describe the techniques used for calculating nitrogen, fluid and electrolyte balances (§8.2.13), and gas-exchange (§8.2.14). I will give details of the blood-samples collected (§8.2.15) and assays performed on them (§8.2.16). I will describe the techniques used to assess glucose tolerance (§8.2.17). Finally, I will describe the analysis of the results and the statistical tests used (§8.2.18).

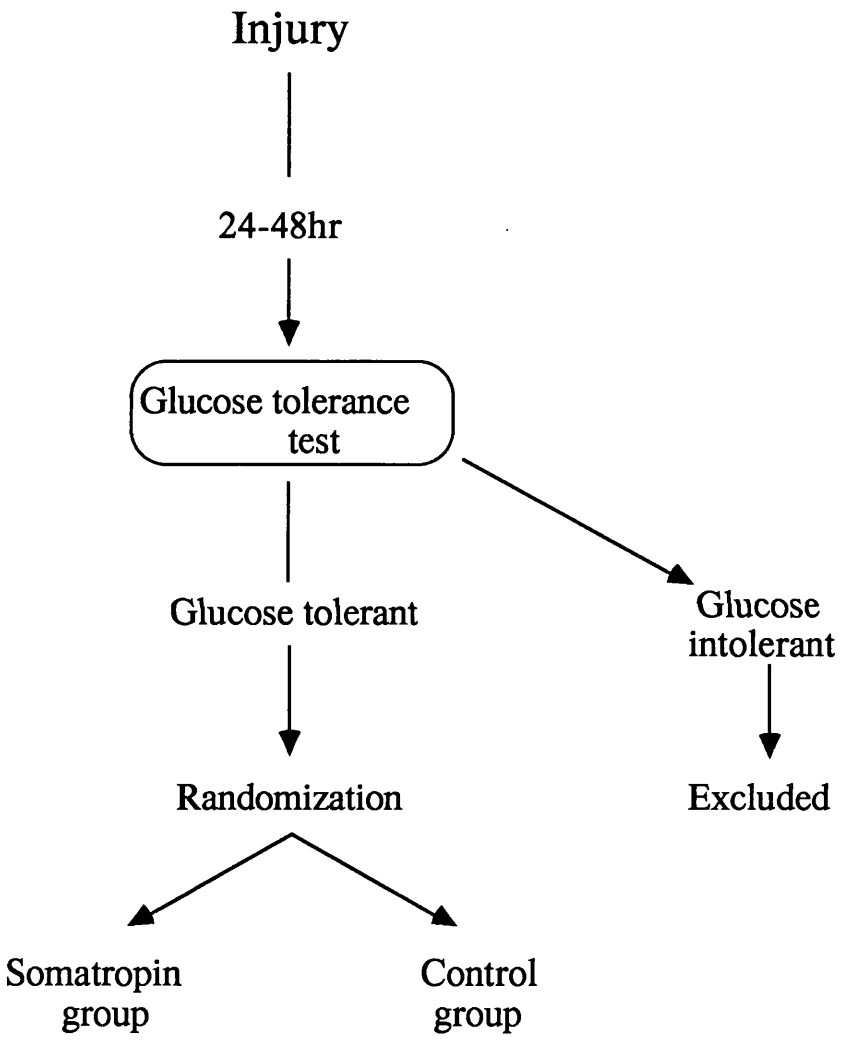
8.2.2 Ethical approval and consent

The study was approved by the district ethical committee and each patient signed a consent form after they and their relatives had been given a full explanation of the study.

8.2.3 Selection of patients

1. Entry into the study occurred at 08.00am, between 24 and 48 hours after injury (Figure 8.1).
2. Inclusion criteria for patients.
   (a) Male or female.
   (b) Sixteen to 70 years old inclusive.
   (c) TBSA assessed on admission to be between 15 and 45% inclusive.
   (d) Predicted mortality of less than 60% [Zawacki et al. 1979]
   (e) The patients were to be able and willing to sign a consent form.
3. Exclusion criteria for patients.
   (a) Admission to the Burns Unit later than 12 hours after injury.
   (b) Clinical evidence of inadequate fluid resuscitation at the time of entry into the study, such as hypotension, oliguria or the presence of a haematocrit of 55% or greater.
   (c) Chemical and electrical contact burns or a coexistent respiratory burn.
   (d) Altered consciousness or the requirement for mechanical ventilation.
   (e) Unsuitability for investigation on psychiatric grounds.
   (f) Pregnancy.

Figure 8.1 Timing of entry into the clinical study of the patients and their allocation to the treatment-groups.



(g) A history of clinically significant cardiac, renal or hepatic disease.

(h) The administration of ß-adrenergic blockers or corticosteroids.

(i) Pre-existing diabetes mellitus.

(j) Glucose intolerance, at the time of entry into the study (see §8.2.17), defined as a baseline blood glucose in excess of 10mmol/l or a peak level, during an intravenous glucose tolerance test, greater than 20mmol/l.

8.2.4 Randomization

At the time of entry into the study, the patients were allocated to control or somatropin-treatment groups by the minimization technique [Taves 1974] (see appendix 1.). The criteria chosen for their allocation to the two study groups were TBSA, age and sex. Equal weighting was given to each criterion. No attempt was made to conceal the group to which each patient had been allocated from them or any Burns Centre staff.

8.2.5 Drug administration

Somatropin (recombinant DNA-derived biosynthetic human growth hormone, Humatrope, Lilly Research Ltd., Windlesham, Surrey) was provided in lyophylised form in vials containing 5.92mg (16iu) of somatropin, 29.6mg mannitol, 5.92mg glycine and 1.36mg dibasic sodium phosphate. The somatropin was reconstituted with 8ml water containing 0.3% metacresol and 0.2% phenol and was administered at 10.00am daily by subcutaneous injection.

The patients allocated to the somatropin group received 0.03mg/kg/day (0.08iu/kg/day) of somatropin between the first and fifth days, 0.06mg/kg/day (0.16iu/kg/day) between the sixth and tenth days and no somatropin during the last five days of the study (Figure 8.2). The control patients received no somatropin at any time.

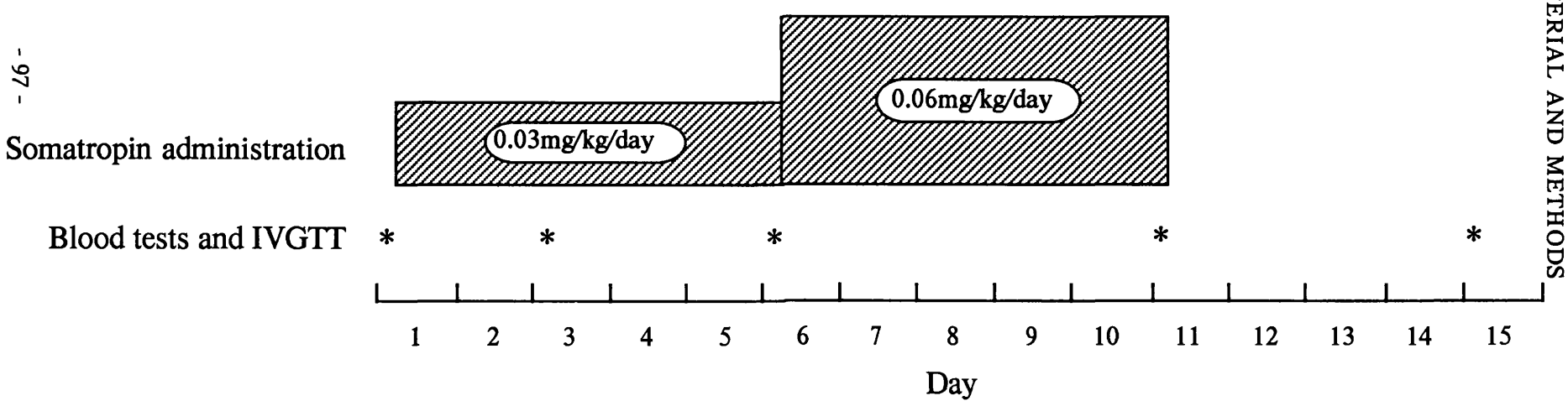
8.2.6 Withdrawal criteria

1. Request of the patient to leave the study.
2. Unsatisfactory glucose tolerance, defined as a baseline blood glucose in excess of 12mmol/l, random capillary glucose values in excess of 15mmol/l on two consecutive days, or the requirement for insulin therapy.
3. Occurrence of clinically significant fluid retention.
4. Decision of the Burns Centre staff to withhold the study drug.

8.2.7 Completion of study

The study was to finish as soon as at least six patients in each group had completed the 15-day period. Patients who failed to complete the study for any reason were to be excluded from analysis.

Figure 8.2 Timing of somatropin administration and investigations during the study.



8.2.8 Patients

Six patients in each group completed the study (see appendix 2.). One patient (#C5) was excluded from entry into the study because of unsatisfactory glucose tolerance. She subsequently required insulin administration prior to her death. One patient in each group was withdrawn during the study, one at his own request (#C8), the other because of hyperglycaemia (#GH4).

8.2.9 Initial management

All patients were managed according to the established Burns Centre protocol.

1. Naked body weight was measured immediately on admission.
2. A peripheral intravenous cannula was inserted.
3. Baseline blood samples were obtained (full blood count, urea and electrolytes, blood glucose, a sample for cross-matching and blood gases when indicated).
4. Intravenous pethidine was administered.
5. Plasma protein fraction (PPF) and dextrose-saline infusions were started intravenously.
6. The burn was examined, the TBSA assessed using Lund and Browder [1944] charts, the depth of burn was estimated and swabs were taken for microbiological culture. Examination was made of the oropharynx and nares for evidence of a respiratory injury, the eyes for corneal burns and areas distal to burns to exclude ischaemia.

7. Escharotomies were performed where necessary.

8. The PPF requirements were calculated and resuscitation performed as recommended by Muir and Barclay [1974]. The total crystalloid volume provided was 1.5 to 2.0ml/kg/hr, orally, nasogastrically or intravenously as clinically indicated.

9. A urinary catheter was inserted and hourly output measurements started.

10. A fine-bore silk nasogastric tube was inserted and feeding started with Pre-Fortison (Cow and Gate Ltd., Trowbridge, Wiltshire) (see appendix 3.) if bowel sounds were present.

11. The burns were dressed.

12. The patients were moved from the resuscitation suite to their rooms immediately these procedures had been performed.

8.2.10 General management

The patients were managed in single rooms which had an environmental temperature of 25^0C maintained thermostatically. The burned areas were dressed with tulle gras, wool and crepe except for facial and perineal burns which were left exposed. Dressings were changed every two to three days, either under a general anaesthetic in patients with large burns or using opiate analgesia. Major dressing changes were covered by a single intravenous dose of an antibiotic which was chosen according to microbiological culture reports.

Patients received their regular medications with the addition of analgesics, antiemetics and antidiarrhoeal drugs as needed. In addition, all were started on ranitidine, folic acid, ascorbic acid and multivitamin supplements.

8.2.11 Nutrition

Enteral feeding was started, as soon as possible after admission and administered via electric rotary pumps. Energy requirements were calculated using the Curreri formula from the TBSA and admission weight [Curreri et al. 1974] (see appendix 3.). The starter feed solution, pre-Fortison, was changed to the full-strength solution, Fortison Standard (Cow and Gate Ltd., Trowbridge, Wiltshire), and the rate of administration increased as quickly as could be tolerated until the calculated energy requirement had been achieved. Patients were provided with standard hospital meals or supplements in addition to their enteral feed. None was fed intravenously.

8.2.12 Clinical data

The following data were recorded daily:

1. Oral fluid intake and output.
2. Enteral feed input.
3. Intravenous fluid input.
4. Food intake.
5. Urine output.

The food intake was recorded on diet-sheets and its nitrogen and electrolyte content calculated by the Burns Centre dietician using a microcomputer and the Microdiet nutritional analysis program (Department of Mathematics and Computer Science, Salford University, Salford, Greater Manchester). Calculation of daily nitrogen and electrolyte intakes included food intake, enteral feed and intravenous fluids including blood products, except when given as part of the initial resuscitation or to replace operative blood-loss (see appendix 3.). Twenty-four hour collections of urine were performed and analysed for urea and electrolytes and their total outputs calculated.

8.2.13 Balance calculations

Daily urinary nitrogen excretion was determined by the technique described by Lee and Hartley [1975] (see appendix 4.) and the daily nitrogen, sodium, fluid and potassium balances were calculated by subtraction of the urinary losses from the total intake. There were no episodes of diarrhoea during the study period and the mean TBSA were comparable, therefore faecal and integumental losses have been assumed to be similar in each group and have not been included in the daily balance.

The body surface area (BSA) of each patient was calculated from their stated height and measured admission weight [Du Bois and Du Bois 1916]. Predicted insensible losses of water [Harrison et al. 1964] and sodium [Davies 1967] were calculated for each patient from their TBSA and BSA (see appendix 4.).

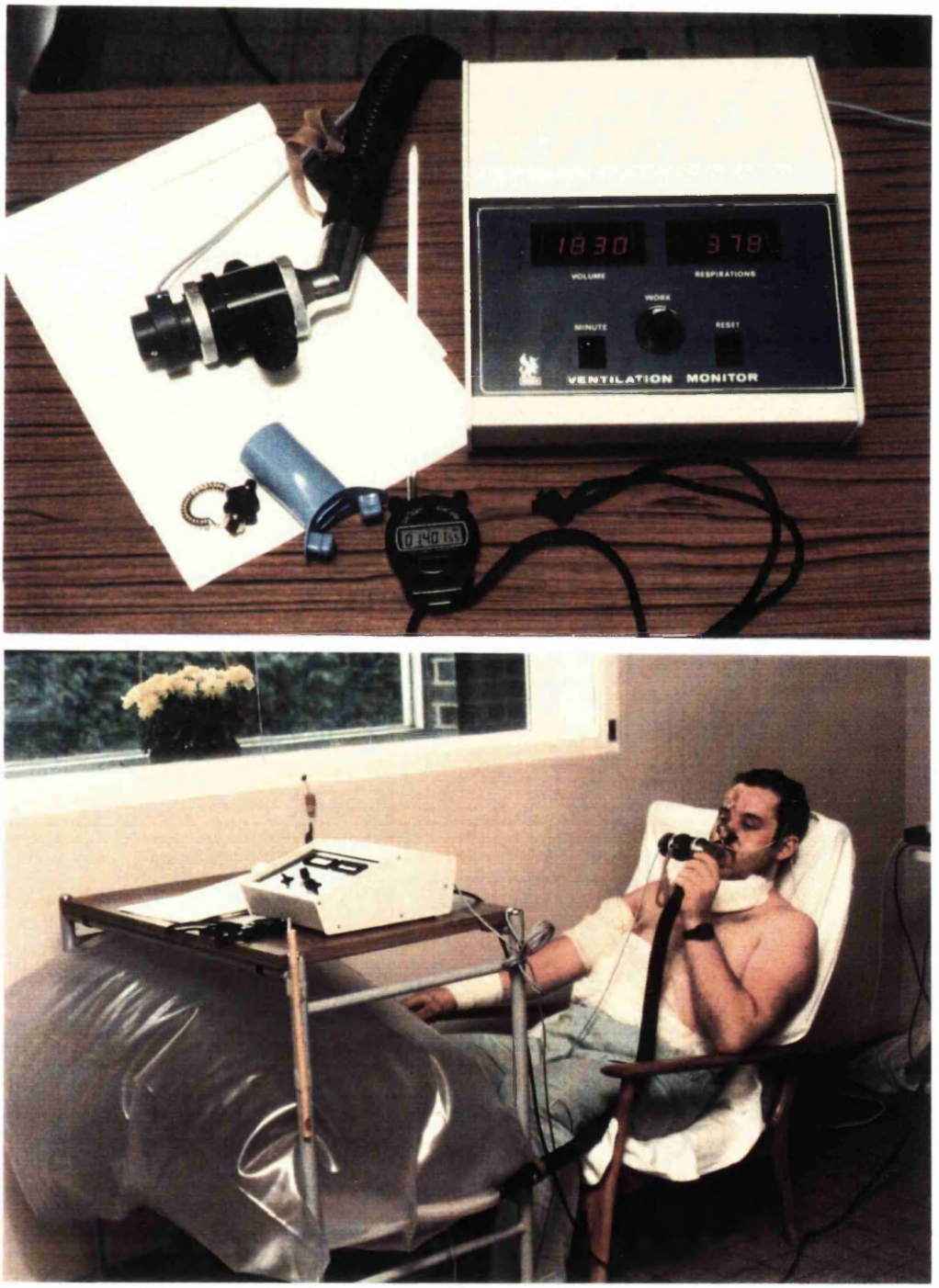
8.2.14 Calorimetry

Resting energy expenditure was measured by indirect calorimetry between the sixth and tenth days (inclusive) over ten to 15 minutes while the patients were supine. Enteral feeding was not stopped but calorimetry was performed at least four hours after meals. At least one measurement had been performed in each patient before the sixth day to familiarize them with the technique.

Inspired volume was measured using a turbine ventilometer (Mark II Ventilometer, Morgan Scientific Instruments, Gillingham, Kent) and expired gas was collected in a 200l plastic Douglas bag and analysed immediately for oxygen and carbon dioxide content (Instrumentation Laboratory 1312 Blood Gas Manager, Warrington, Cheshire) (Figure 8.3). Gas volumes were corrected for the ambient temperature and the REE and substrate oxidation rates were calculated using the Bursztein Formula [Bursztein et al. 1980] (see appendix 5.).

Results presented are the mean values of calorimetry runs in which the ventilation equivalent (VE) was less or equal to 2.9. If the VE exceeded 2.9 in all runs then the result with the lowest VE was used.

Figure 8.3 The equipment used for calorimetry. Top: stopwatch, mouthpiece, nose-clips, "breathing head" including one-way valve (outflow connected to the Douglas-bag) and turbine (connected to ventilometer), thermometer and digital ventilometer. Bottom: gas-exchange being measured in patient #GH1 (who is seated here to show the Douglas-bag).



8.2.15 Blood samples

Peripheral venous blood samples were obtained on the first, third, sixth, eleventh and fifteenth days of the study in all patients. Samples for hormone and plasma protein assays were chilled immediately, centrifuged and the plasma or serum was stored at $-40^{0}C$ within an hour of collection.

The following investigations were performed on these days:

1. Full blood count.
2. Urea and electrolytes.
3. Albumin.
4. Thyroxine-binding pre-albumin (TBPA).
5. Retinol-binding protein (RBP).
6. Total immunoglobulin-G (IgG).
7. Insulin-like growth factor-I (IGF-I).
8. Glucose.
9. Insulin.

8.2.16 Assay techniques

Full blood counts were performed on a Coulter-S auto-analyser (Coulter Electronics Ltd., Luton, Bedfordshire). Blood and urine urea and electrolytes were measured using a SMA II auto-analyser (Technicon Intruments Ltd, Basingstoke, Hampshire) [Marsh et al. 1965] and blood glucose was estimated using a glucose-oxidase technique using a Monarch 2000 Analyser (Instrumentation Laboratory, Warrington, Cheshire). Serum albumin, TBPA, and total IgG concentrations were estimated by rate

nephelometry (Beckman Array Protein System, Beckman Instruments, Brea, California, USA) [Ward 1986]. Retinol-binding protein [Beetham et al. 1985], insulin, and IGF-I [Morrell et al. 1986] were measured by competitive radioimmunoassays.

8.2.17 Glucose tolerance tests

Intravenous glucose tolerance tests (IVGTT) were performed on the first, third, sixth, eleventh and fifteenth days of the study, while the patients were recumbent. All IVGTT were done 12 hours after food and six hours after intravenous dextrose solutions had been stopped. Those on the first day were done six hours after the enteral feed had been stopped but the feed was continued throughout the subsequent IVGTT. A 19-gauge cannula was inserted into a peripheral vein and after collection of two baseline blood samples (T_0), 50ml of 50% dextrose (25g) was injected over 90 to 120 seconds and timing started mid-way through the injection. Blood samples were collected at five (T_5), ten (T_{10}), twenty (T_{20}) and sixty (T_{60}) minutes after glucose injection.

Basal plasma insulin values were corrected for the prevailing blood glucose concentration by calculation of the basal insulinogenic index (basal insulin/basal glucose). The blood glucose and insulin concentrations were converted to their delta-equivalents by subtraction of the baseline values.

Early insulin release was assessed by the incremental increase in insulin concentration between T_0 and T_5 (0-5' delta-insulin) (see appendix 6.). This was corrected for the size of glycaemic stimulus by calculation

of the 0-5' insulinogenic index (0-5' delta-insulin/0-5' delta-glucose). Total glycaemic stimulus and insulin response were estimated by calculation of the areas under the delta-glucose and delta-insulin curves (AUC) from T_0 until T_{60} or until such time that blood glucose returned to the baseline value.

The total insulinogenic index or index of insulinogenic reserve was calculated by dividing the area under the delta-insulin curve by the corresponding delta-glucose area [Seltzer et al. 1967]. The glucose disappearance constant (k) was derived from the slope between the T_{20} to T_{60} glucose values plotted semilogarithmically [Samols and Marks 1965].

8.2.18 Analysis and statistics

The results presented are confined to those patients who completed the study. Those who were withdrawn from the study for whatever reason or in whom data is absent are not included on an "intention to treat" basis. This decision was made before starting the study. It was considered that any aberration from the protocol or missing data were liable to obscure the effects of somatropin because of the small sample size.

All results are presented as mean±SE unless otherwise stated. Where appropriate, the data has been corrected for BSA. Both parametric and non-parametric tests have been used to compare sets of data (see appendix 7.). In instances where there is a difference in the statistical significance between test types, the heterogeneity of the variances has been calculated. Where there is a significant difference between the

variances, the non-parametric test is preferred. The rejected test-result is indicated in the tables by brackets and the variance ratio noted at the foot of the table.

8.3 - RESULTS

8.3.1 Patients and severity of injury

The twelve patients who completed the study formed the two study-groups. These two groups were well-matched, there being no significant differences in either the sex ratio, mean age, BSA, TBSA or median predicted mortality between them (Figure 8.4) (Table 8.1). The median times to discharge from the burns unit were comparable in the two groups (control 22 days (range 17-50), somatropin 27 days (range 21-49), ns) (see appendix 2.).

8.3.2 Haematology

The mean haemoglobin concentrations were normal on the first day but subsequently fell and remained below the normal range for males in both groups (Table 8.2). Two patients in each group required blood transfusion to correct anaemia, other than during surgery (see appendix 2.). The mean total white blood cell count exceeded the normal range in both groups on all but the third study day but there was no significant difference between the two groups at any time (Table 8.3).

8.3.3 Calorimetry and energy expenditure

There was no significant difference in REE between the two groups (Figure 8.5) (Tables 8.4, 8.5 and 8.6). The mean REE, overall, exceeded the predicted BEE by a mean of 36.9±4.1%. The energy expenditure calculated from the Curreri formula (CEE) overestimated the REE by an overall mean of 15.6±3.8%.

Figure 8.4a The distribution of the burns in the twelve patients who completed the study.

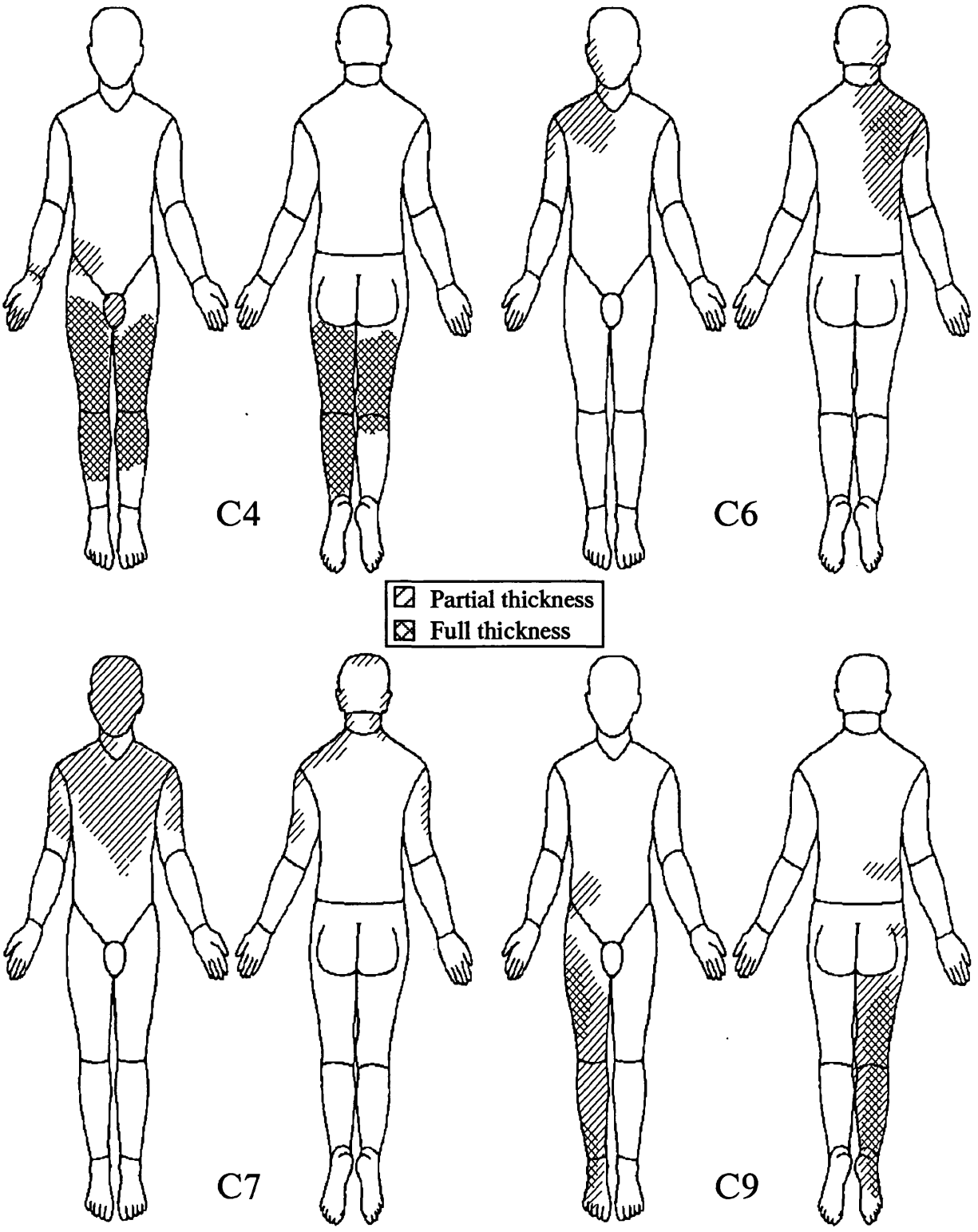


Figure 8.4b The distribution of the burns in the twelve patients who completed the study.

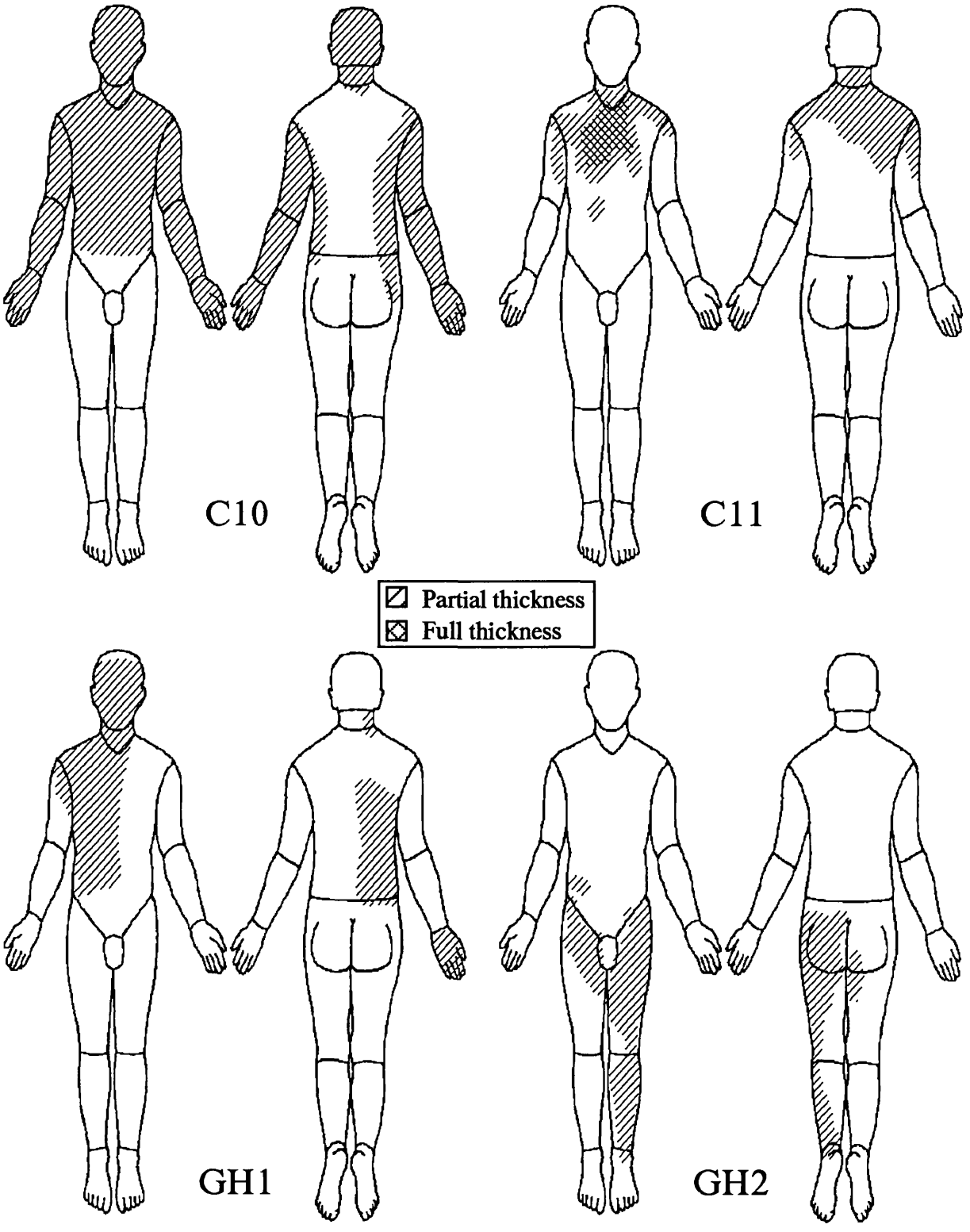


Figure 8.4c The distribution of the burns in the twelve patients who completed the study.

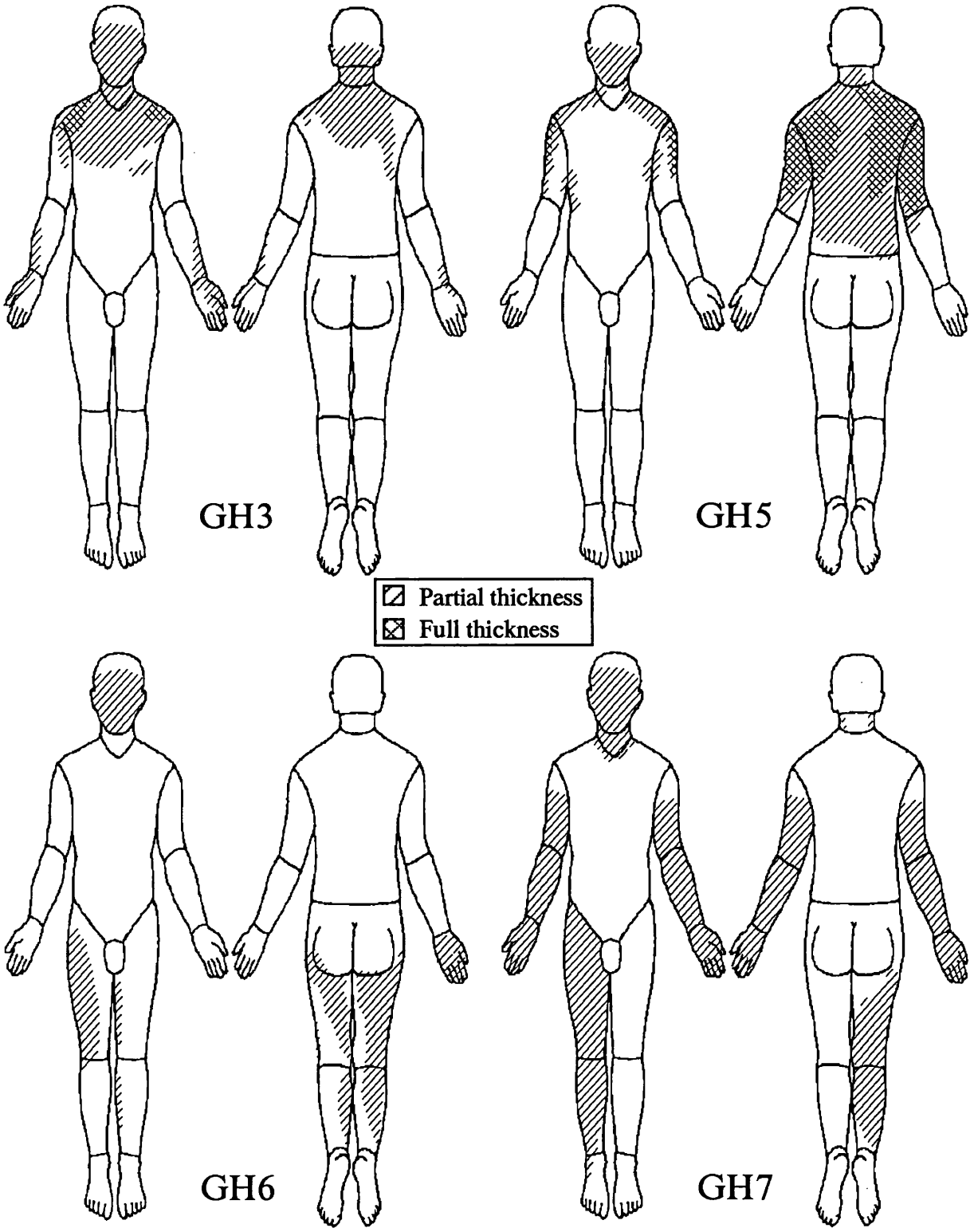
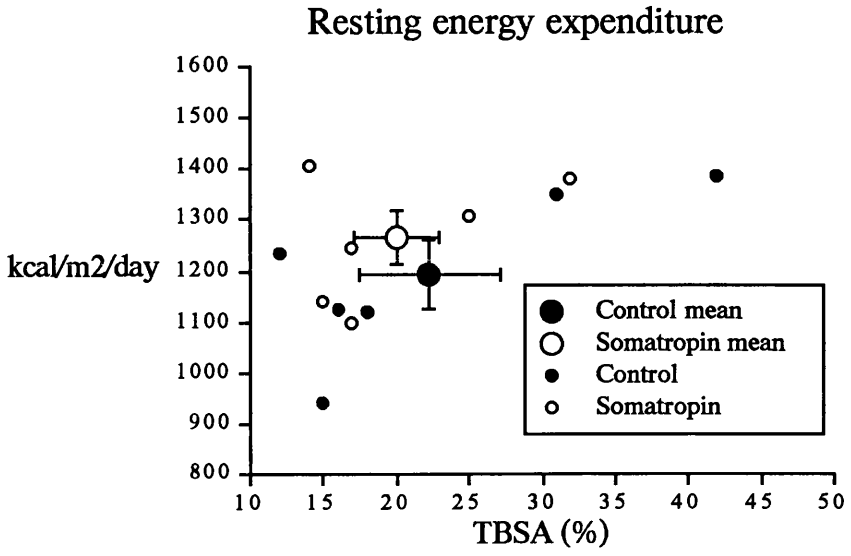


Figure 8.5 Resting energy expenditure and TBSA in the twelve patients studied. Individual values and means for each group are shown.



There were no significant differences in the mean rates of substrate oxidation between the two groups or in the percentage of energy derived from protein (PEC) (control 21.4±2.0%, somatropin 16.3±3.3%, ns).

8.3.4 Dietary intake

Enteral feeding was well tolerated by all patients and there were no episodes of diarrhoea, defined as the passage of more than three loose stools each day. There was a gradual increase in the energy and nitrogen intakes during the study (Figures 8.6 and 8.7) (Tables 8.7, 8.8 and 8.10). The mean energy intakes exceeded the CEEs on all but the first two days in both groups (Table 8.8). The actual energy intakes between the fifth and tenth days exceeded the REE by an overall mean of 61.0±9.3% (Table 8.6).

8.3.5 Nitrogen metabolism

The mean serum urea concentration was significantly lower in patients treated with somatropin on the first, third and eleventh days (Table 8.9) and there was a significant increase in its concentration after cessation of somatropin treatment in this group.

There was no difference in the mean absolute protein oxidation rate (Table 8.5), PEC (see 8.3.3) or nitrogen excretion (Table 8.11) between the two groups. The overall mean nitrogen excretion rate was 9.9±0.9g/m^2/day for the fifteen days. The mean nitrogen balance, excluding faecal and integumental losses, was positive in both groups during each of the three

Figure 8.6 Mean energy intake in the two groups during the study.

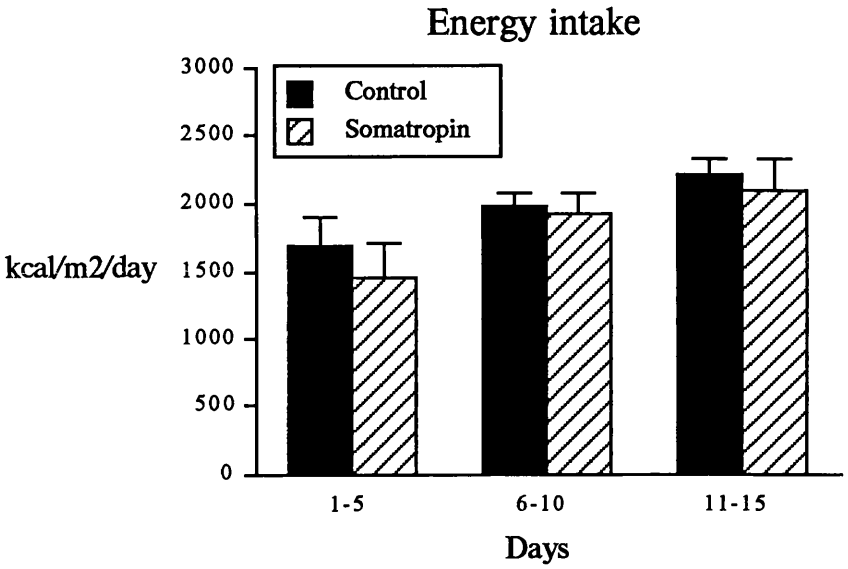
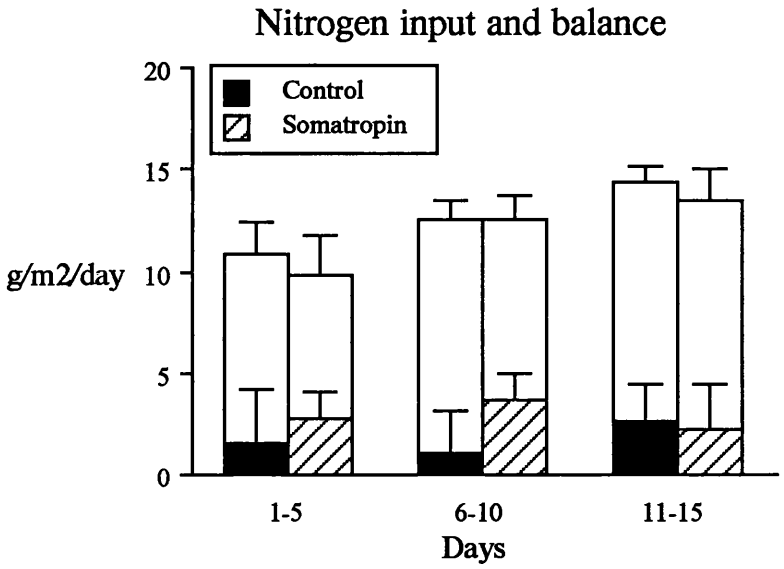


Figure 8.7 Mean nitrogen input and balance in the two groups during the study.



treatment periods but there was no significant difference between groups at any time (Figure 8.7) (Table 8.12).

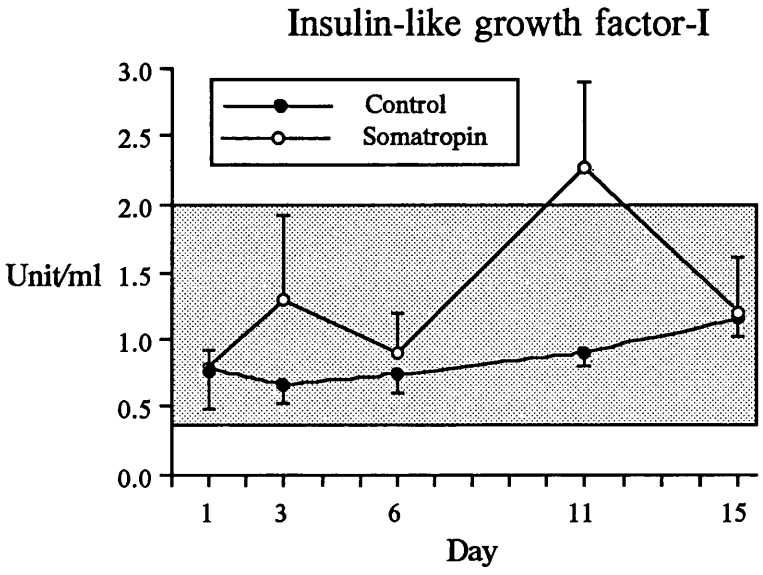
8.3.6 Plasma proteins

The mean serum IGF-I concentration was within the normal range, in both groups on the first day of the study (Figure 8.8) (Table 8.13). There was a gradual and significant increase in its mean concentration from this time until the final study day in the control patients. It was consistently higher in the treated patients during the period of somatropin administration but not significantly so, although it exceeded the normal adult range on the eleventh day. It fell significantly in the treated patients following the cessation of somatropin treatment in contrast to the control patients in whom there was a small but significant rise in its concentration between the eleventh and fifteenth day of the study.

The mean serum albumin concentrations were within the normal range on the first day but fell significantly to a nadir on the sixth day in both groups (Figure 8.9) (Table 8.14). The fall was greater in somatropin-treated patients than controls, significantly so on the third day. Following this no significant recovery occurred in control patients but there was a small and significant rise in the mean concentration in the treated ones.

The mean serum TBPA and RBP concentrations had fallen to the lower limits of, or below, their normal ranges by the first day of the study although their nadirs occurred on the third and sixth days respectively

Figure 8.8 Mean insulin-like growth factor-I concentration in the two groups during the study. The normal range is shown by the stippled area.



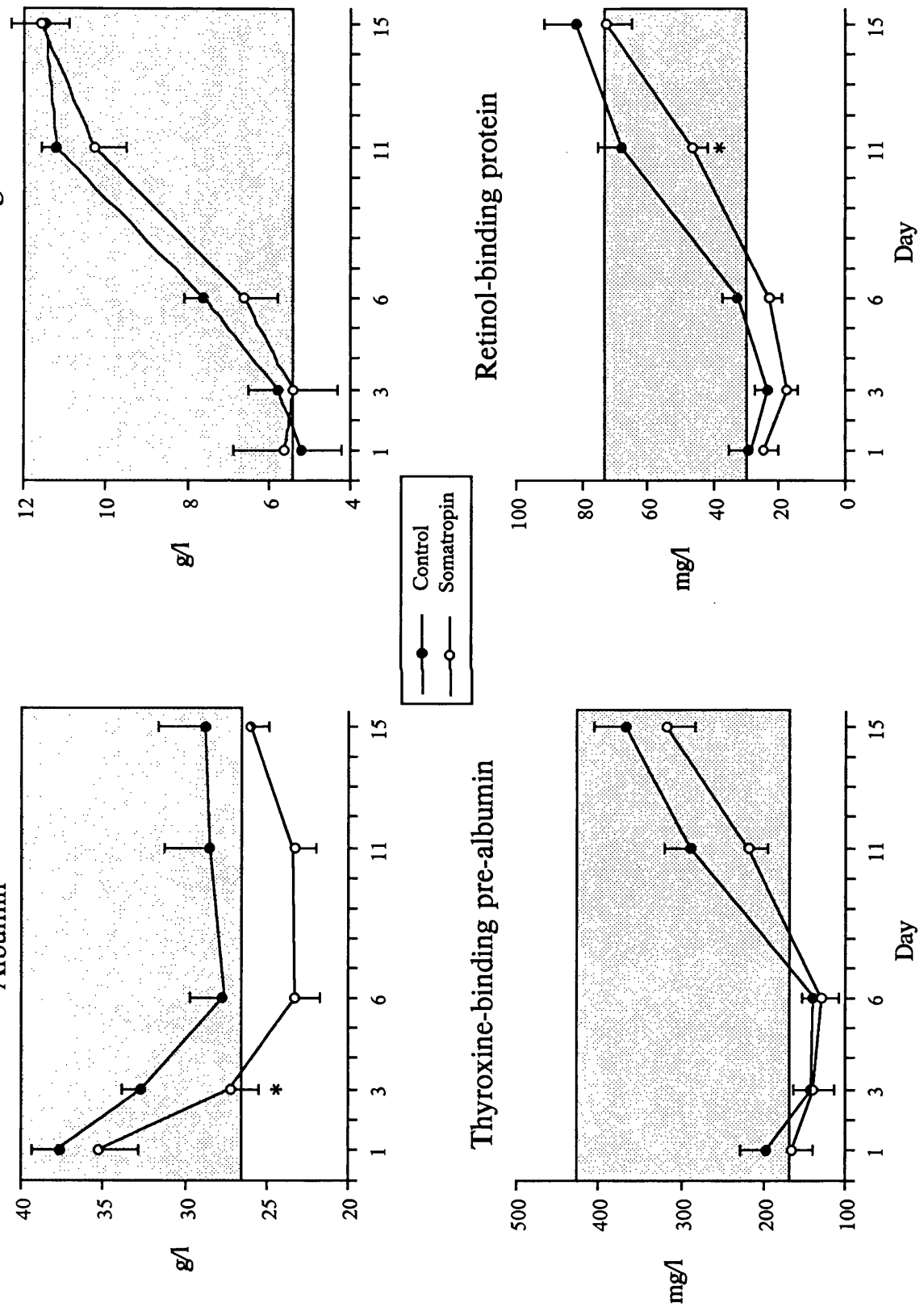


Figure 8.9 Mean plasma protein concentrations in the two groups during the study. The normal ranges are shown by the stippled areas. *p<0.05; somatropin treated patients versus control ones.

(Tables 8.15 and 8.16). The concentrations of both recovered significantly by the final day of the study. The mean concentrations of both were consistently lower in the somatropin-treated group, although only significantly so for RBP on the eleventh day.

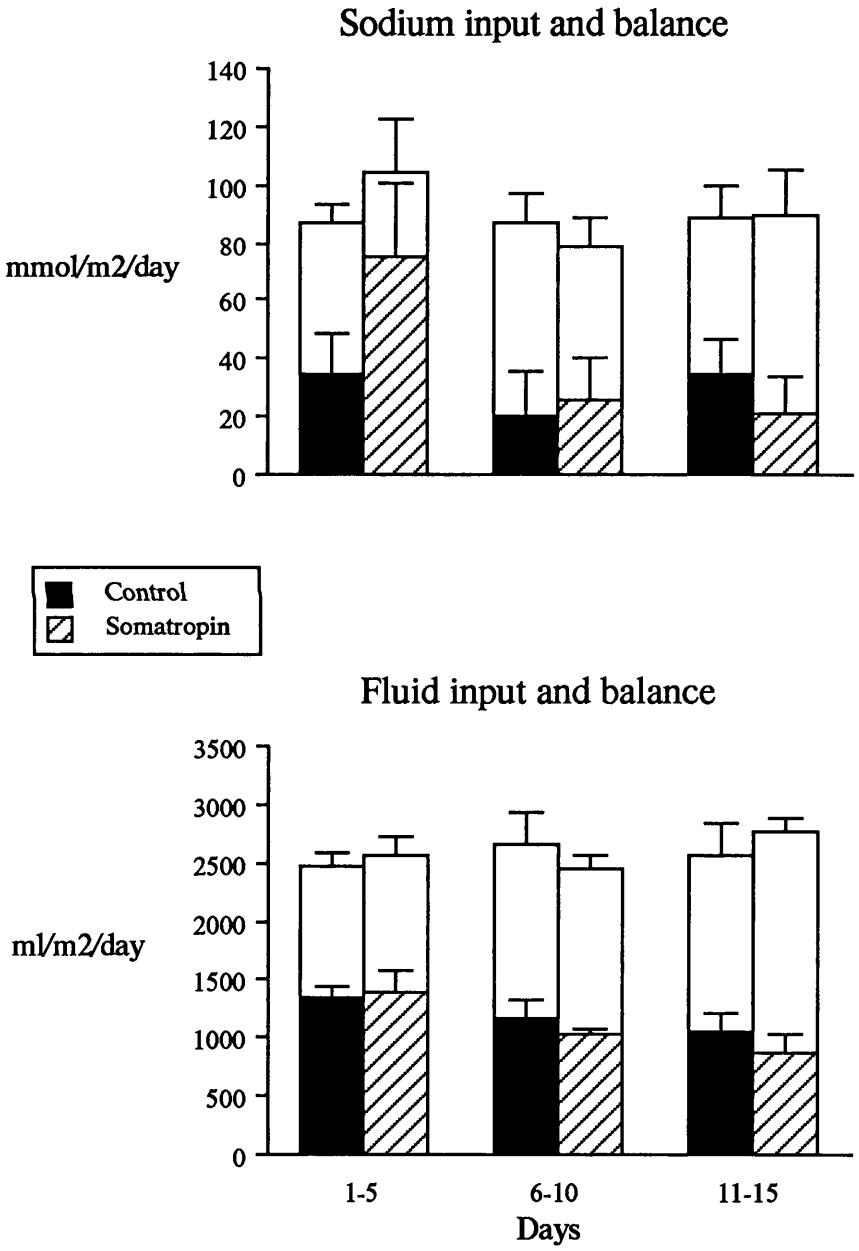
The mean total IgG concentration had fallen either below or to the lower limit of the normal range in both groups by the first day of the study (Table 8.17). It recovered significantly in both groups by the fifteenth day. There was no significant difference between control or somatropin-treated patients.

8.3.7 Fluid and electrolytes

The sodium, fluid and potassium inputs were comparable in each group (Figure 8.10) (Tables 8.18, 8.20 and 8.22) and there were no significant differences in the balances of these between groups at any time during the study (Table 8.19, 8.21 and 8.23). The mean sodium and water balances, over the 15 days, were comparable to the predicted insensible losses (Table 8.24).

There were no significant differences in the mean serum sodium or potassium concentrations between the two groups of patients at any time (Table 8.25 and 8.26).

Figure 8.10 Mean sodium and fluid inputs and balances in the two groups during the study.



8.3.8 Basal glucose and insulin concentrations

The mean rates of enteral feed administration during the IVGTT were comparable in the two groups throughout the study (Table 8.27). The fasting blood glucose concentration exceeded the upper limit of the normal range in four patients on the first day (Table 8.28). There were no significant differences in the fasting or basal glucose levels between the two groups at any time (Figure 8.11) (Table 8.29).

The mean basal plasma insulin concentration (Figure 8.12) (Tables 8.30 and 8.31) and basal insulinogenic index (Table 8.32) remained constant in the control patients throughout the study. In contrast, both increased rapidly and significantly between the first and third days in the patients treated with somatropin and were significantly greater than in the control patients on the eleventh day. Cessation of somatropin treatment was not followed by a significant fall in either although both declined to comparable levels to those in control patients.

8.3.9 Glucose responses during IVGTT

There were no significant differences in mean blood glucose concentration during the IVGTT between the two groups of patients on any day of the study except the final day when it was significantly higher in the somatropin group ten minutes after glucose administration (Figure 8.13) (Table 8.28).

The mean incremental rise in the blood glucose concentration five minutes after injection (0-5' delta-glucose) was consistently higher in the somatropin-treated patients, significantly so on the first day of the study

Figure 8.11 Mean basal glucose concentration in the two groups during the study.

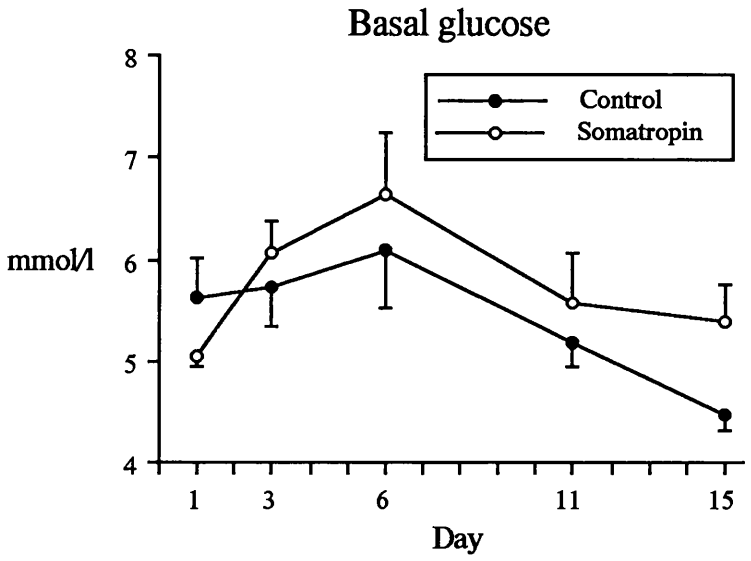
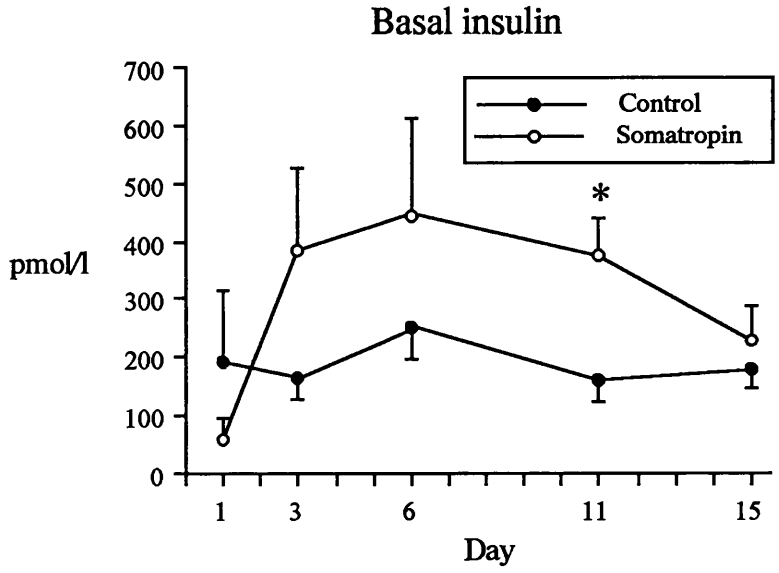


Figure 8.12 Mean basal insulin concentration in the two groups during the study. *p<0.05; somatropin treated patients versus control ones.



(Table 8.33). The mean incremental glucose AUC (delta-glucose area) was significantly greater in somatropin-treated patients on the eleventh day (Figure 8.14) (Table 8.34). On the first day of the study, the glucose disappearance constants (k) were comparable in each group but the combined mean k-value of 1.10±0.07 was low compared with healthy subjects [Marks and Marrack 1962] (Table 8.35). In the control group there was a small but significant decline in k-value between the first and third day of the study coincident with the change in test technique but this did not occur in the patients treated with somatropin. There was no significant difference in k-value between groups during the study but following the cessation of somatropin treatment there was an increase in k-value in the treated group which approached statistical significance ($0.05<p<0.1$).

8.3.10 Insulin responses during IVGTT

The pattern of insulin release following intravenous glucose administration was normal in each of the groups throughout the study with the maximum plasma insulin concentration occurring at T_5 (Figure 8.15) (Table 8.30). There was no difference in the mean plasma insulin concentration between groups at any time after glucose injection on any day. There were no differences in the early or total insulin release in absolute terms (Tables 8.36 and 8.38) or when indexed against the glycaemic stimulus (Tables 8.37 and 8.39). The change in test technique was accompanied by increases in both the 0-5' and total insulinogenic indices which were either significant or approached statistical significance ($0.05<p<0.1$) in the control and somatropin-treated patients respectively (Tables 8.37 and 8.39).

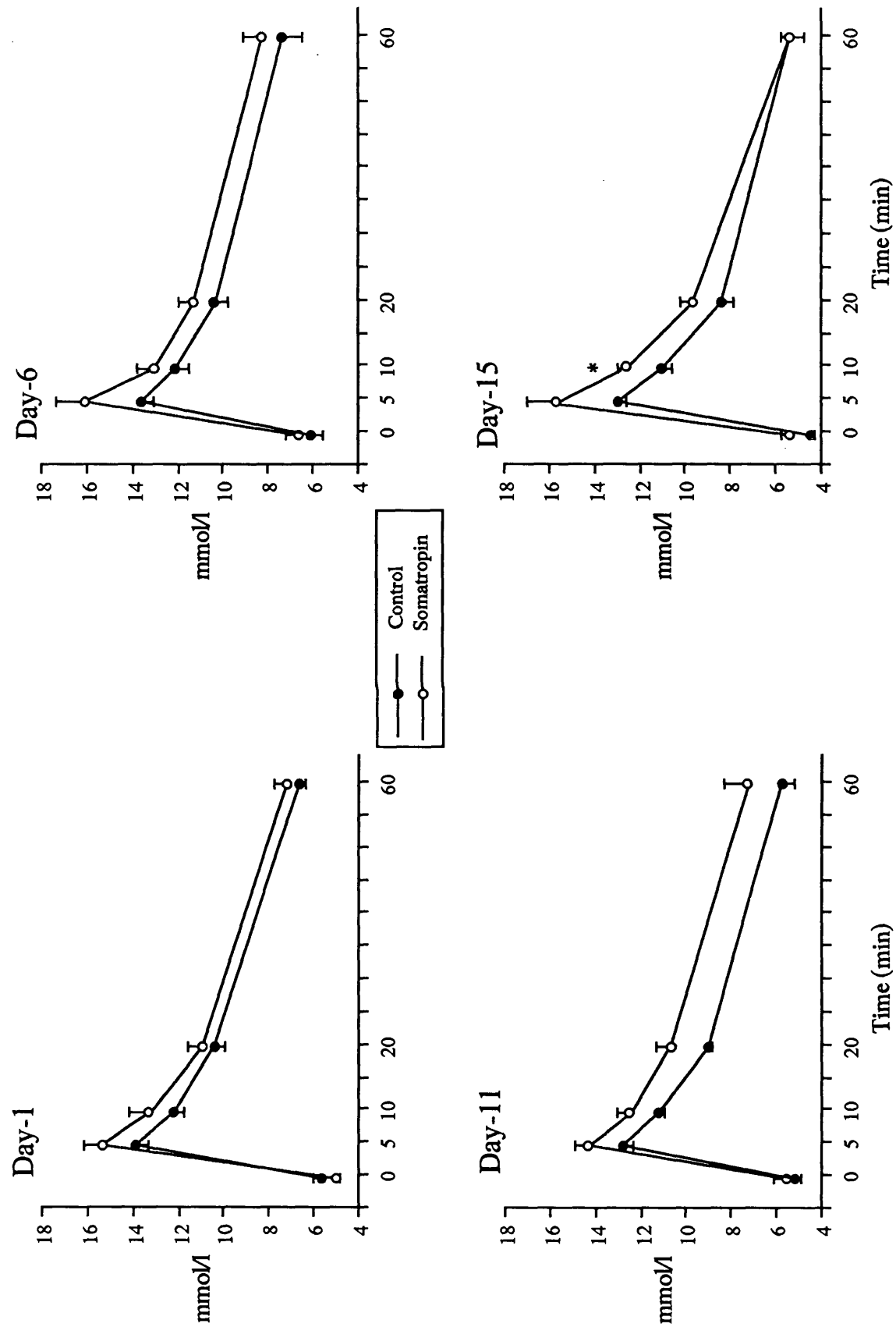


Figure 8.13 Mean glucose concentrations in the two groups during the IVGTT.
*p<0.05; somatropin treated patients versus control ones.

Figure 8.14 Mean delta-glucose area in the two groups during the IVGTT. *p<0.05; somatropin treated patients versus control ones.

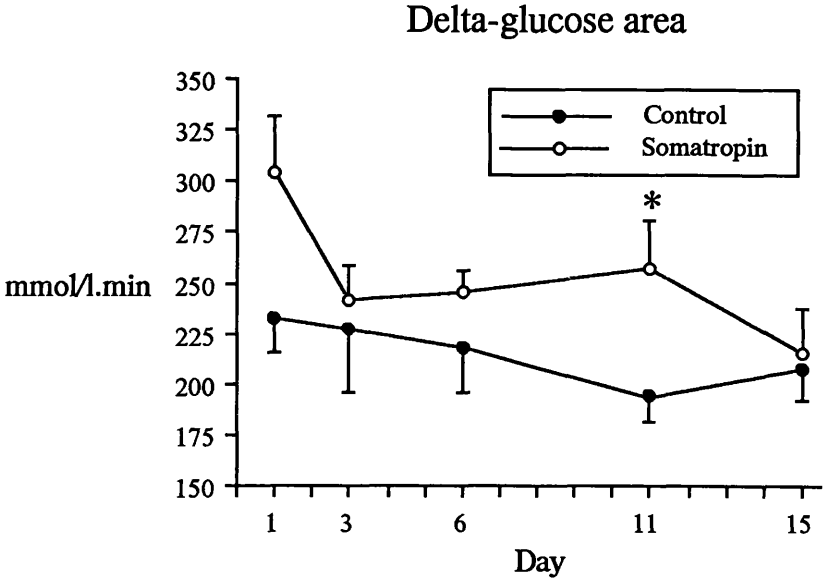
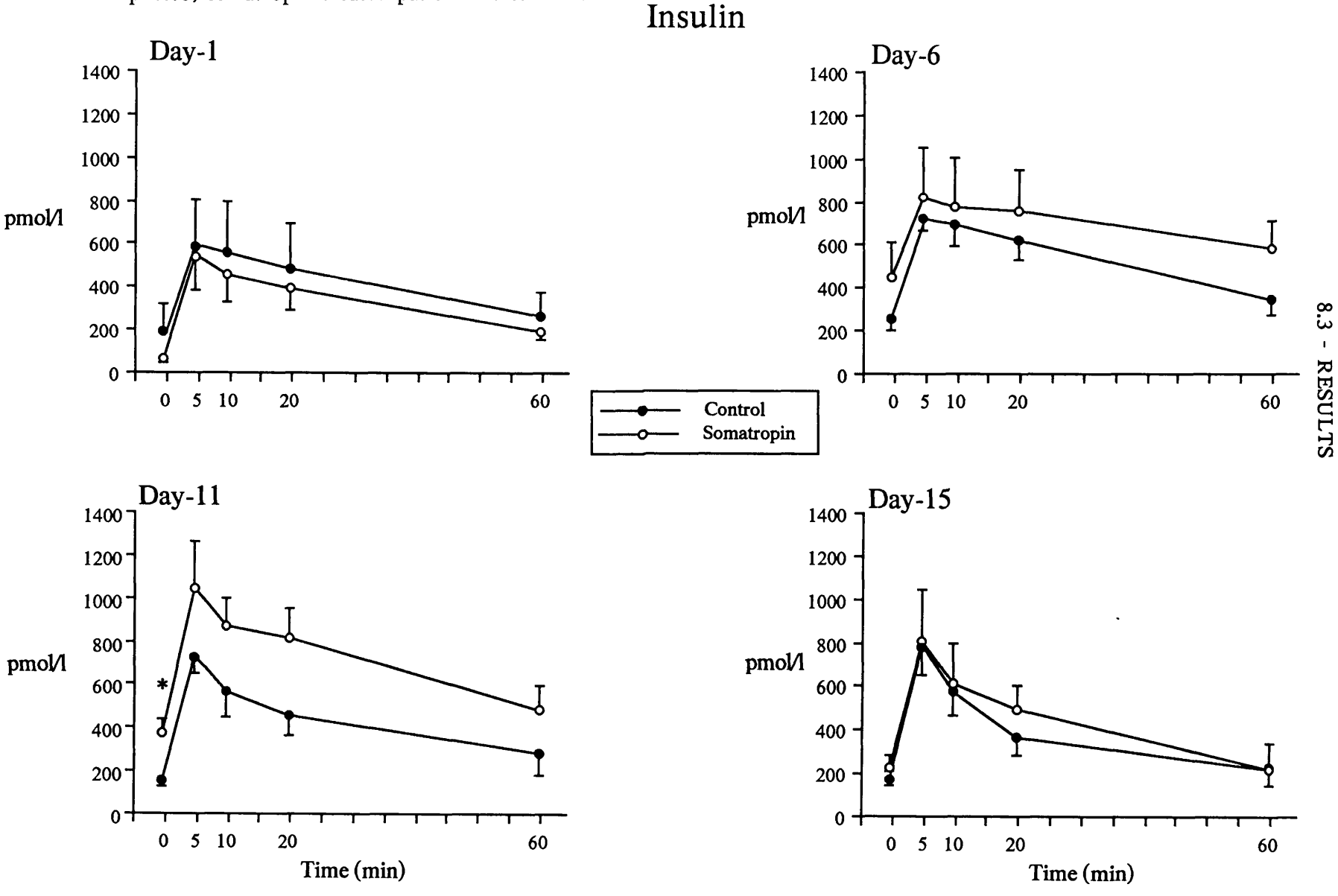


Figure 8.15 Mean insulin concentrations in the two groups during the IVGTT.
*p<0.05; somatropin treated patients versus control ones.



8.3.11 Side-effects of somatropin

The somatropin injections were well tolerated and no local or systemic reactions were noted. Somatropin treatment was withheld from one patient because of hyperglycaemia (see appendix 2.).

8.4 - DISCUSSION

8.4.1 Introduction

The present study, conducted over 15 days in adult burned patients, has shown that injury by burning is followed by rises in REE and nitrogen excretion accompanied by a fall in plasma protein concentrations and insulin-resistance. Somatropin administration fails to cause a significant rise in plasma somatomedin levels. Its administration does not decrease the rate of protein breakdown or increase either the positivity of nitrogen balance or plasma protein concentrations. Somatropin administration does not cause any sodium or fluid retention. It causes no alteration in insulin release in response to injected glucose but it exacerbates the insulin-resistant state present in burned patients.

In the discussion to follow, I will summarise the clinical aspects of the present study (§8.4.2). I will consider the causes for the haematological changes that are observed after injury by burning (§8.4.3). I will comment on the ease of providing the patients with their calculated energy requirements by the enteral route (§8.4.4). I will high-light the problems that I encountered in measuring gas-exchange (§8.4.5). I will compare the increase in REE found in the present study with those found in previous ones and discuss the effect of energy intake on the pattern of energy-substrate utilization (§8.4.6). I will compare the effects of somatropin on energy expenditure found in the present study with those found previously and speculate on the reasons for its lack of effect on both REE and the rate of fat oxidation (§8.4.7). I will note the increase in urinary nitrogen excretion seen after injury by burning and review the problems associated with balance estimation (§8.4.8). I will comment on the alteration in the normal relationship between nitrogen and potassium

retention seen in the present study (§8.4.9). I will examine the effects of somatropin on nitrogen metabolism and speculate on the reasons why no anabolic effect was demonstrated (§8.4.10). I will discuss the changes in plasma somatomedin concentrations that occurred, the failure of somatropin to elicit a significant rise in levels and suggest that these occurrences contributed to the failure of somatropin to exert a significant anabolic effect (§8.4.11). I will review the mechanisms controlling plasma protein concentrations and discuss the changes seen in the present study (§8.4.12). I will go into the causes of the increased insensible sodium and water losses in burned patients and contrast the effects of somatropin on their balances with those found previously (§8.4.13). I will examine the effects of injury by burning (§8.4.14) and somatropin (§8.4.15) on glucose metabolism and suggest that somatropin exacerbates the insulin-resistant state seen in burned patients. I will expand on the effects of somatropin on glucose metabolism with reference to a patient who was withdrawn from the study (§8.4.16). I will discuss the choice of dosage of somatropin and caution that the use of larger doses in hypermetabolic subjects is precluded by its effects on glucose metabolism (§8.4.17). Finally, I will conclude that the present study has failed to demonstrate that somatropin has any anabolic activity in patients with burns and will indicate how shortcomings in it can be overcome (§8.4.18).

8.4.2 Patients and general management

The present study was completed in twelve adult patients who had moderately-sized burns and low predicted mortalities. They were managed in a specialised Burns Centre according to established guide-lines. Their

surgical management was conservative, early grafting being indicated in only two patients who had large, predominantly full-thickness, burns. Two patients were treated successfully with intravenous antibiotics for septicaemia diagnosed on clinical grounds. All made satisfactory recoveries and were discharged from the Burns Centre no later than the fiftieth day after burning.

8.4.3 Haematological responses

Anaemia is common in burned subjects for a number of reasons. Erythrocytes are haemolysed during the first 48 hours after injury as a result of heating in the burn-wound [Baar 1979, Heideman 1979] and frank haemoglobinuria is evident within hours of injury in patients with large burns. An immediate fall in the haemoglobin concentrations was not seen in the present study because the patients were resuscitated according to the protocol of Muir and Barclay [1974] which requires the rate of plasma administration to be adjusted to maintain a normal haematocrit. The subsequent fall in the mean haemoglobin concentration was due to a continued decrease in red cell volume which has been shown to amount to one to two percent per day. This results from a reduction in the half-life of erythrocytes [Loebl et al. 1973] and inadequate synthesis of new erythrocytes, despite the presence of raised erythropoietin levels [Sheldon et al. 1978]. These effects are exacerbated by blood loss from the burn-wound. There was no difference between groups in the extent of the anaemia or requirements for blood.

There was a mild leucocytosis present in both groups during most of the study. Experiments have shown that this occurs within hours of injury

[Newsome and Eurenius 1973, Heideman 1979] and that white cell counts usually remain mildly elevated. This response is probably mediated by both counter-regulatory hormones and inflammatory mediators derived from the burn-wound (see §1.3) and predictably, there was no difference in this response between groups. No patients became leucopenic as may occur in patients with very large burns or with severe sepsis.

8.4.4 Energy intake

The present study has shown patients with moderate burns can be satisfactorily fed via the enteral route. The calculated energy requirements were achieved quickly in most patients as a result of early feeding. The feeds were well tolerated and no patients had diarrhoea which may have resulted from our policy of early feeding (see §4.5), our selective use of antibiotics and the administration of both analgesics and anti-diarrhoeal drugs. Only one patient failed to achieve, overall, his calculated requirements for the 15 days of the study.

8.4.5 Calorimetry

Some difficulties were encountered in measuring gas-exchange using the present system. Consistent measurements could not be made in a number of patients in the first five days of the study, particularly in those with facial burns. This was due to the anxiety and discomfort associated with the use of a mouthpiece and nose-clip, and air-leakage around the mouthpiece in those with burned lips. These problems lessened with practice and with the rapid healing of the facial burns. Despite

these problems, the mean ventilation equivalents (VE) were within normal limits [Bartlett et al. 1982]. Two patients continued to hyperventilate, despite repeated practice runs, and this was reflected in their relatively high VEs. One was unwell and spoke poor English (#GH5), the other was an epileptic woman with low intelligence (#C11).

The present system had the merit of being cheap and simple to use but was poorly tolerated by most patients. It was evident that it was unsuitable for patients who either had facial burns or who were unable or unwilling to cooperate. Comparative studies, in healthy subjects, between hood systems and mask or mouthpiece systems have shown that hood systems are no more accurate or better tolerated [McAnena et al. 1986, Segal 1987]. Nevertheless, it may be concluded that calorimetric systems using a ventilated hood are more suitable in burned patients.

8.4.6 Energy expenditure

The patients were hypermetabolic and the mean rise in REE above the calculated BEE was comparable to other series in which increases of between approximately 30 and 47% have been seen in fed patients with burns [Turner et al. 1985, Saffle et al. 1985, Matsuda et al. 1987, Allard et al. 1988]. The RQ were high in both groups as a result of the concurrent administration of enteral feed which provided an energy intake calculated from the Curreri formula (CEE). The CEE exceeded the REE as in previous studies in which it has been shown to overestimate the REE by between 13 and approximately 22% in patients with similar TBSA [Saffle et al. 1985, Matsuda et al. 1987].

Although nitrogen retention can be improved by the increased administration of carbohydrate-derived energy (see §4.3), little or no improvement can be demonstrated once the REE has been exceeded (see §4.2). Carbohydrate intakes in excess of 10g/kg/day are not oxidised but are stored as fat. The carbohydrate oxidation rates in the present study were approaching this level in a number of patients who also had negative fat oxidation rates indicative of net lipogenesis [Frayn 1983]. The total energy inputs, including meals and high energy supplements were in excess of those during the gas exchange measurements and it can be concluded that some of the patients were overfed. Many Burns Centres continually adjust the dietary target according to the open wound size, which takes account of wound-healing and skin-graft donor sites. This modification was not used in the present study and its use may have limited the extent of overfeeding.

8.4.7 Somatropin and energy expenditure

There was no significant differences in the REE or rates of substrate oxidation resulting from somatropin administration unlike recent studies in which somatrem administration increased the REE and rate of fat oxidation (see §6.8). These studies differ in a number of ways from the present one. They were performed in patients who had little or no rise in REE and who were receiving either hypocaloric or eucaloric diets. In one, gas exchange was measured in the fasting state [Manson and Wilmore 1986]. In the present study, the calorigenic effect of somatropin may, therefore, have been obscured by the existing hypermetabolism or other clinical variables such as sepsis, dressing changes and pain. Furthermore, the

treated group can be predicted to have had a 4% lower REE than the control group because of their smaller TBSA and energy intake [Allard et al. 1988].

Little fat oxidation occurred in either group in the present study, because of the high energy intakes, in contrast to these previous studies in which patients were already deriving a significant proportion of energy from fat oxidation. Recently, somatrem has been administered to post-operative patients who were receiving full intravenous nutritional support and although there was a significant increase in REE, there was no increase in lipolysis [G. Ponting et al., unpublished work]. It may be postulated that increased insulin levels, associated with high energy intakes, counteract the lipolytic activity of GH preparations.

8.4.8 Nitrogen metabolism

The mean rate of nitrogen excretion and therefore protein oxidation, was high in the present study and was very similar to that seen previously in patients who had similar TBSA and who were receiving an almost identical dietary regimen (see §2.6).

The nitrogen balances were positive in both groups despite this increase in the rate of nitrogen excretion. This may indicate the adequacy of the feeding regimen but also may reflect some of the limitations of nitrogen balance techniques. In general, intakes tend to be overestimated and losses underestimated [Kopple 1987]. In the present study, I have estimated total urinary nitrogen excretion from urinary urea excretion using the technique described by Lee and Hartley [1975]. This technique

assumes that urea comprises 80% of the total nitrogen excreted in urine. This assumption is reasonably accurate in burned patients (Figure 7.1) but variation may occur as a result of changes in feeding regimens and clinical condition and it is possible that this technique underestimates the total nitrogen excretion. It is, however, a very convenient approximation and is the routine method of assessing nitrogen excretion in Burns Centres in this country. The measurement of total urinary nitrogen would have been preferable but the technique is expensive, slow and not widely available.

Faecal and cutaneous losses were omitted from the balance calculations for reasons of practicability. Faecal losses in enterally fed burned patients are comparable to the 1.2g/day losses seen in normal subjects [Serog et al. 1983]. It has been shown that the nitrogen losses from a burn wound, between the fourth and sixteenth days after burning, are 0.1g/TBSA(%)/BSA(m^2)/day [Waxman et al. 1987] and therefore, the losses for all the patients can be estimated to have been 4.2g/day (2.1±0.3g/m^2/day). It can therefore be supposed that the patients in the present study were approximately in nitrogen equilibrium during the 15 days of the study. However, Hegsted [1976] has shown that there is an "apparent retention" of nitrogen at high intake levels amounting to approximately 20% of the intake exceeding 5g/day and therefore this supposition may be unfounded.

8.4.9 Potassium balance

Classically, 3mmols of potassium are retained with every gram of nitrogen, which corresponds to their relative distribution in muscle. In the present study, approximately 7mmol of potassium were retained with

each gram of nitrogen. This is consistent with previous studies in burned patients, in whom positive potassium balances occurred despite negative nitrogen balances and in whom five to 7mmols of potassium were retained with each gram of nitrogen at a comparable time after injury [Pearson et al. 1961]. This probably is the result of potassium losses from the burn-wound.

Kopple [1987] stated that "a comparison of nitrogen and mineral balances is often helpful for confirming results of a balance study". There was no evidence that somatropin administration caused any significant potassium retention consistent with its effects on nitrogen balance.

8.4.10 Somatropin and nitrogen metabolism

The changes in the mean serum urea concentration, in the absence of significant differences in fluid balance between groups, are suggestive of a decreased rate of protein breakdown. This difference, however, preceded the administration of somatropin and was not reflected by a significant decrease in the rate of either protein oxidation or nitrogen and potassium balance in treated patients.

The findings of the present study differ from previous studies using human pit-GH in burned patients and biosynthetic GH preparations in normal, malnourished and obese subjects and postoperative patients, all of which showed that the administration of GH caused a significant fall in nitrogen excretion (see §7.3). The previously observed improvement in mean nitrogen balance resulting from somatrem administration has amounted to

between 0.9g/day and 2.9g/m^2/day in uninjured subjects and between approximately 1.9 and 2.7g/day in post-operative patients. In the present study, there was greater variation in nitrogen balance amongst patients, as indicated by larger standard error of means. This reflects the individual differences in their severity of injury, clinical management and the presence of sepsis in some. It is doubtful therefore if improvements of this magnitude can be reliably detected in patients such as these in whom so many clinical variables are present. Human pit-GH has been shown to improve nitrogen retention in two previous studies in burned patients but these were performed later after injury, when they are usually stable and nutrition is fully established (see §7.2).

Kopple [1987] noted that the accuracy of balance measurements is limited, particularly in the clinical situation and commented that "investigators use a more comprehensive approach to metabolic or nutritional studies in which balances are measured in association with other parameters of nutritional or metabolic status". It is possible that the nitrogen-sparing effect of somatropin was obscured by methodological errors (see §8.4.8) and clinical variables but gas-exchange measurements and plasma protein estimates also indicate that somatropin does not exert a significant anabolic effect in burned adults.

8.4.11 Somatomedin responses

The mean serum IGF-I concentration was within the wide normal adult range at the start of the study in both groups [Teale and Marks 1986]. Between this time and fifteenth day, it recovered, significantly so in control patients, which suggests that a fall may have occurred as a result

of injury. Previous studies have shown that it falls after injury, in common with the other plasma proteins, and that the size and duration of the fall correlates with the "injury severity score" and TBSA respectively [Coates et al. 1981a, 1981b, Frayn et al. 1984]. In control patients, the lowest levels and poorest recoveries were seen in those with the largest burns.

The response to somatropin administration was very variable, levels being well in excess of the normal range in some patients whilst those with burns of 25% and over, one of whom had sepsis, showed little response. There was no significant difference in serum concentrations between groups as a result of this variability but there was a significant fall after cessation of treatment. These changes suggest that some response to somatropin occurs in burned patients but that this is diminished, particularly in the more severely injured. This is the first study in which GH administration has not been shown to cause a significant rise in plasma somatomedin levels. All previous ones in uninjured subjects [Hintz et al. 1982, Manson et al. 1986, Clemmons et al. 1987, Wilton and Sietnieks 1987, Binnerts et al. 1988, Snyder et al. 1988, Ziegler et al. 1988] and post-operative patients [Ward et al. 1987, Ponting et al. 1988, G. Ponting et al. unpublished work] have shown that the administration of comparable doses of both somatropin and somatrem, which have similar biological activities, have caused significant rises in somatomedin levels.

The somatomedins are thought to mediate the anabolic actions of GH (see §6.5). It can be postulated that the failure of somatropin to exert a significant anabolic effect in the present study reflects the diminished somatomedin response. This may explain the previous findings of Soroff et

al. [1967] who stated that: "The most striking nitrogen retention occurred early in the anabolic phase. Growth hormone did not exert any nitrogen-sparing effect during the height of the catabolic phase". It may, therefore, be postulated that the immediate period after burning is an unsuitable time to administer somatropin.

8.4.12 Plasma proteins

The fall in plasma protein concentrations in the present study were comparable to previous ones in burned patients [Munster et al. 1970, Daniels et al. 1974, Ninnemann et al. 1978, Batstone et al. 1982, Moody et al. 1982]. The control of plasma protein concentrations is described by the two-compartment model [Fleck 1985a]. The plasma pool is replenished by synthesis and lymphatic return from the extravascular space and decreased by catabolism and leakage into the extravascular space. The major cause of the fall in plasma protein concentrations, including somatomedins, in the present study was a rapid and short-lived increase in capillary permeability. The escape of albumin to the extravascular space has been shown to increase by at least twofold in patients with septic shock and by up to threefold following cardiopulmonary bypass surgery [Fleck et al. 1985b]. Furthermore, there is a 12-fold increase in albumin extravasation into burned areas within 30 minutes of injury [Carvajal et al. 1979].

A significant proportion of the redistributed protein is lost from the burn. This amounts to 235 and 142g/m^2 burn/day from full-thickness and partial thickness burns respectively in the first three days after injury [Waxman et al. 1987]. Plasma protein catabolism is also increased after

injury [Davies et al. 1969, Davies 1970]. The rate of albumin catabolism may increase to three-times normal in burned patients treated in a cool environment. This change is however offset by an increase in the rate of plasma protein synthesis soon after injury [Davies et al. 1969] and therefore it probably does not contribute greatly to the fall in plasma protein concentrations in burned patients.

The albumin concentration was well within the normal range on the first study day in both groups unlike the other proteins, although it subsequently fell. This initial preservation of its concentration resulted from the use of plasma protein fraction (PPF) during resuscitation [Demling et al. 1984]. The fall in plasma protein concentrations was consistently greater in the somatropin-treated group. There is no obvious explanation for this but it may indicate that this group received a greater physiological injury.

Plasma protein levels may quickly return to normal levels, following the return of capillary integrity and the establishment of adequate nutrition. The speed of recovery reflects the half-lives and pool-sizes of individual proteins. Albumin recovery in the present study was negligible as in previous studies [Daniels et al. 1974, Batstone et al. 1982] but it is recognised not to reflect short-term changes in nutritional status because of its large pool-size and long half-life [Ingenbleek et al. 1975, Shetty et al. 1979, Church and Hill 1987]. In contrast, the concentrations of TBPA and RBP returned swiftly to the upper limits of their normal ranges. Both proteins are sensitive markers of nutritional status and respond quickly to dietary repletion because of their short half-lives and small pool-sizes [Ingenbleek et al. 1975, Shetty et al. 1979, Cavarocchi et al. 1986, Church

and Hill 1987]. The speed of their recovery in the present study was greater than previously observed in burned patients [Moody 1982] and probably reflects the nutritional regimen used.

There was no evidence that somatropin administration improved the rate of plasma protein recovery indicating that it results in no nutritional benefit.

8.4.13 Sodium and fluid balance

In the present study, the overall sodium and fluid balances for both groups were more positive than those seen in normal man. Under normal circumstances insensible water losses in adults amount to approximately 1200ml/day. In contrast, insensible sodium losses are minimal, the kidney being the only route for eliminating the dietary intake [Earley and Daugharty 1969]. It has been shown that the total insensible fluid and sodium losses in burned patients are proportional to the TBSA [Harrison et al. 1964, Davies 1967]. The increased insensible losses occur largely from the burn-wound, although losses from other routes are also increased as a result of fever and hyperventilation. Harrison [1964] has calculated that the total insensible water losses of patients in the first 10 days after injury are 517g/m^2 BSA/day plus 2832g/m^2 TBSA/day. Using data published by Davies [1967], it may be estimated that the non-renal sodium losses of burned patients are in the order of 170 mmol/m^2 TBSA/day in the first 15 days after injury. Although insensible losses vary according to wound management and environmental conditions, those seen in the present study were close to those predicted from these estimates.

8.4 - DISCUSSION

There was no significant alteration in sodium and fluid balances or serum sodium concentrations as a result of somatropin treatment. The apparent increase in sodium retention in the treated patients during the first five days of the study was predominantly due to patient #C2 who had become sodium-depleted during resuscitation and retained all but 20mmol/m^2 of the 883mmol/m^2 sodium administered to her during this time. These findings contrast with recent studies which have suggested that fluid retention may occur during the administration of biosynthetic GH preparations (see §6.9). It is possible that small changes in fluid balance may be obscured by the larger fluid shifts that occur in burned patients, nevertheless, it may be concluded from the present study that there is no evidence that somatropin causes fluid retention.

8.4.14 Glucose metabolism

A modest rise in fasting blood glucose concentration was present in four patients at the time of entry into the study which is commonly observed in burned patients (see §2.2). There was no impairment of early or total insulin release following glucose injection, as occurs in the initial few hours after injury [Allison et al. 1968], but glucose disposal was impaired as indicated by the low mean k-value. This pattern of response is identical to that observed in Allison's study in which the mean k-value in burned adults was 1.20±0.11 in IVGTT performed from two days after injury onwards. Although these findings suggest a decrease in the rate of cellular glucose uptake, such an interpretation is suspect. Glucose uptake is increased in hypermetabolic patients (see §2.2) but endogenous glucose production is also increased, even during glucose

administration (see §2.1). The low k-value in burned patients indicates insulin-resistance but it is probably due as much to a failure of insulin to suppress endogenous glucose production as to an impairment of peripheral insulin-dependent glucose uptake.

The initial IVGTT were performed in the usual manner under fasting conditions. Subsequent tests were superimposed on a background of continuous enteral feeding for two reasons. First, further fasting periods, in addition to those necessary for surgery or dressing changes, would have prejudiced the achievement of the nutritional targets. Secondly, continuous feeding is the "normal" state for patients with significant burns and the present study was designed to assess the effect of somatropin on glucose tolerance within a clinical setting. The change in methodology resulted in no change in basal glucose or insulin concentrations in the control patients but coincided with a further fall in their k-value despite increased early and total insulin release. This probably represents increasing insulin-resistance in this group rather than a response to the altered test background.

8.4.15 Somatropin and glucose metabolism

The administration of somatropin caused no significant change in the mean basal glucose concentration but resulted in a rise in the mean basal insulin concentration and insulinogenic index which were maintained only during the period of treatment. This is consistent with previous studies which have shown that when near-physiological doses of GH are administered, insulin levels typically double [Rosenfeld et al. 1982, Rizza et al. 1982]. Viewed teleologically, more insulin is required during

treatment to maintain a given basal glucose level. These findings indicate insulin-resistance.

The early and total insulin release in response to intravenous glucose were well-matched in both groups throughout the study, despite the increase in basal insulin levels due to somatropin. This suggests that insulin responsiveness to changes in blood glucose is unaltered by somatropin, as has a recent study in normal subjects [Bratusch-Marrain et al. 1982]. There was a significant rise in the glucose AUC on the eleventh day of the study in patients receiving somatropin which is indicative of a decrease in glucose tolerance. The k-value did not deteriorate further at this time but the mean 0-5' delta-glucose values were consistently higher in the treated group, probably as a result of their smaller surface area [Lerner and Porte 1971]. The administration of this relatively larger glucose load may have obscured any decrease in k-value due to somatropin because k-values increase in proportion to glucose load [Moorhouse et al. 1963]. Following the cessation of somatropin treatment, there was an increase in the k-value approaching statistical significance which again suggests that somatropin impaired glucose tolerance.

It can be concluded that somatropin treatment exacerbated the insulin-resistance seen in burned patients. These findings are consistent with the known effects of GH on glucose metabolism (see §6.6) and are similar to those observed previously in burned patients [Wilmore et al. 1974c]. Human pit-GH administration resulted in a twofold increase in the basal insulin concentration, despite an insignificant rise in blood glucose. Following a standard 25g IVGTT, the blood glucose concentration declined

to a significantly higher asymptote despite an increase in insulin release. There was no difference in the glucose disappearance constant.

8.4.16 Trial withdrawals

Two patients who failed to complete the study were excluded from statistical analysis (see §8.2.18). One left the study at his own request and another was withdrawn because of the occurrence of clinically significant hyperglycaemia during somatropin treatment (see appendix 2.). In the latter, the pattern of insulin release in response to glucose, after the first day of the study was atypical both during and after withdrawal of somatropin-treatment. Early insulin release was either absent or very poor, although total insulin release was well maintained. This is similar to the pattern seen in non-insulin dependent diabetics (Type-II diabetes) [Seltzer et al. 1967]. The failure of glucose disposal to improve after withdrawal of treatment, as judged by the continuing low k-values, is consistent with the known correlation of this constant with early insulin release [Lerner and Porte 1971]. The failure of early release is unlikely to have been directly due to somatropin as there was no alteration in the response in the other treated patients and there was little improvement after withdrawal of treatment. It is most likely that extra insulin-demands due to the onset of sepsis, which is associated with a further rise in hepatic glucose output in burned patients [Wilmore et al. 1980], exceeded his insulin secretory capacity which may have already been genetically compromised.

This side-effect has been noted in two patients in a previous study although both had had surgical procedures that involved the pancreas

[Ponting et al. 1988]. Its occurrence, together with the evidence of increased insulin-resistance in the present study, indicate that the potential benefits of somatropin treatment on nitrogen metabolism are offset by glucose intolerance, particularly in injured patients and those with sepsis in whom some insulin-resistance is inevitable. It may be concluded that the blood glucose concentration should be monitored carefully during the administration of somatropin and that the presence of a family history of diabetes mellitus is a relative contraindication its administration.

8.4.17 Dosage

The maximum somatropin dose (0.06mg/kg/day, 0.16iu/kg/day) used in the present study was twice that recommended for physiological replacement in children with GH-deficiency. The administration of comparable doses of both somatrem and somatropin has resulted in significant anabolic activity in man (see §7.3). It could be argued that a larger dose should be used in injured subjects but the administration of 0.14iu/kg/day [Sherwin et al. 1983] and approximately 0.22iu/kg/day of human pit-GH [Rosenfeld et al. 1982] has been shown to impair markedly glucose tolerance in normal subjects. Furthermore, the administration of 0.14iu/kg/day to normal subjects receiving a continuous infusion of cortisol, glucagon and adrenalin accentuated the effects of these three hormones on glucose metabolism (see §1.3). These findings and the occurrence of glucose intolerance in the present and previous clinical studies would appear to preclude the use of larger doses, particularly in hypermetabolic subjects in whom glucose intolerance is common.

8.4.18 Conclusions

The present study is the first examination of the effects of a biosynthetic human GH preparation in hypermetabolic patients. It fails to demonstrate that somatropin has any anabolic activity in patients with burns, possibly as a result of an impaired somatomedin response. It is possible, however, that the effects of somatropin were obscured by methodological errors and clinical variables. It is therefore desirable to re-examine the anabolic effects of somatropin in an experimental model that allows standardisation of severity of injury, management and the measurement of total nitrogen excretion.

8.5 - PRINCIPAL FINDINGS

1. Injury by burning is followed by rises in REE and nitrogen excretion, accompanied by insulin-resistance.

2. Injury by burning causes a fall in the levels of all measured plasma proteins.

3. Somatropin administration to burned patients does not decrease the rate of protein breakdown or increase either the positivity of nitrogen balance or plasma protein concentrations.

4. Somatropin administration fails to cause a significant rise in the plasma somatomedin concentration.

5. Somatropin administration does not cause an increase in retention of sodium or water.

6. Somatropin administration causes no alteration in insulin release in response to injected glucose.

7. Somatropin administration exacerbates the insulin-resistant state present in burned patients.

PART III

ANIMAL STUDIES

9.1 - OBJECTIVES

The study to be described was designed to investigate the effect of somatropin on serum somatomedin concentrations and nitrogen balance of both uninjured rats and rats with a standardised burn-injury. It was intended to overcome the methodological problems noted in the preceding clinical study.

9.2 - GENERAL MATERIAL AND METHODS

9.2.1 Introduction

The two studies described in this and the subsequent chapter were done in the Surgical Unit at the Westminster Hospital on mature rats. I will describe below the material and methods that are common to both studies. Material and methods that are unique to each study are described separately (see §9.3 and §10.2).

In the material and methods section below, I will quote my Home Office licence numbers (§9.2.2). I will detail the animals used and their preliminary care (§9.2.3). I will describe the anaesthetic techniques used (§9.2.4), the preparation of the rats (§9.2.5) and the method of randomization (§9.2.6). I will describe the technique of burning the rats (§9.2.7), their fluid resuscitation (§9.2.8) and analgesia (§9.2.9). I will give details of the somatropin and placebo preparations used (§9.2.10). I will then describe the diet administered to the rats (§9.2.11), their general care (§9.2.12) and finally, the method of their sacrifice (§9.2.13).

9.2.2 Home Office

The author held Licence number PPL70/00986, Home Office number PIL 70/03003 under the Animal Scientific Procedures Act 1986, valid at the Westminster Hospital, during the conduct of all animal experiments.

9.2.3 Animals

The animal experiments were performed on male, Sprague-Dawley rats (purchased from Harlan-Olac Ltd., Bicester, Oxfordshire). They were delivered to the Westminster Hospital Surgical Unit at the age of 11

weeks. They were then individually caged for six days before the start of each experiment. During this time they had free access to 41B rat maintenance-feed (Pilsbury's Ltd., Birmingham, England) (see appendix 8.).

9.2.4 Anaesthetic techniques

For major operative procedures, such as laparotomy or injury by burning, anaesthesia was induced with an intraperitoneal injection of 40mg/kg sodium pentobarbitone (Sagatal) and 0.05mg/kg atropine sulphate and maintained with a 50/50% mixture of oxygen and nitrous oxide with the addition of between 0.5 and 2.0% of Halothane (Fluothane) using an open-circuit technique.

For terminal or minor procedures, such as removal of skin clips, anaesthesia was both induced and maintained with oxygen/nitrous-oxide and halothane.

9.2.5 Preparation

Following the induction of anaesthesia, all the rats were shaved circumferentially with electric clippers from the nape of the neck to the base of the tail dorsally and the axillae to pubis ventrally.

9.2.6 Randomization

The rats were allocated to the experimental groups in each experiment by selection of cards which were marked and shuffled before the experiment. These were handed to the laboratory technician who was

helping me. She selected a card, at random, from the pack after I had completed any operative procedures and administered pethidine to each rat. The rats were then burned if allocated to the appropriate group.

It was anticipated that injury by burning would result in some deaths and therefore more rats were allocated to be burned than were put in the control groups.

9.2.7 Burn technique

A 13×11cm oblong was marked on the dorsum of those rats randomized to be burned (Figure 9.1). These rats were placed in a net which was tightened in order to flex them and allow clear access to the dorsum. The marked areas were then immersed for 30 seconds in water heated to 90^0C in a thermostatically controlled water bath (Figure 9.2).

The length and breadth of the burns were measured after six days, when they were fully demarcated (Figure 9.3) and the burn area (BA) calculated. The body surface area (BSA) of each animal was calculated from its weight ($BSA(cm^2) = 10.65 \times (weight(g)^2)^{1/3}$) [San Roman et al. 1985] and the TBSA was then calculated by dividing the burn area by the body surface area of the rat (BA/BSA).

This method resulted in burns covering approximately 20% of the body surface which were confirmed histologically to be full-thickness.

Figure 9.1 Marking of the rats before burning. Top: dorsal view. Bottom: side view.

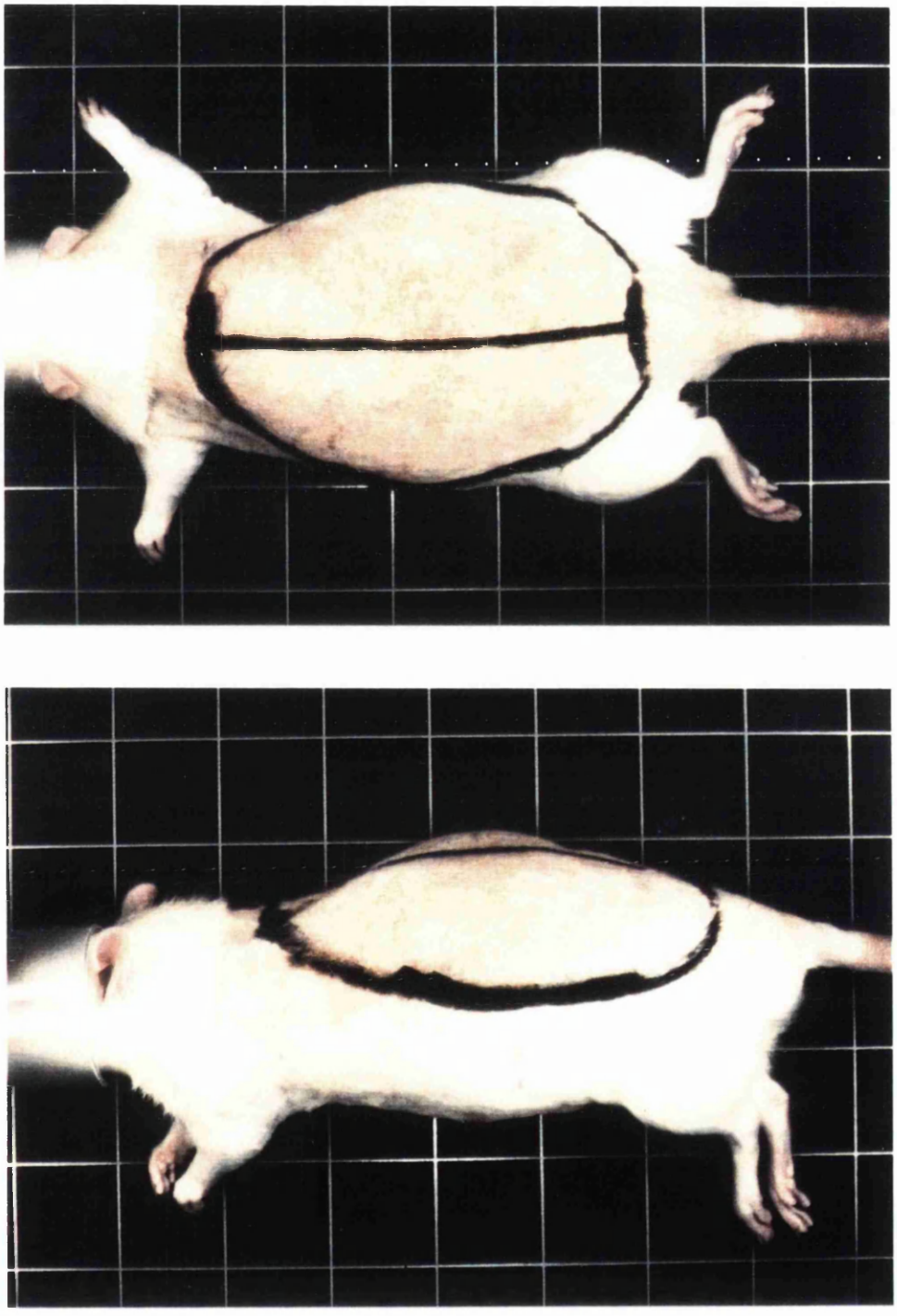


Figure 9.2 Burning of an anaesthetised rat in a water bath.

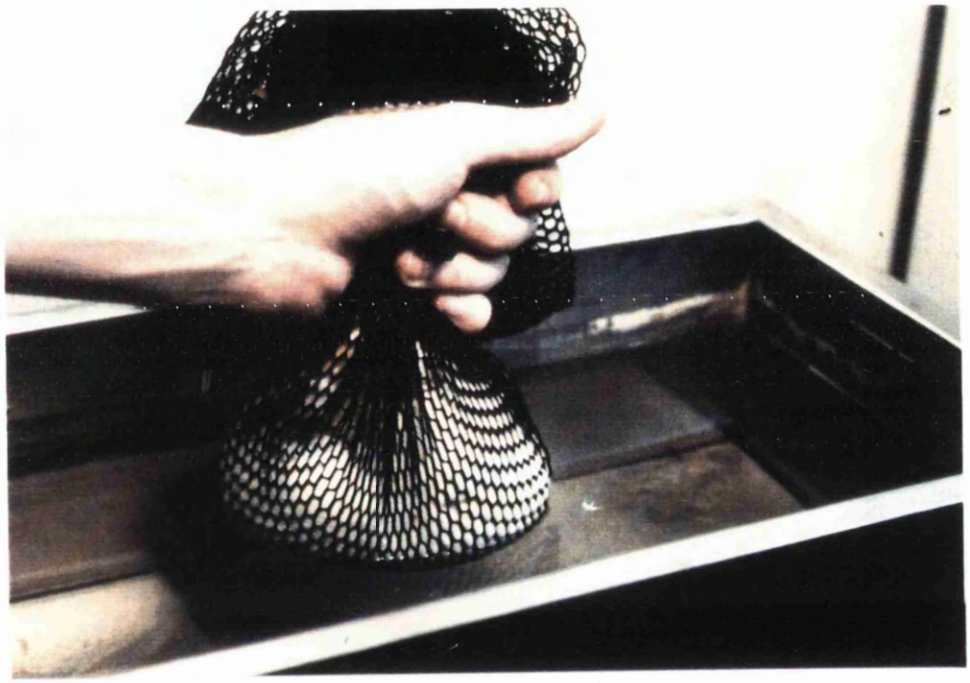
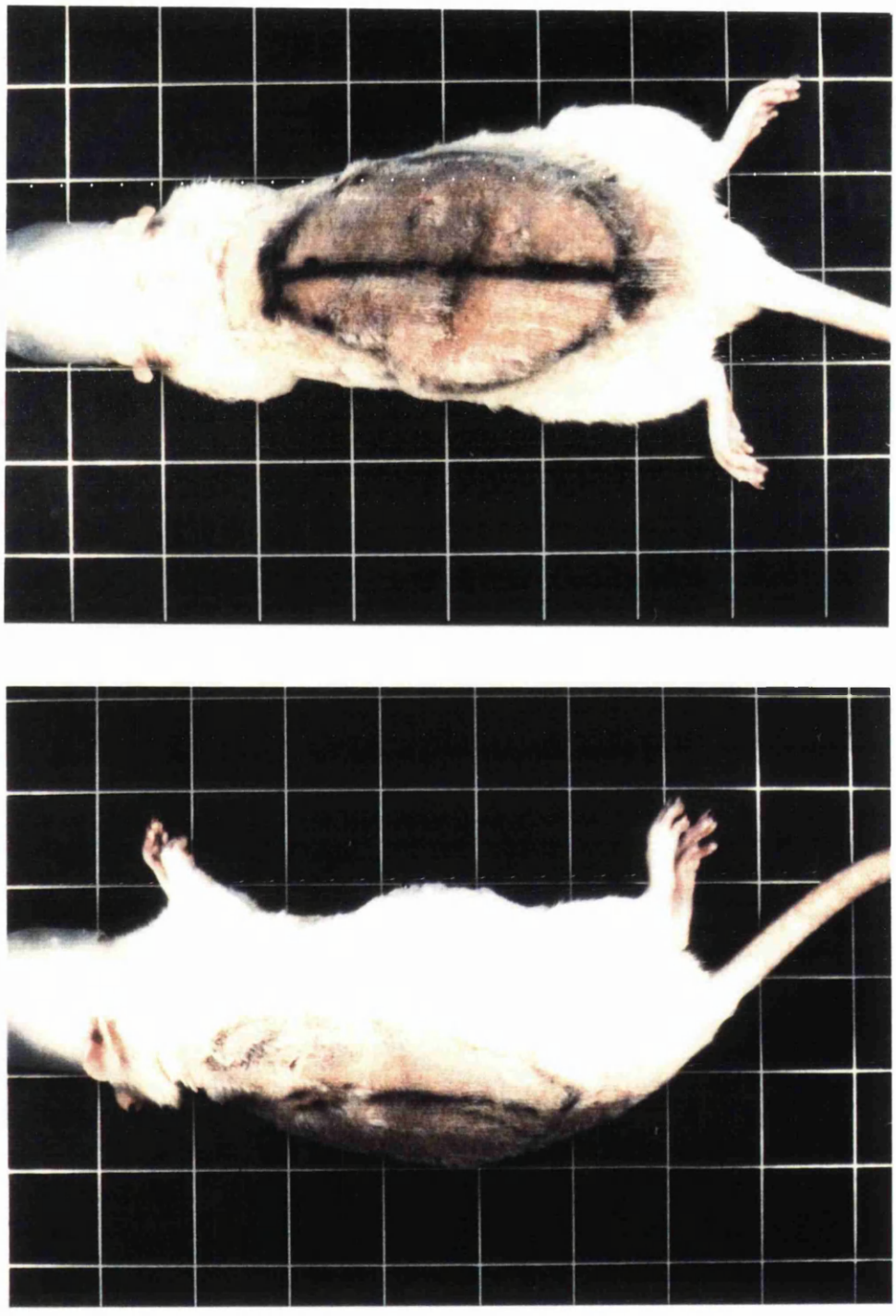


Figure 9.3 Demarcation of a burn after six days. Top: dorsal view. Bottom: side view.



9.2.8 Fluid resuscitation

Fluid resuscitation was administered only to burned rats by the intraperitoneal injection of 3ml/kg/% burn of Hartmann's solution (compound sodium lactate) (or 60ml/kg as the TBSA was assumed to be 20%). This was performed immediately before the rats were burned.

9.2.9 Analgesia

At the completion of the major operative procedures, all rats received a single subcutaneous injection of 10mg/kg of Pethidine. Thereafter, all were provided with approximately 100mg/kg/day aspirin in their drinking water (1000mg aspirin dissolved in 1000ml tap-water).

9.2.10 Somatropin

Somatropin (Humatrope) and a placebo preparation were supplied in lyophilised form (Lilly Research Ltd., Windlesham, Surrey). The active preparation contained 1.48mg somatropin (4iu), 7.4mg mannitol, 1.48mg glycine and 0.34mg dibasic sodium phosphate. The placebo preparation was identical except for the absence of somatropin. Both preparations were reconstituted with 4ml water containing 0.3% metacresol and 0.2% phenol.

The preparations were relabelled by the Pharmacy Department on their arrival from the Lilly Research Laboratories. The identity of the active preparation was not revealed to anyone by the pharmacist until the experiments had been completed. Different coding was used for each of the two studies.

Somatropin/placebo was administered by subcutaneous injection at a dose of 0.1mg/kg/day (0.27iu/kg/day) adjusted according to animal-weight.

9.2.11 Diet

The rats were provided with 85g/kg/day of 41B rat and mouse maintenance feed (Pilsbury's Ltd.) throughout the experiments, which was adjusted for changes in body weight (see appendix 8.).

9.2.12 General care

All the rats were housed in individual cages and kept in thermostatically controlled rooms at a temperature of 20^0C throughout the experimental periods. Their weights and food consumption were measured to the nearest gram.

9.2.13 Sacrifice

The rats were sacrificed under general anaesthetic. An incision was made in the abdomen, a needle inserted into the inferior vena cava and the rat exsanguinated to provide terminal blood samples. The procedure was completed by cervical dislocation.

9.3 - MATERIAL AND METHODS

9.3.1 Introduction

The experiment described below was performed on both uninjured rats, designated as control, and burned rats. It used a cross-over design and lasted 16 days. The rats were randomly allocated to receive both somatropin and placebo for five days in a random order.

Much of the material and methods have been described previously (see §9.2). In the material and methods section below I will detail the composition of the four experimental groups (§9.3.2), the study periods and the timing of administration of the drug preparations (§9.3.3). I will describe the general care of the animals and the method of their pairing (§9.3.4). I will give an account of the collection urine and faeces samples, the estimation of their nitrogen content and the calculation of nitrogen balance (§9.3.5). I will describe the collection of blood samples and the assay of their IGF-I concentrations (§9.3.6). Finally, I will describe the composition of the four treatment groups and the statistical tests used for analysis of the results (§9.3.7).

9.3.2 Study groups

On the first day of the experiment, 22 rats were anaesthetised, shaved and then randomly allocated to four **experimental groups**:

1. CPS; control rats to receive placebo and then somatropin (n=5),
2. CSP; control rats to receive somatropin and then placebo (n=5)
3. BPS; burned rats to receive placebo and then somatropin (n=6),
4. BSP; burned rats to receive somatropin and then placebo (n=6).

All were injected with pethidine before randomization. The control rats were allowed to recover immediately but those allocated to the burn groups were injected with Hartmann's solution, burned and then allowed to recover.

9.3.3 Study periods

The 16 days of the study were divided into four periods (Figure 9.4):

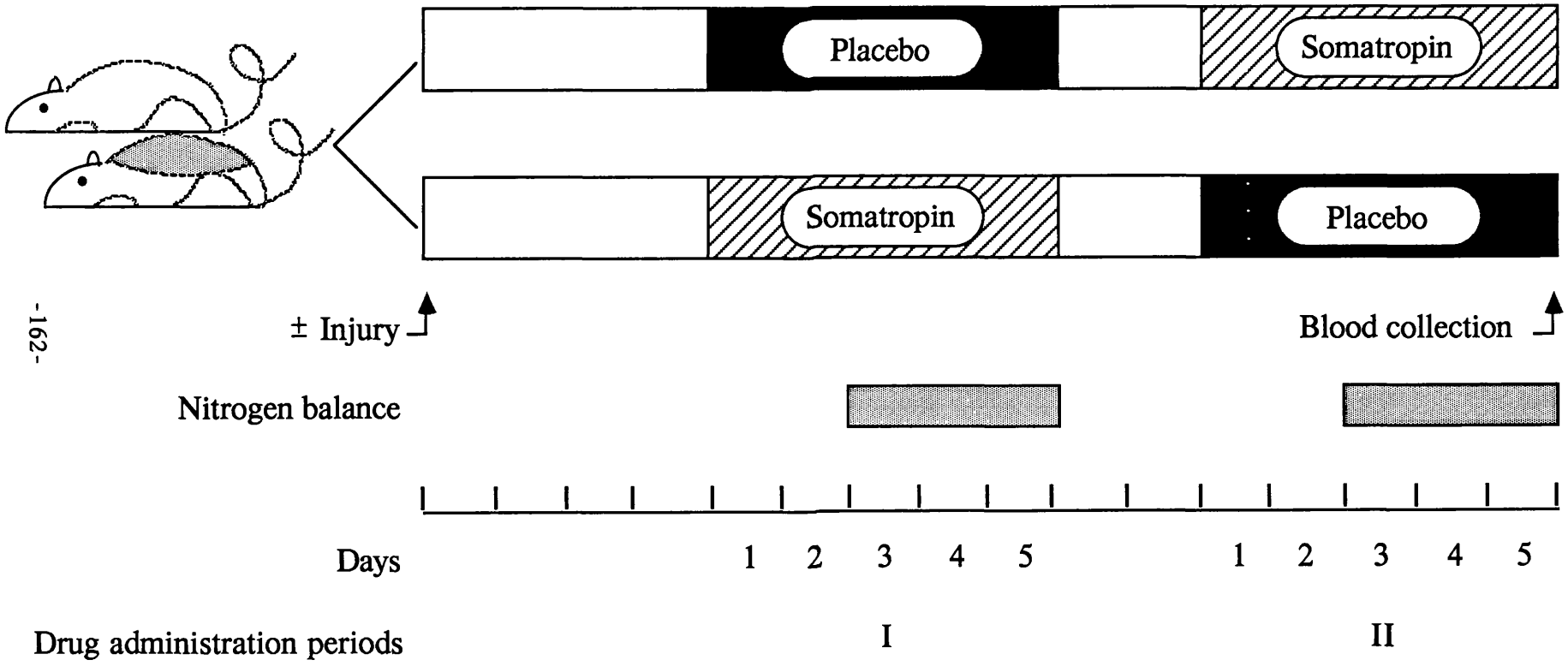
1. Recovery period (4 days),
2. First drug administration period (5 days) (Period I),
3. Rest period (2 days),
4. Second drug administration period (5 days) (Period II).

The drug preparations, having been relabelled by the Pharmacy Department, were injected subcutaneously on each day of the drug-administration periods. Control rats in the CPS group, therefore, received placebo during the first administration period, somatropin during the second. Neither preparation was administered during the recovery or rest periods. The individual dosages were adjusted according to the weight of each rat at the beginning of the relevant administration period.

9.3.4 General care

All the rats were housed individually in "metabolic cages" until the end of the study. They were weighed at the beginning and end of each period and were provided with 85g/kg/day of 41B feed throughout each, adjusted according to their weight.

Figure 9.4 An outline of the study showing the timing of injury, drug administration periods and investigations.



The CPS and BPS rats were paired with their CSP and BSP counterparts respectively, according to their initial weights. The cages of each pair were placed together in order to limit variation in the timing of manipulation, injection and collection of samples.

9.3.5 Nitrogen excretion and balance

On the last three days of each drug administration period, urine and faeces were collected daily from each rat in separate containers. These samples were stored at -20^{0}C until analysis at the end of the study. The total nitrogen content of these samples were measured by the Kjeldahl technique (Tecator 1030 auto-analyser, Hoganas, Sweden) [Suhre et al. 1982]. Daily nitrogen balances were calculated (dietary intake - urinary excretion - faecal excretion). The mean balance was calculated for these three days (g/day) and was then corrected for body-weight (g/kg/day).

9.3.6 Blood collection

At the end of the study, the rats were sacrificed and terminal blood samples collected. The blood samples, having clotted, were centrifuged and the serum stored at -20^{0}C within one hour of collection.

Serum was also collected and pooled from three additional rats of identical strain and age. These rats had not been anaesthetised or shaved and had received 41B feed *ad libitum* during the study period.

The serum samples were assayed for insulin-like growth factor-I (IGF-I) by a competitive radioimmunoassay [Morrell et al. 1986]. The serum

IGF-I concentration was expressed as Units/ml, using the pooled rat-serum as a standard (1U/ml).

9.3.7 Analysis and statistics

The cross-over design of the study allowed the creation of four **treatment groups:**

1. CP; control rats receiving placebo
3. CS; control rats receiving somatropin
3. BP; burned rats receiving placebo
4. BS; burned rats receiving somatropin

For example, the CP results were derived from data obtained during the first drug administration period of the CPS group and the second administration period of the CSP group (n=10).

The results of the terminal blood samples were assigned according to the drug administered second; for example, blood samples taken from BSP group were designated as BP (n=6).

All results are presented as mean±SE. The paired t-test and Wilcoxon's signed-ranks test (see appendix 7.) were used to compare placebo with somatropin (ie. CP vs. CS and BP vs. BS). The unpaired t-test and Mann-Whitney test were used to compare control and burned groups (ie. CP vs. BP and CS vs. BS).

9.4 - RESULTS

9.4.1 Injury groups

The weight of the control and burned rats were well-matched at the beginning of the study (control 414.6±6.3g, burned 409.7±5.6g, ns) (Table 9.1). The mean TBSA of the twelve burned rats was 20.7±0.6% (Table 9.2). No rats died during the study.

9.4.2 Weight-changes

The control rats gained weight slowly during the drug administration periods unlike the burned rats which lost weight rapidly (Figure 9.5) (Tables 9.1 and 9.3). There was no significant difference between placebo or somatropin-treated groups.

9.4.3 Food consumption and nitrogen intake

The mean food intake was greater in the control rats but this reflected their greater body-weight (Figure 9.6) (Tables 9.4 and 9.5) because there was no significant difference in the mean food consumption of the four groups when corrected for body weight. Similarly, the nitrogen intake during the period of nitrogen balance measurement, was comparable in the four experimental groups when corrected for body weight (Figure 9.7) (Tables 9.6 and 9.7).

9.4.4 Nitrogen excretion and balance

The rate of urinary nitrogen excretion was significantly greater in the two burned groups compared with their control counterparts (Figure

9.7) (Tables 9.6 and 9.8). The faecal nitrogen losses were indistinguishable in the four groups (Table 9.9). All four groups were in positive nitrogen balance (Table 9.10) but the balances were significantly less positive in the burned rats compared with control rats. There were no significant differences in the rates of urinary or faecal nitrogen excretion between rats receiving placebo or somatropin, nor was there any difference in their nitrogen balances.

9.4.5 Somatomedin responses

The mean serum IGF-I concentration was low in all the four groups compared with the pooled normal rat serum which was used as a standard (1U/ml) (Figure 9.8) (Table 9.11). Burned rats had a significantly lower mean level than their control counterparts. The CS group had a significantly higher level than the CP but there was no difference between BP and BS groups.

Figure 9.5 Mean food intake in the four treatment groups during the drug administration periods.

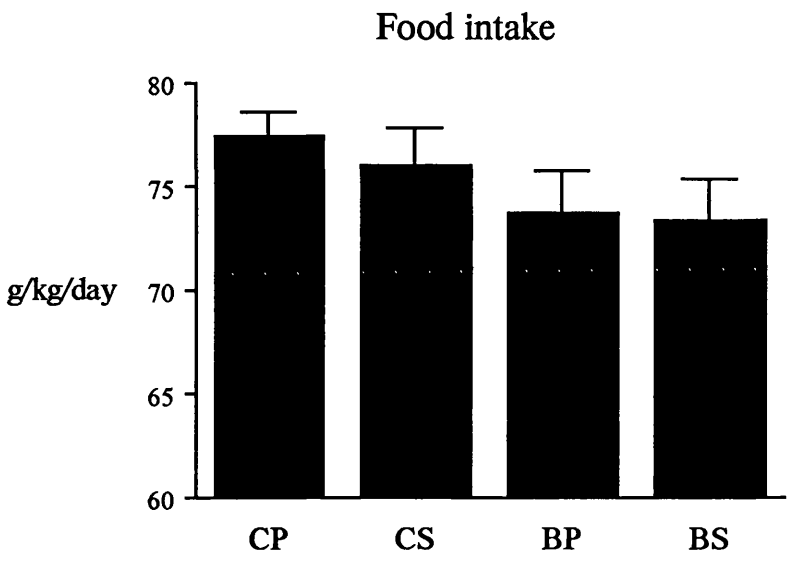


Figure 9.6 Mean weight-change in the four treatment groups during the drug administration periods. CP versus BP and CS versus BS, $p<0.0001$.

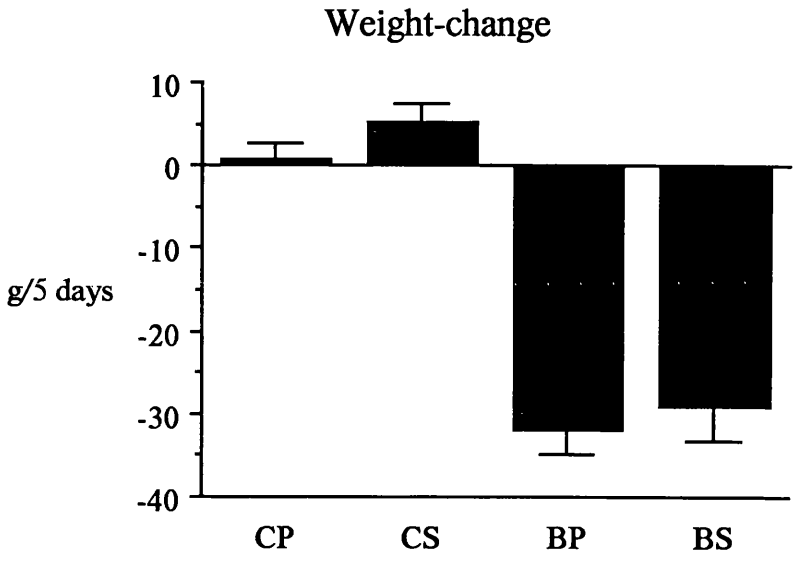


Figure 9.7 Mean nitrogen balance in the four treatment groups during the last three days of the drug administration periods. Urinary nitrogen excretion: CP versus BP and CS versus BS, $p<0.01$. Nitrogen balance: CP versus BP, $p<0.05$; CS versus BS, $p<0.01$.

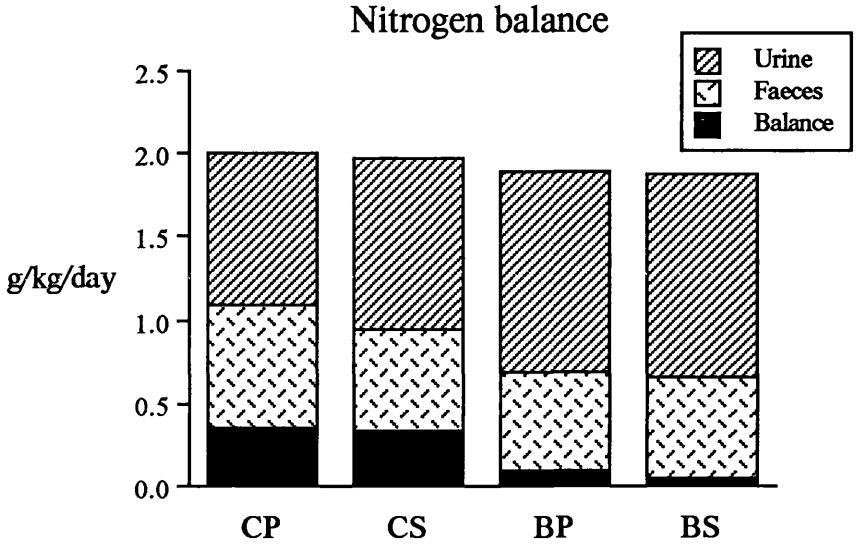
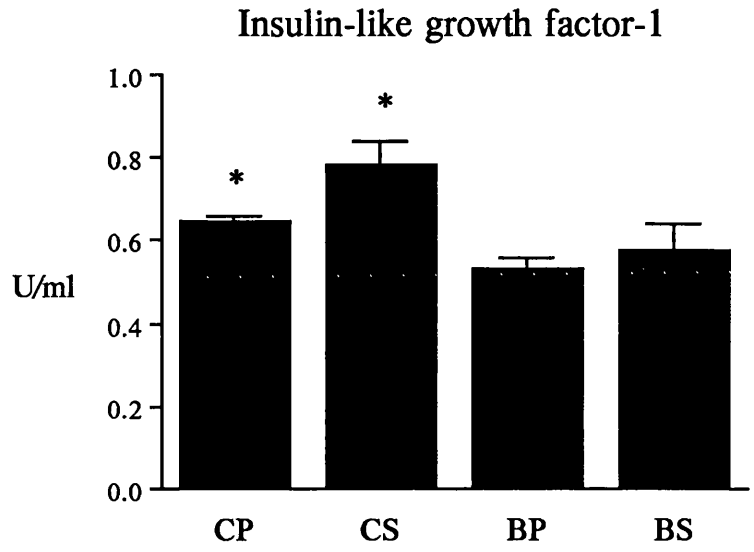


Figure 9.8 Mean insulin-like growth factor-I concentration in the four treatment groups at the time of sacrifice. * CP versus CS, $p<0.05$. CP versus BP, $p<0.01$; CS versus BS, $p<0.05$.



9.5 - DISCUSSION

9.5.1 Introduction

The present blind cross-over study shows that injury by burning in rats is followed by a rise in urinary nitrogen excretion, resulting in a fall in the positivity of nitrogen balance and rapid weight-loss. Injury by burning also causes a fall in the serum somatomedin concentration. Somatropin administration causes a rise in the somatomedin concentration in uninjured rats only, no rise occurring in burned rats. Somatropin administration causes no increase in the positivity of nitrogen balance or weight-gain.

In the discussion to follow, I will comment on design of the study (§9.5.2). I will describe the effects of injury by burning on the rat, particularly on nitrogen balance (§9.5.3). I will discuss the failure of somatropin to exert any anabolic activity in the present study and contrast these findings with those of previous ones (§9.5.4). I will discuss the changes observed in the serum somatomedin concentrations and conclude that the present study provides further evidence that injury by burning impairs the somatomedin response to somatropin (§9.5.5). I will defend the choice of the dose of somatropin used in the present study (§9.5.6) and discuss the possible effects of altering the frequency and route of its administration (§9.5.7). I will review the evidence suggesting that rats with normal pituitary function are less sensitive to exogenous GH than are hypophysectomized rats (§9.5.8). Finally, I will conclude that the present study has failed to demonstrate that somatropin has any anabolic activity in either uninjured or injured rats and will indicate further directions of investigation (§9.5.9).

9.5.2 Study design

The present study was designed to limit individual variation and to exclude the methodological errors which may have occurred in the preceding study (see §8.4.18). Individual variations in severity of injury and response to injury were limited by the use of a cross-over design. The total nitrogen losses in both urine and faeces were measured by a Kjeldahl technique in order to increase the accuracy of their estimation. Although no measure or estimate of nitrogen losses from the burn-wound were included, the effect of this omission was limited by standardisation of the injury and the use of a cross-over design.

9.5.3 Effects of injury

In the present study, a 20% full-thickness burn caused rapid weight-loss and a significant reduction in nitrogen retention due to an increase in urinary nitrogen excretion. This is consistent with previous animal studies that have used similar experimental protocols [Caldwell 1962, Caldwell and Levitsky 1963]. In these and the present one, the nitrogen balances remained positive in the burned rats despite the increase in urinary nitrogen excretion but this does not include losses from the burn-wound. It is likely that the burned rats were in negative nitrogen balance in view of their rapid weight-loss.

9.5.4 Somatropin and anabolism

Somatropin administration failed to result in a significant improvement in nitrogen balance or weight-gain in either control or burned

rats. Previous studies have shown that the administration of 1mg/kg/day of bovine pit-GH to rats with cutaneous wounds [Kowalewski and Yong 1968] and 4mg/day bovine pit-GH to rats with burns [Gump et al. 1960] resulted in a marked increase in weight compared with controls. Although the biological activity of these GH preparations are not stated, it is likely that the dose used was much greater than in the present study. Furthermore, the use of such large quantities of pituitary-derived preparations may have caused fluid retention due to contamination with other pituitary hormones (see §6.9). Jorgensen and Andreassen recently administered 0.5, 2.0 and 8.0mg/kg/day of somatropin to normal 11 week old rats for seven days causing an increase in weight which was proportional to the dose administered [Jorgensen and Andreassen 1987]. However, again, the authors failed to state the activity of their preparation. More recently, Damm Jorgensen et al. [1988] administered 0.5, 3.3 and 25iu/kg/day somatropin to normal male rats over a 90 day period. The body-weights of the low-dose group remained comparable to rats treated with saline and although those of the intermediate and high dose groups increased significantly, no increase was apparent before 24 days.

9.5.5 Somatomedin responses

The serum IGF-I levels were low in all the groups, compared with the normal pooled rat serum. Somatomedin levels are sensitive to nutritional status in man (see §6.4). Similarly, in twelve week old rats, the serum somatomedin-C concentration (now called IGF-I) was shown to be sensitive to the level of both energy and protein intake [Prewitt et al. 1982]. Furthermore the somatomedin-C concentration was found to correlate with

the rate of growth. The findings of the present study suggest that even the control rats were less well nourished than the normal ones which had not been shaved and which had had free access to food. It has been shown that shaving increases the food intake of rats housed at 20^0C by approximately 30% [Caldwell 1962]. Although, the control rats were provided with a dietary intake that had been observed to be their normal one in a preliminary study (see appendix 8.), it is evident that the use of a fixed dietary intake did not allow for this increase in dietary requirements. A fixed intake is, however, advantageous because it prevents inequalities occurring between groups. Burned animals, once they have recovered from the injury, consume more food than uninjured animals and this tends to obscure the effects of the injury on nitrogen balance and weight-loss [Caldwell 1962].

Somatropin administration caused a small but significant increase in the serum somatomedin concentration in the control rats which was comparable in size to that seen previously in rats with normal pituitary function receiving ovine pit-GH [Stred et al. 1987] and confirms that the dose used was physiologically active.

Injury by burning caused a significant decrease in the somatomedin concentration, consistent with previous studies in man (see §8.4.11). Somatropin administration caused no increase the somatomedin levels in burned rats, unlike the control ones. The absence of a somatomedin response following a burn is consistent with the findings in burned man (see §8.4.11) and provides further evidence that a severe injury impairs the normal response to somatropin.

9.5.6 Choice of somatropin dose

The dose of somatropin used in the present study is the same as that in a number of recent human studies in which biosynthetic human GH preparations have been shown to increase nitrogen retention (see §7.3). This dose was chosen in order to assess the effects of a clinically usable dose of somatropin, as the use of higher doses is limited by their effect on glucose metabolism, particularly in burned patients. The administration of comparable doses of human pit-GH [Jansson et al. 1982b, Clark et al. 1985, Schoenle et al. 1985, Guler et al. 1988] and somatropin [Moore et al. 1988] to hypophysectomized rats has been shown to result in a significant increase in the rate of growth. The dose of somatropin used in the present study is therefore analogous to those used in human studies by the author (see §8.4.17) and others (see §7.3), in approximating to doses used for correcting hypopituitarism.

9.5.7 Route and frequency of administration

The insensitivity of rats to somatropin in the present study and humans in the preceding study (see §8.4.10) may reflect the route and frequency of administration. Endogenous GH is secreted episodically in rodents [Tannenbaum and Martin 1976], as in man, there being six to eight peaks in a day (see §6.2). The administration of somatropin once each day by the subcutaneous route produces a broad, low amplitude peak in the plasma GH concentration [Jansson et al. 1982a, 1982b, Russo and Moore 1982] which is not physiological. Subcutaneous administration of GH four or eight times per day enhances the effectiveness of a given dose of GH, compared with a single injection [Jansson et al. 1982a, 1982b]. Further

studies, using chronically indwelling cannulae, have shown that the intravenous route is more effective than the subcutaneous one and confirm that pulsatile delivery of GH results in greater weight-gain [Clark et al. 1985]. Intravenous administration is followed by a shorter, sharper increase in plasma GH concentration, mimicking the normal pattern of GH release. All these studies, however, were performed in hypophysectomized rats and therefore their relevance to either rats or human subjects with normal pituitary function is questionable.

9.5.8 Pituitary function and sensitivity to exogenous GH

There is evidence that mature rats with both normal pituitary function and growth rates are relatively insensitive to the anabolic effects of exogenous GH whilst remaining sensitive to its effects on glucose metabolism [Stred et al. 1987]. *In vitro* experiments have shown that the exposure of muscle from hypophysectomized rats to GH causes an increase in amino-acid uptake and protein synthesis, whereas muscles taken from juvenile (from 22 days old onwards) or mature normal rats fail to respond to GH [Albertsson-Wikland and Isaksson 1976, Nutting 1976]. The responsiveness of muscle to GH can be rapidly restored by the prior administration of anti-GH serum to the rats [Schwartz 1982]. These experiments suggest that muscle tissue from normal rats is already stimulated by endogenous GH and therefore is refractory to exogenous GH. In contrast, there is ample evidence that GH administration to normal rats can enhance wound-healing (see §7.6). Therefore, various types of cell differ in their response to GH. Nitrogen balance predominantly reflects events occurring in muscle (see §2.4). It may, therefore, be more

profitable to examine the effect of GH on protein metabolism in the fibroblast.

9.5.9 Conclusions

The present study is the first to examine the effects of GH in the injured rat. It confirms that injury by burning causes a fall in serum somatomedin levels and a loss of somatomedin responsiveness to somatropin. It is also the first to examine the effect of GH on nitrogen balance in the rat and it shows that rats with normal pituitary function are insensitive to the anabolic actions of exogenous GH. It is possible that neither body-weight nor nitrogen balance are sensitive measures of the effect of exogenous GH in the presence of normal pituitary function. It may be useful to re-examine the anabolic effects of somatropin in a system known to be sensitive to exogenous GH.

9.5 - PRINCIPAL FINDINGS

1. Injury by burning in the rat is followed by a rise in urinary nitrogen excretion, resulting in a fall in the positivity of nitrogen balance and rapid weight-loss.

2. Injury by burning in the rat causes a fall in the serum somatomedin concentration.

3. Somatropin administration causes a rise in the somatomedin concentration in uninjured rats.

4. Somatropin administration causes no rise in somatomedin concentration in burned rats.

5. Somatropin administration causes no increase in the positivity of nitrogen balance or weight-gain in either uninjured or burned rats.

10.1 - OBJECTIVES

The study to be described was designed to investigate the effect of somatropin on wound-healing in both unburned rats and those with a standardised burn.

10.2 - MATERIAL AND METHODS

10.2.1 Introduction

The experiment described below was performed on both uninjured rats, designated as control, and burned rats. The rats underwent a midline laparotomy at the beginning of the study and then received either somatropin or placebo. The healing of the wounds was assessed by tensiometry after either six or fourteen days.

Much of the material and methods have been described previously (see §9.2). In the material and methods section below, I will detail the composition of the experimental and treatment groups (§10.2.2). I will describe the performance of the laparotomies (§10.2.3), the general care of the animals (§10.2.4), the collection of blood samples (§10.2.5) and the technique of tensiometry (§10.2.6). Finally, I will describe the analysis of the results and the statistical tests used (§10.2.7).

10.2.2 Study groups

Eighty rats were randomly allocated to four **treatment groups** which formed eight experimental groups:

1. CP; control rats to receive placebo (n=16),
2. CS; control rats to receive somatropin (n=16),
3. BP; rats to be burned and to receive placebo (n=24),
4. BS; rats to be burned and to receive somatropin (n=24).

Half the rats in each of these four groups were sacrificed on the sixth post-operative day (groups CP6, CS6, BP6 and BS6) and the remainder on the fourteenth (groups CP14, CS14, BP14 and BS14), thereby creating eight **experimental groups.**

10.2.3 Laparotomy

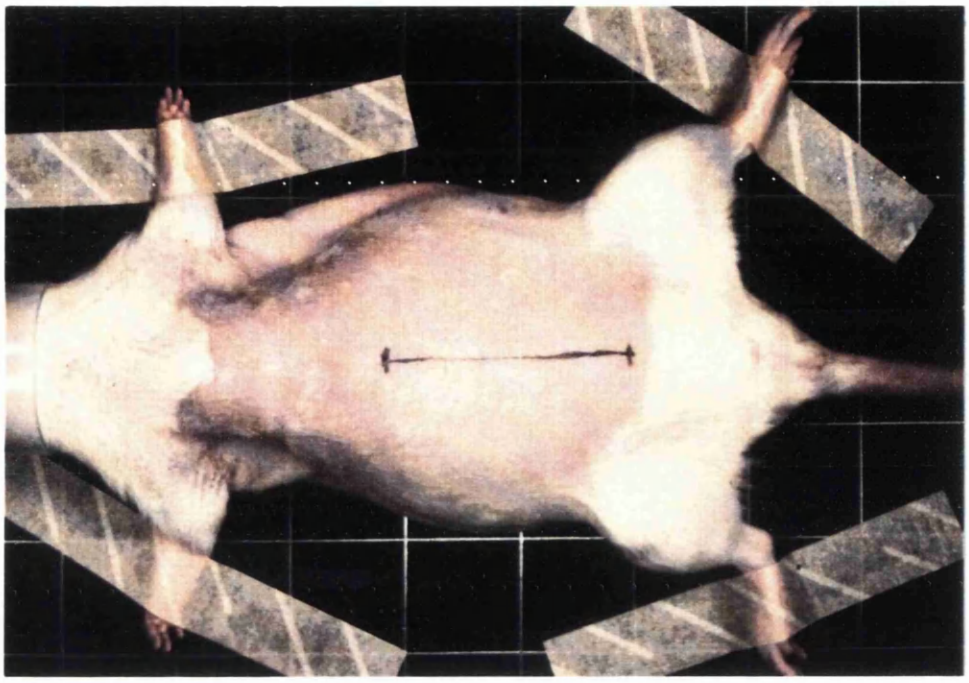
Following the induction of anaesthesia and shaving (see §9.2.4 and §9.2.5), the anterior abdominal wall of each rat was cleaned with 0.5% chlorhexidene solution. A six centimetre midline incision was made in the skin, linea alba and peritoneum (Figure 10.1). The abdominal wall was repaired with a continuous *over and over* suture technique using 0000 monofilament nylon (Ethilon, Ethicon Ltd., Edinburgh, Scotland)). The skin was closed with six millimetre stainless-steel skin clips (DS-25, 3M Ltd., Loughborough, Leicestershire) which were removed on the sixth post-operative day.

10.2.4 General management

At the completion of the laparotomy, whilst still anaesthetised, the rats were injected with pethidine and then randomly allocated to one of the eight experimental groups. Following randomization, each rat was injected with the allotted somatropin/placebo preparation. The control rats were allowed to recover immediately but those allocated to the burned groups were injected with Hartmann's solution, burned and then allowed to recover. All were then caged individually until the end of the study.

On alternate days, the rats were weighed and injected with the allotted somatropin/placebo preparation at a dose of 0.2mg/kg (0.1mg/kg/day, 0.27iu/kg/day) which was adjusted continuously for changes in weight. The rats were given 85g/kg/day of 41B feed throughout the study, adjusted continuously for changes in weight.

Figure 10.1a Performance and closure of the laparotomy wound. Top: the midline incision marked. Bottom: the incision through skin, the linea alba and peritoneum into the abdominal cavity.



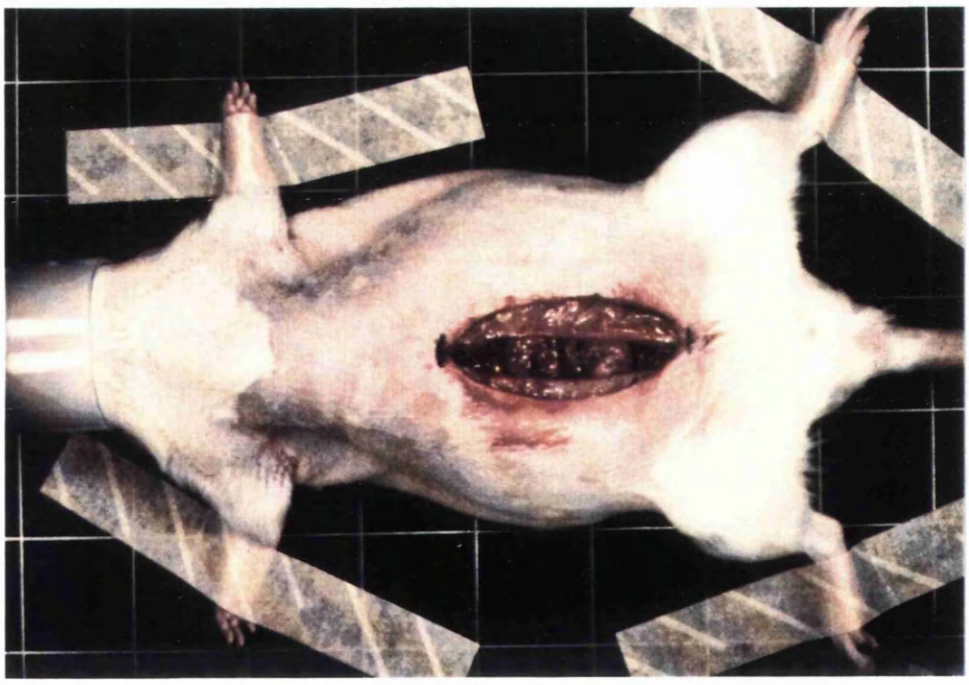


Figure 10.1b Performance and closure of the laparotomy wound. Top: Closure of the abdominal wall with 0000 nylon. Bottom: at the completion of the closure.

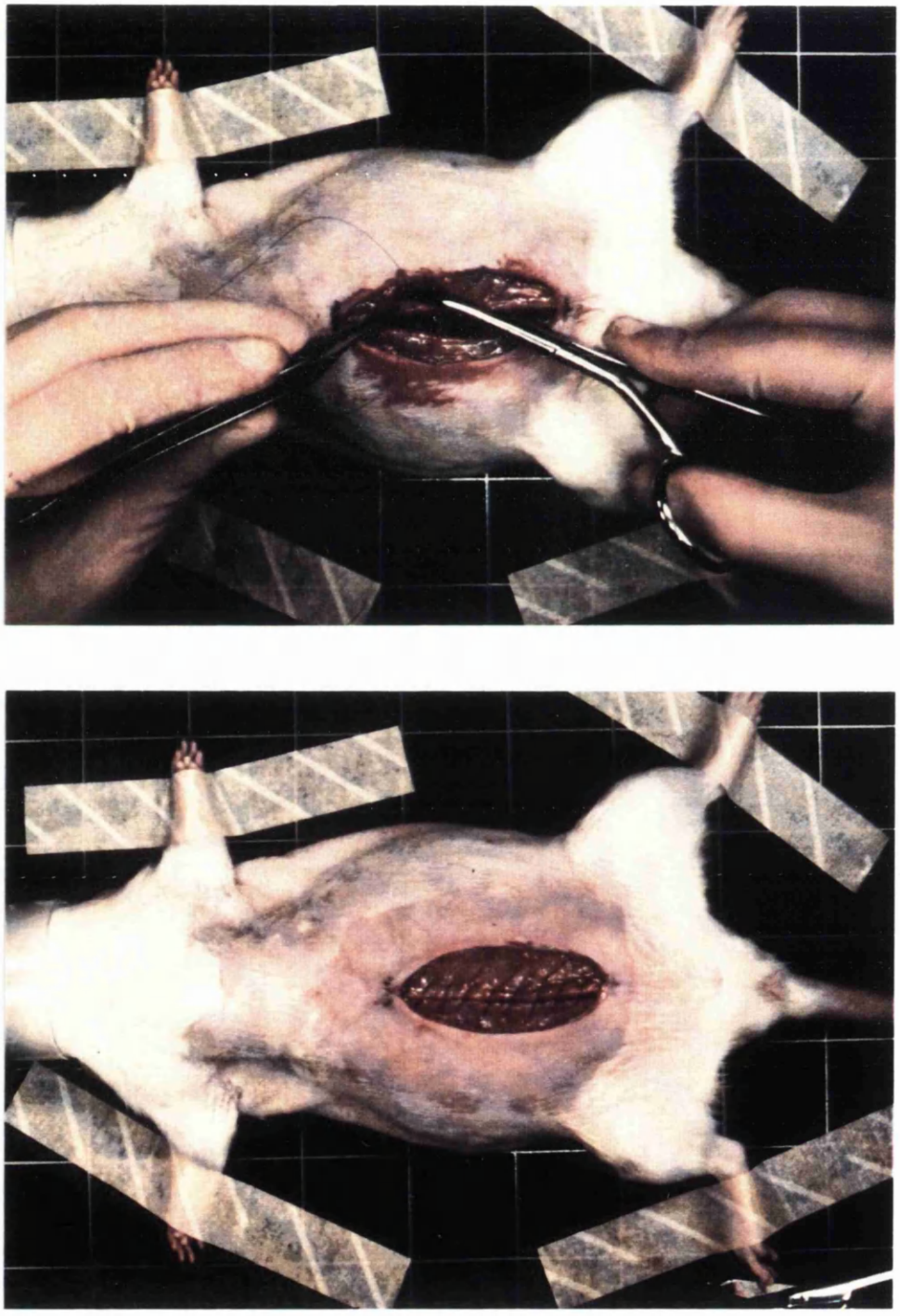
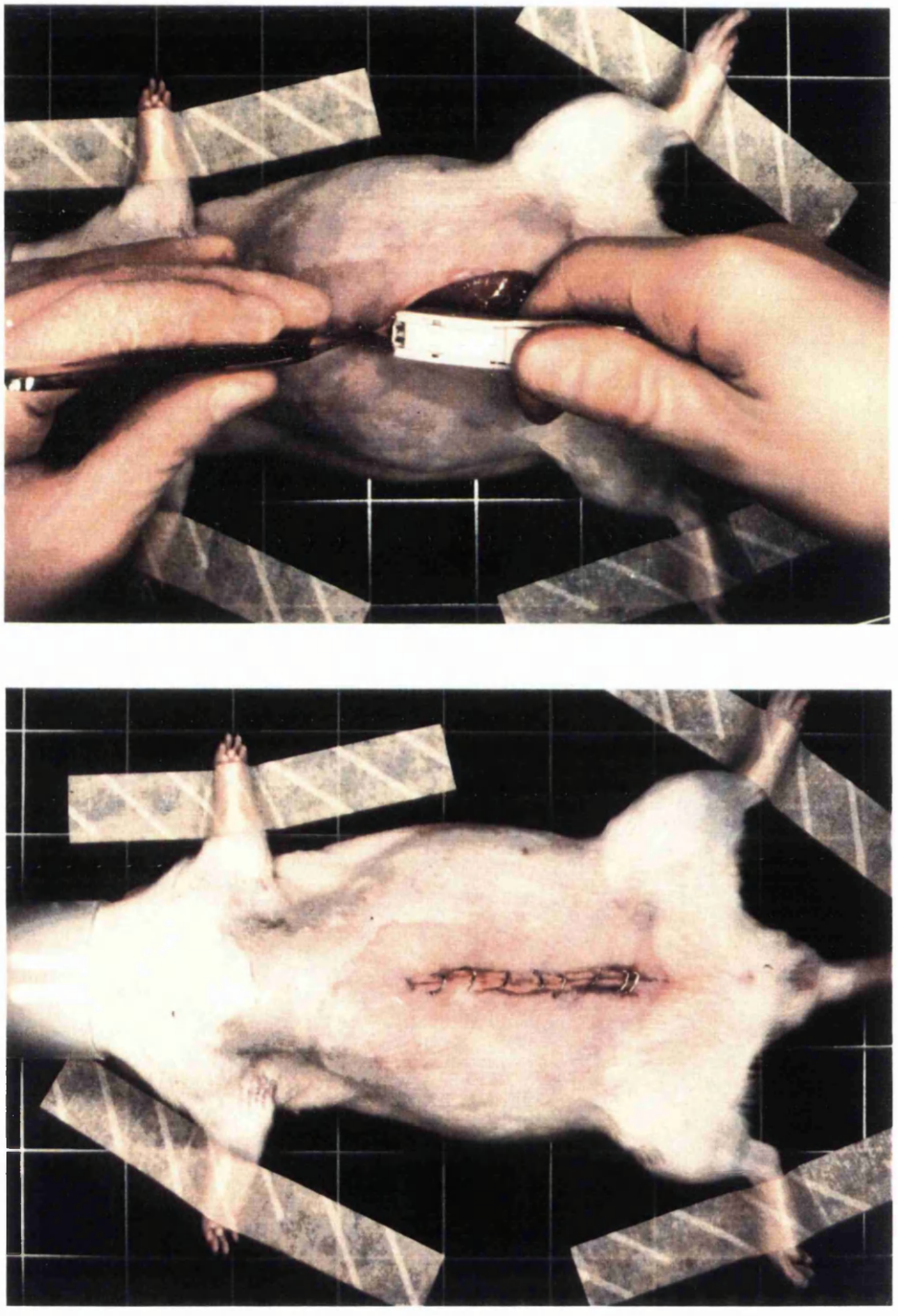


Figure 10.1c Performance and closure of the laparotomy wound. Top: closure of the skin with clips. Bottom: the final appearance of the wound.



10.2.5 Blood collection and analysis

The rats were sacrificed immediately prior to tensiometry. The terminal blood samples were collected in sodium edetate (EDTA) tubes for haemoglobin estimation and the remainder, having clotted, was centrifuged and the serum stored at -20^0C within one hour of collection. The haemoglobin concentration was estimated using a Coulter-S auto-analyser, (Coulter Electronics Ltd., Luton, Bedfordshire). The serum samples were assayed for albumin by radial immunodiffusion using rabbit anti-rat albumin anti-serum and the results were expressed as the percentage of the pooled normal albumin concentration [Mancini et al. 1964].

10.2.6 Tensiometry

The anterior abdominal wall was excised and tensiometry was performed immediately after sacrifice. The skin and muscle layers were separated by sharp dissection (Figure 10.2). Both layers were pinned out without tension onto a cork board and, using a metal template, two one-centimetre wide, dumb-bell shaped strips were prepared for tensiometry from both layers.

Wound-healing was assessed using a tensiometer (4301 Material Testing Machine, Instron Ltd., High Wycombe, England) immediately after preparation of the specimens (Figure 10.3). The tissue for measurement was placed with the wound equidistant between and at a right-angle to two smooth, rubber-faced, pneumatic jaws. The specimens were extended at a rate of 20mm/min from an initial jaw-separation distance of 15mm. Wound-healing was assessed by measurement of the maximum force (N/cm) applied to a

wound before disruption. The peak forces for skin and muscle in each rat were calculated as the mean of the two measurements made on each layer.

10.2.7 Analysis and statistics

The design of the study allowed collection of independent data for each of the four treatment groups (CP, CS, BP and BS) on two study days (day-6 and day-14). The weight and food consumption data from rats sacrificed on the sixth day were combined with that from the animals in the same treatment group sacrificed on the fourteenth day (for example CS6 was combined with CS14 and therefore, between day-0 and 6, n=16 and subsequently, following sacrifice of the CS6 group, n=8 for the CS group).

All results are presented as mean±SE. Both parametric and non-parametric tests have been used to compare sets of data (see appendix 7.).

Figure 10.2a Preparation of the muscle and skin strips for tensiometry. Top: the incision re-marked. Bottom: the excised section of the anterior abdominal wall laid out on a cork board.

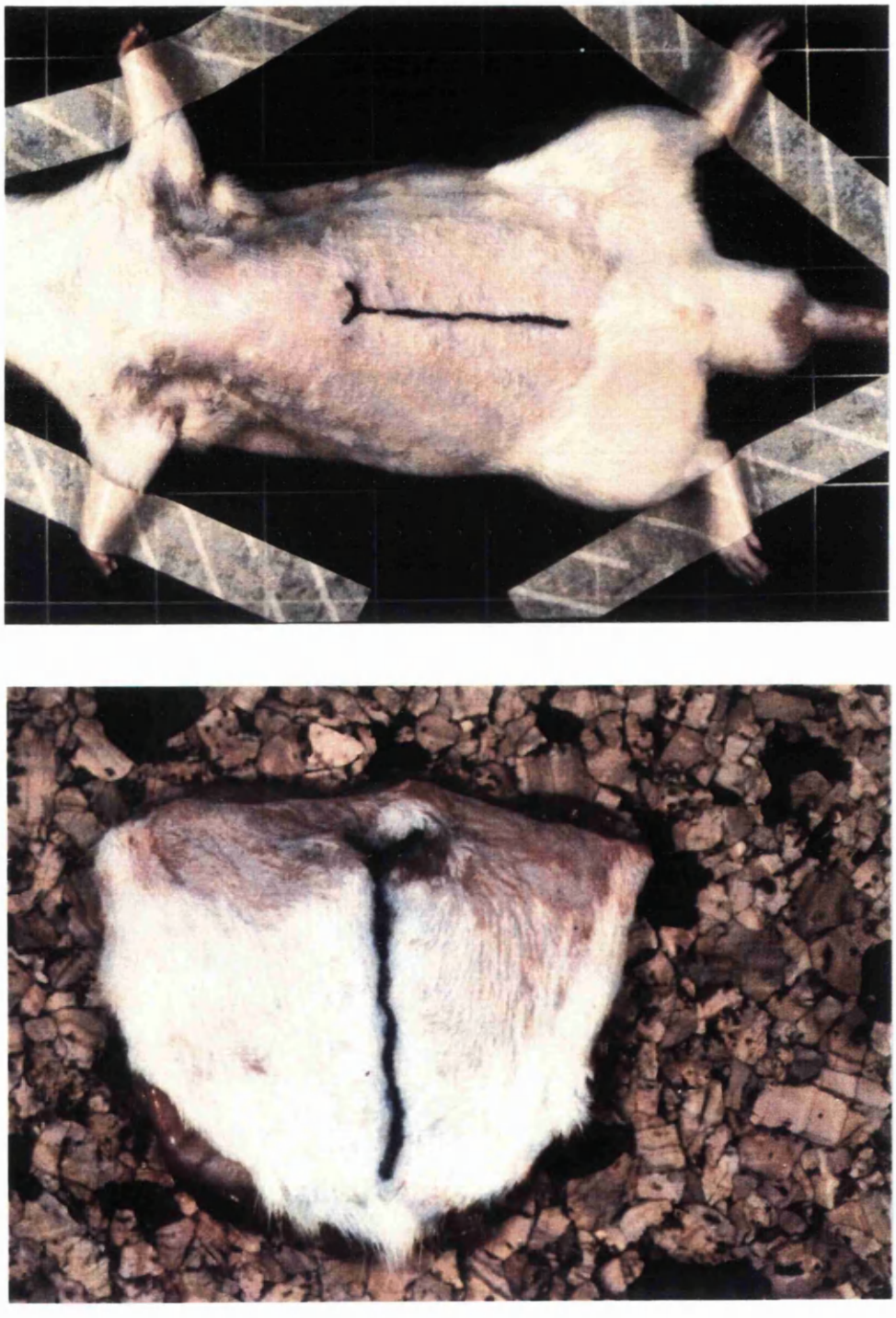


Figure 10.2b Preparation of the muscle and skin strips for tensiometry. Top: the two layers being separated by sharp dissection. Bottom: the muscle layer pinned out showing the wound with the sutures still in place.

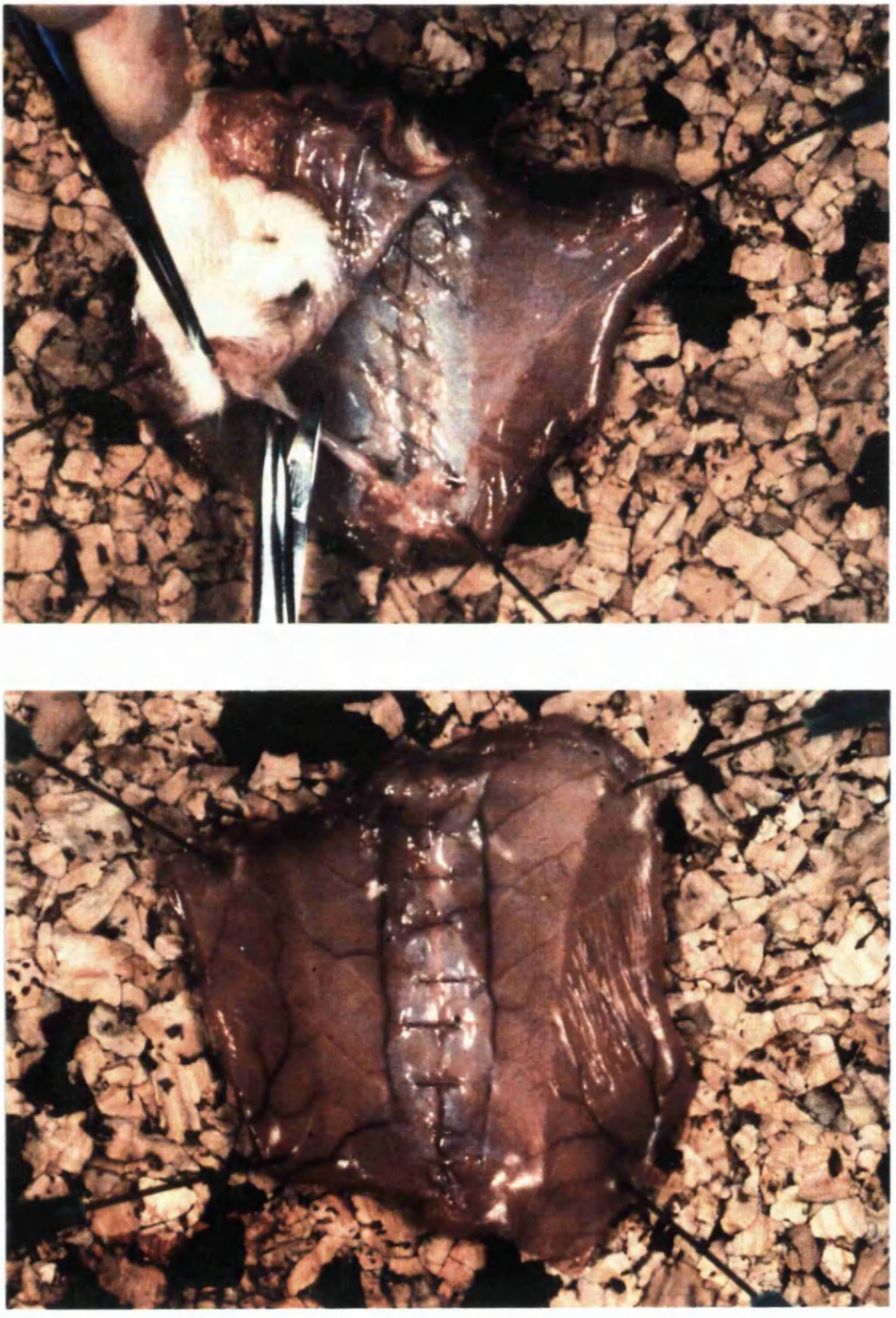


Figure 10.2c Preparation of the muscle and skin strips for tensiometry. Top: the metal template pinned over the wound following removal of the sutures. Bottom: the preparation of two strips of muscle.

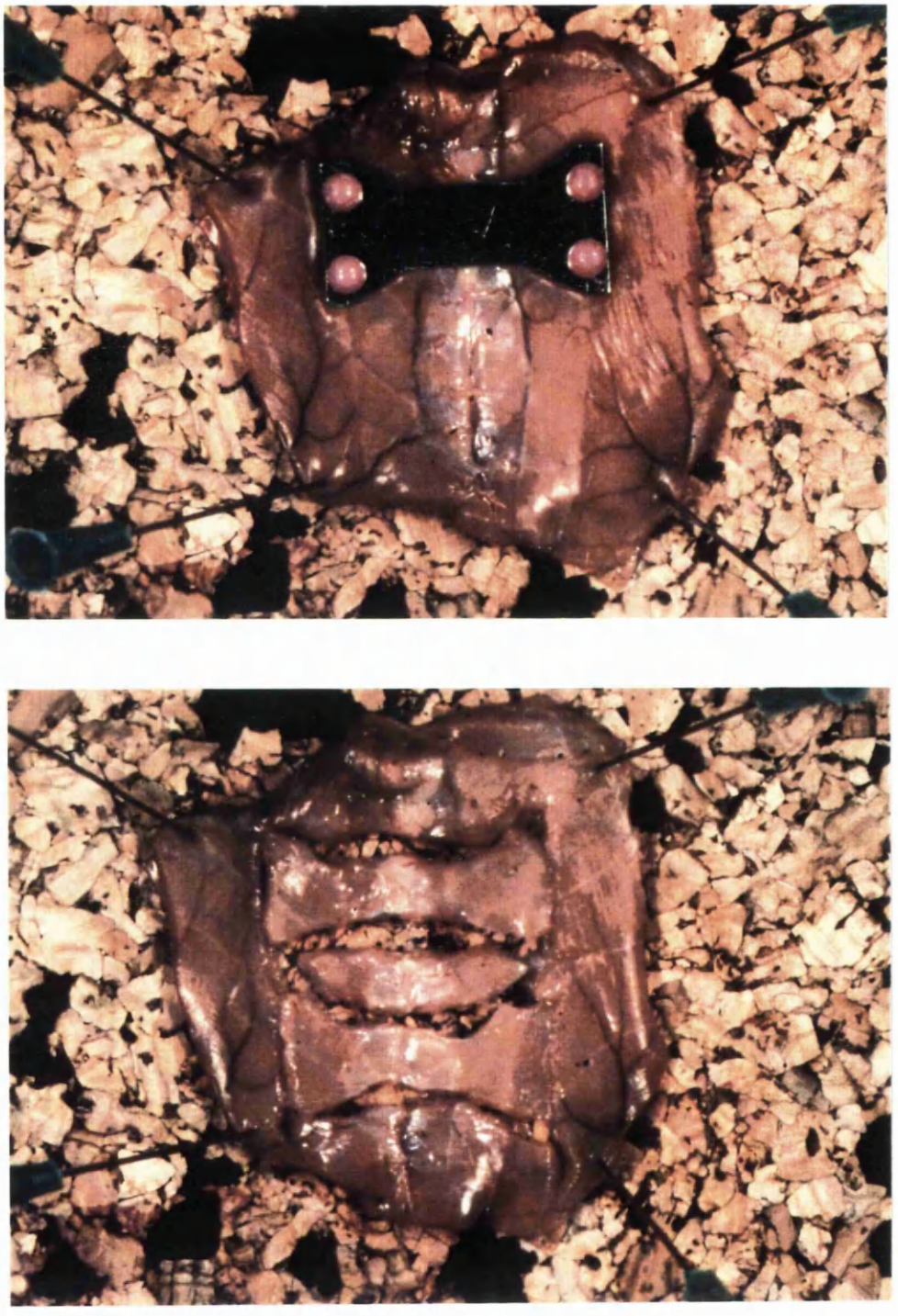


Figure 10.2d Preparation of the muscle and skin strips for tensiometry. Top: the template placed on the skin. Bottom: the four specimens ready for tensiometry.

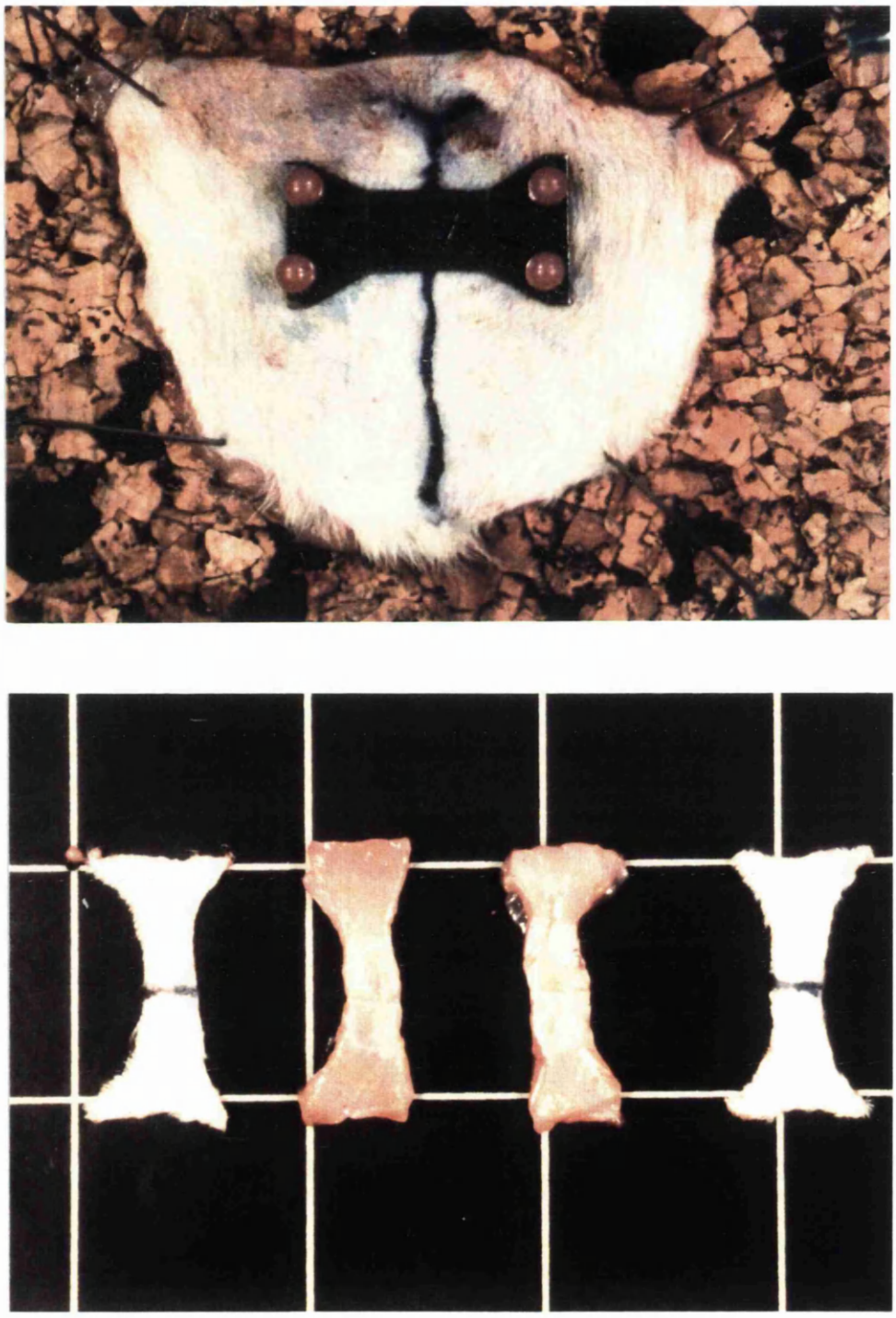
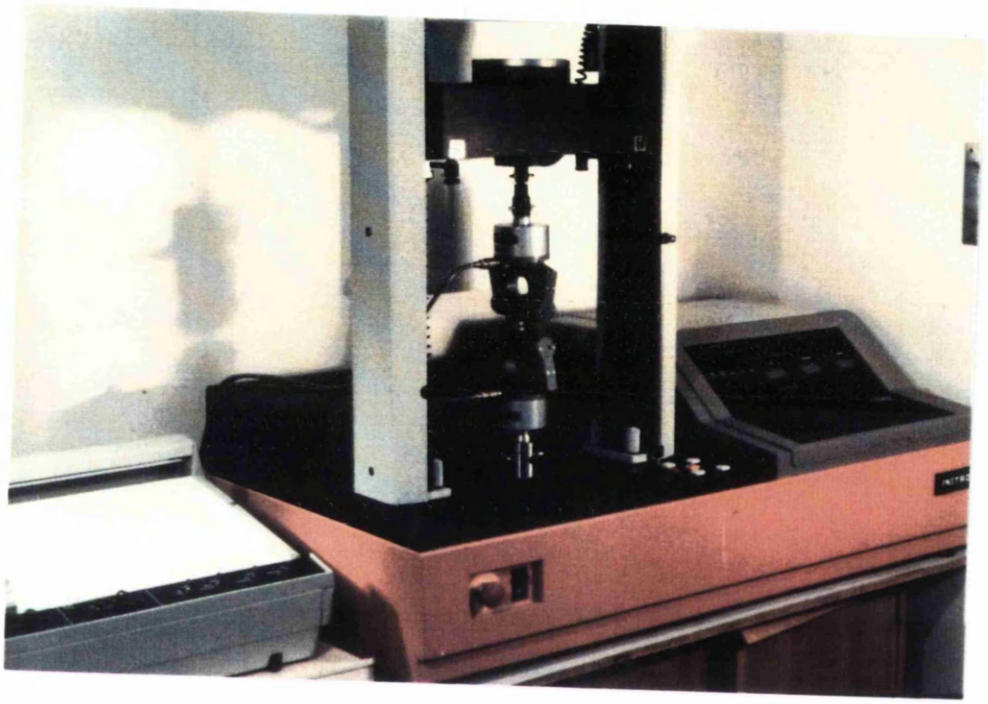


Figure 10.3a Tensiometry. The Instron 4301 tensiometer.



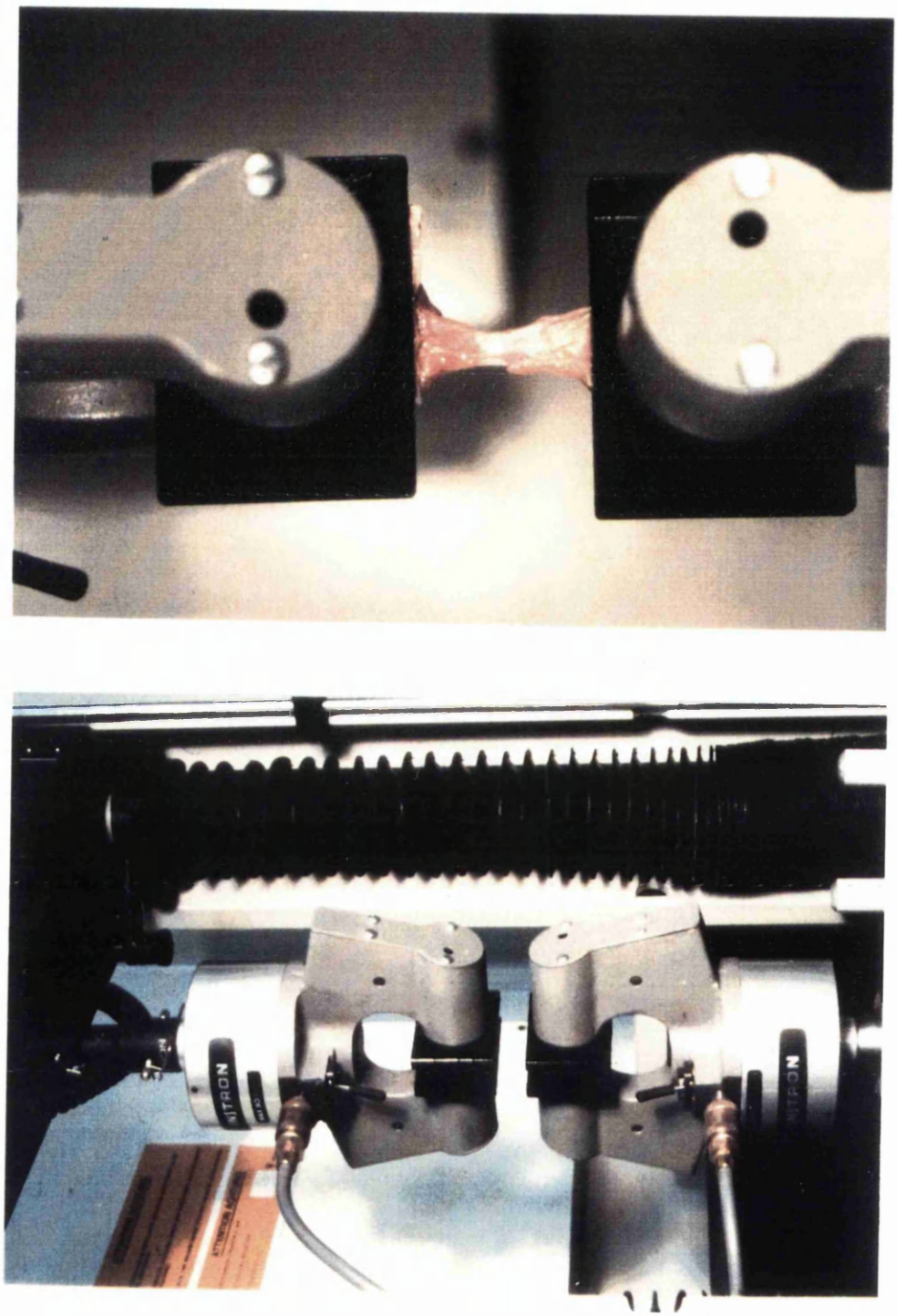


Figure 10.3b Tensiometry. Left: its pneumatic jaws prior to insertion of specimens. Right: a muscle strip being distracted.

10.3 - RESULTS

10.3.1 Experimental groups

The mean weights of the four treatment groups were well-matched at the beginning of the study (Tables 10.1 and 10.2). The mean TBSA were comparable in the four experimental groups which had been burned (Table 10.3). One rat out of the 24 (4%) in the each of the burned treatment groups died within the first three days of injury.

10.3.2 Dietary intake

The food intake of the burned rats was significantly less than controls in the first two days after injury (Figure 10.4) (Tables 10.4 and 10.5). The intakes of all four treatment groups, corrected for changes in body weight, were comparable thereafter, although the actual intake was significantly lower in the two burned groups on the last two days of the study (Table 10.4).

10.3.3 Weight-changes

The mean weight of all four treatment groups fell in the first two days of the study (Figure 10.5) (Table 10.2). Following this, the control groups gained weight (Table 10.1), whereas the burned rats continued to lose weight rapidly and by the end of the study had lost 25.2±2.2% of their initial weight. There was no significant difference between rats receiving somatropin or placebo.

10.3.4 Haemoglobin

The mean haemoglobin concentrations were significantly lower in the two burned groups compared with their control counterparts (Figure 10.6) (Tables 10.6 and 10.7). There were no changes in concentration between the two days in any treatment group and there was no difference between rats receiving somatropin or placebo.

10.3.5 Albumin

The mean serum albumin concentrations of the control groups were comparable to the normal pooled-serum value on both the sixth and fourteenth days of the study (Figure 10.7) (Tables 10.8 and 10.9). The burned rats had significantly lower concentrations than the control rats on both days. There were no significant differences between somatropin or placebo-treated rats. The concentration in control rats receiving somatropin was significantly lower on the fourteenth day than that on the sixth but there were no differences between days in the other groups.

10.3.6 Wound-healing

On the sixth post-operative day, the mean peak forces applied to the musculofascial (Figure 10.8) (Tables 10.10 and 10.11) and skin wounds (Figure 10.9) (Tables 10.12 and 10.13) were significantly greater in control rats than those that had been burned. The musculofascial wounds were significantly stronger in the control rats receiving somatropin than those receiving placebo but this difference was not evident in their skin

wounds. There was no difference in the strength of either the musculofascial or skin wounds between the BP and BS rats.

On the fourteenth day, both the musculofascial and skin wounds were significantly stronger than those on the sixth. There were no significant differences in the strength of the wounds between the four experimental groups.

Figure 10.4 Mean food consumption in the four treatment groups during the study. * Days 0-2; CP vs. BP and CS vs. BS, $p<0.001$.

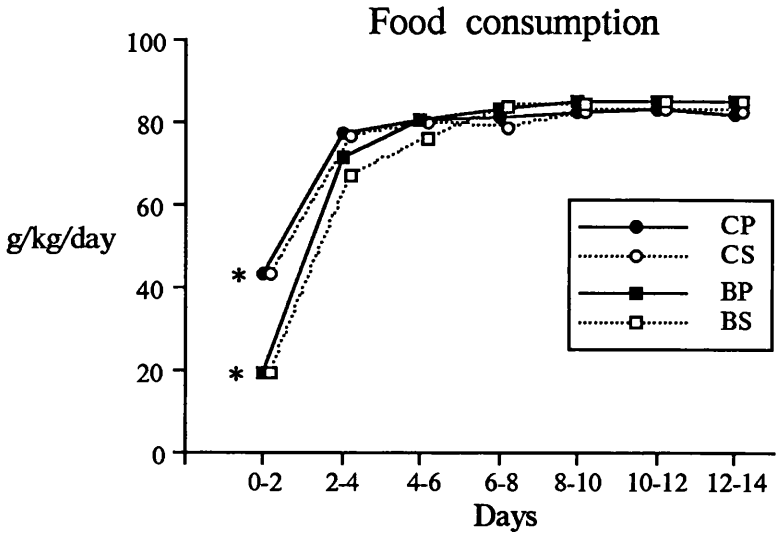


Figure 10.5 Mean weight in the four treatment groups during the study.

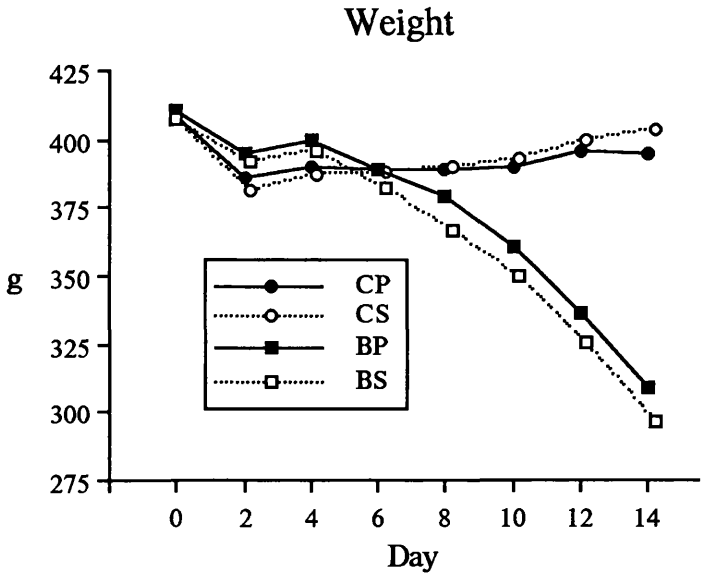


Figure 10.6 Mean haemoglobin concentration in the four treatment groups on the sixth and fourteenth days of the study. Day-6; CP vs. BP, $p<0.001$, CS vs. BS, $p<0.05$. Day-14; CP vs. BP, $p<0.05$, CS vs. BS, ns.

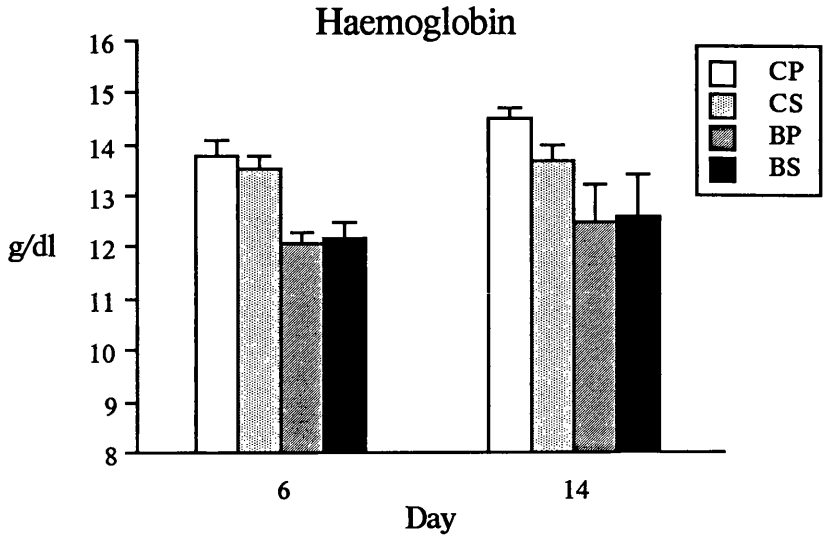


Figure 10.7 Mean albumin concentration in the four treatment groups on the sixth and fourteenth days of the study. Days-6 & 14; CP vs. BP and CS vs. BS, $p<0.001$.

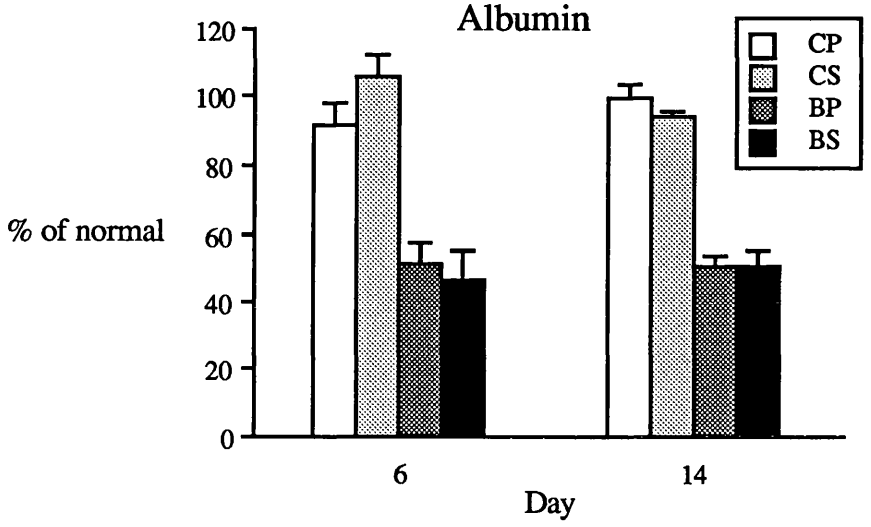


Figure 10.8 Mean muscle peak force in the four treatment groups on the sixth and fourteenth days of the study. * CP vs. CS, $p<0.05$. Day-6; CP vs. BP, $p<0.01$, CS vs. BS, $p<0.001$.

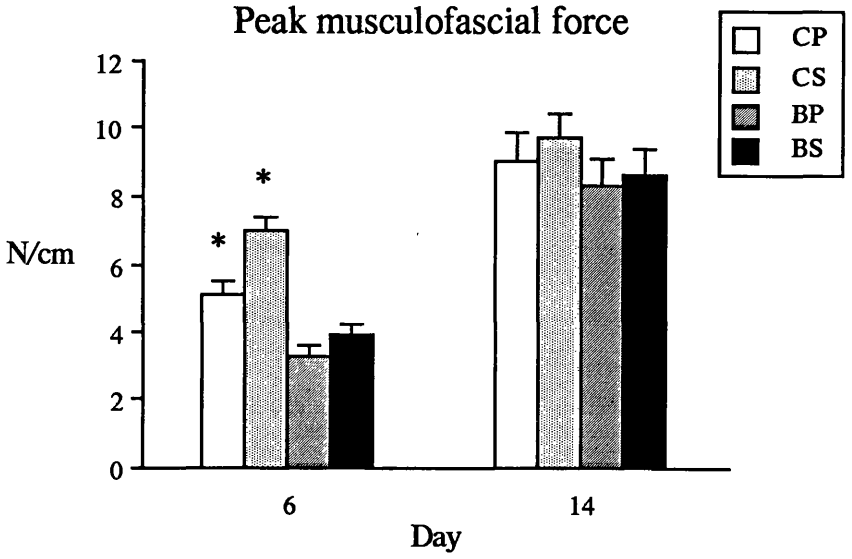
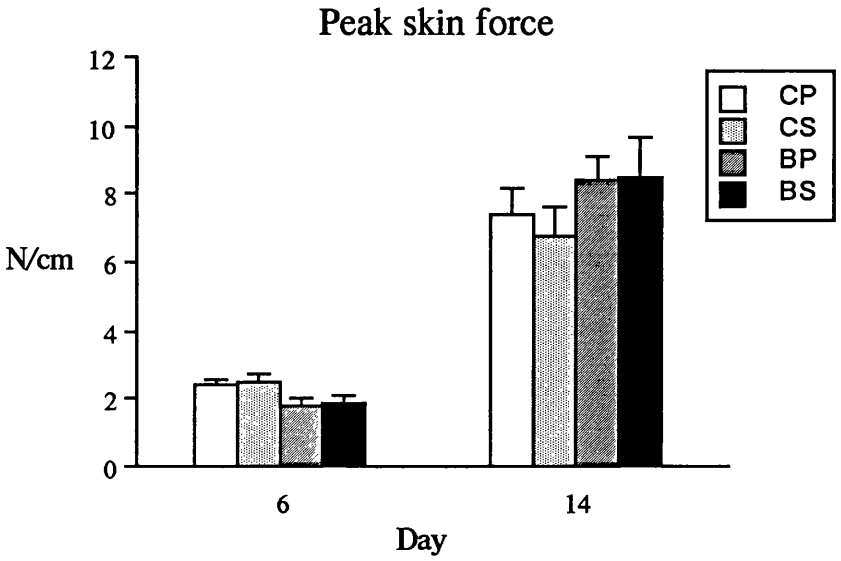


Figure 10.9 Mean skin peak force in the four treatment groups on the sixth and fourteenth days of the study. Day-6; CP vs. BP and CS vs. BS, $p<0.05$.



10.4 - DISCUSSION

10.4.1 Introduction

The present study shows that injury by burning in the rat results in weight-loss, anaemia, hypoalbuminaemia and a transient reduction in the strength of a healing laparotomy wound. Somatropin administration to the rat causes no weight-gain or increase in either haemoglobin or serum albumin concentrations. Its administration to unburned rats causes a transient increase in the strength of healing musculofascial wounds but it causes no increase in wound-strength in burned rats.

In the discussion to follow, I will comment on the effects of both injury by burning (§10.4.2) and somatropin administration (§10.4.3) on body-weight in the present study and note the similarity of these findings with those of the preceding one. I will discuss the changes that occurred in haemoglobin (§10.4.4) and serum albumin concentrations (§10.4.5). I will indicate the importance of wound-healing in the burned patient and discuss the choice of the wound-healing model (§10.4.6). I will review the available techniques for assessing the tensile strength of a wound and discuss the advantages of that used in the present study (§10.4.7). I will discuss the effect of injury on wound-healing (§10.4.8) and go into the possible mechanisms for this effect (§10.4.9). I will discuss the increase in wound-strength observed in unburned rats treated with somatropin and postulate that this reflects increased collagen production in the wound (§10.4.10). I will review recent evidence that suggests that somatomedins are necessary for normal fibroblast function and postulate that the failure of somatropin to enhance wound-healing in burned rats is due to impaired somatomedin responsiveness (§10.4.11). Finally, I will conclude that the findings of the present study support the view that the anabolic

activity of somatropin, although limited in rats with normal pituitary function, is impaired after injury (§10.4.12).

10.4.2 Injury and weight-changes

The food intake of the rats was diminished in the first two days after injury and was associated with a loss of weight in all groups. Following this, the weight of the control rats increased whilst the burned rats lost weight rapidly despite having comparable dietary intakes on all but the first two days of the study. These findings are identical to those seen in the preceding study and are due to the onset of a negative nitrogen balance after a burn (see §9.5.3).

10.4.3 Somatropin and weight-changes

There was no evidence that somatropin administration caused any gain of weight over the fourteen days of the study in either unburned or burned rats. This is consistent with the preceding study and with the recent work of Damm Jorgensen et al. [1988] (see §9.5.4).

10.4.4 Haemoglobin

Injury by burning in the rat resulted in a fall in haemoglobin concentration, consistent with the findings in burned man (see §8.4.3). There was no evidence of either a further decline or a recovery in haemoglobin concentration between the sixth and fourteenth day.

Growth hormone has previously been shown to potentiate erythropoiesis *in vitro* [Golde et al. 1977] and in hypophysectomized rats [Fruhman et al. 1954] but somatropin treatment resulted in no increase in haemoglobin concentration during the present study. However, in the absence of reticulocyte counts, the study was probably too short to draw any conclusions about the effect of somatropin on erythrocyte production.

10.4.5 Albumin

Injury by burning in the rat resulted in a fall in serum albumin concentration, consistent with the findings in burned man (see §8.4.12). This was predominantly due to the increase in capillary permeability that occurs in both burned and unburned tissues following injury. There was no evidence of recovery after the sixth day despite the transience of the changes in capillary permeability after a burn [Carvajal et al. 1979]. This is not surprising, as considerable weight-loss occurred during this time. It may also reflect the large pool-size of albumin which restricts its ability to respond to any short-term changes in synthesis.

There was no evidence that somatropin caused any increase in albumin concentration in either control or burned rats. Conversely, there was a significant decline in concentration between the sixth and fourteenth day in control rats receiving somatropin which did not occur in rats receiving placebo. This finding is not readily explicable.

10.4.6 Wound-healing

Wound-healing is an important process in the burned patient and ultimately determines the length of admission in the majority. Burns heal by secondary intention with the formation of granulation tissue, separation of the eschar and epithelialization both from the wound-margin and from surviving nests of epithelium such as in hair follicles. In superficial burns and skin-graft donor sites, epithelialization is rapid and little granulation tissue forms. In contrast, full-thickness burns form considerable granulation tissue and epithelialization occurs only from the wound-margin. This process is slow and results in unacceptable contracture formation and therefore is usually modified by tangential excision of the eschar and split-skin grafting. In the present study, the burn eschars began to become detached at the wound margins from the tenth post-burn day onwards revealing a raw granulating surface with little evidence of epithelialization.

Assessment of healing in open wounds is difficult in small mammals because much of the reduction in wound-size is due to wound-contraction rather than epithelialization [Modolin et al. 1985]. Assessment is further complicated by infection which can cause wide variation in the speed of healing. An incised wound, such as the midline laparotomy, which heals by primary intention (see §3.3) is much less likely to become infected. Although this wound has little clinical relevance to a burned patient, it provides a more reliable model with which to study healing.

10.4.7 Tensiometry

There are three major techniques available for assessing the tensile-strength of a healing experimental laparotomy wound [Van Winkle 1969]. Measurement can be made of the intra-abdominal pressure required to burst the wound, the force required to pull apart the wound in an intact animal or the force required to break an excised piece of tissue. The latter technique was chosen for use in the present study for several reasons. It is the most widely used and provides the best control of the force applied to a wound. It allows the study of both skin and musculofascial layers separately. Furthermore, more than one sample can be prepared from each layer which permits a more accurate assessment of healing to be made for each animal.

10.4.8 Injury and wound-healing

The strength of both musculofascial and skin wounds were decreased on the sixth day of the study in burned rats compared with controls. The reduction in the strength of the musculofascial wounds can be reproduced by the intraperitoneal administration of Hartmann's solution (compound sodium lactate) to uninjured rats (see appendix 9.). The use of fluid resuscitation in burned rats reduces their mortality and limits hypovolaemia which is also associated with impaired wound-healing (see §10.4.9). Whilst the use of the intraperitoneal route is not ideal under these circumstances it is preferable to other routes which were found to be less satisfactory. It is simple, non-traumatic, reliable and relatively large fluid volumes can be administered.

10.4 - DISCUSSION

The reduction in the strength of the skin wounds in the burned rats cannot be reproduced by fluid resuscitation in uninjured rats (see appendix 9.) and therefore is an effect of injury by burning. Chassin et al. [1953] showed that a wide variety of physical insults, including skin excision, fractures and burns, can impair wound-healing in the rat and that the duration of impairment is related to the severity of the injury. Fracture of the femur in rats results in a significant fall in the breaking strength of skin wounds and a reduction in the accumulation of hydroxyproline in subcutaneously implanted polyvinyl-alcohol sponges [Seifter et al. 1975, Crowley et al. 1977a, 1977b].

The impairment in wound-healing in the present study was only evident on the sixth post-operative day and not on the fourteenth, consistent with previous studies which have indicated that injury by burning causes a transient reduction in wound-strength. Levenson et al. [1954] performed midline laparotomies in adult rats which had been burned one day earlier by a technique similar to that used in the present study. Abdominal bursting strength was measured between two and nine days post-operatively and compared with that of unburned control rats. It was only significantly lower in the burned animals on the second and third days after injury. Histological examination of the laparotomy wounds showed that, after three days, fibroblasts were less numerous and less mature, and capillaries less abundant than in control rats. Subsequently, epithelialization was delayed and collagen maturation was slower in the burned group. Similarly, he showed that collagen formation in the laparotomy wounds of burned guinea pigs was scanty after seven days but

after 14 days, the wounds were indistinguishable from unburned animals [Levenson et al. 1957].

10.4.9 Other factors influencing wound-healing

Injury by burning is associated with a number of factors which could have been expected to impair wound-healing. It is therefore surprising that the impairment was only transient despite their plurality. These factors can broadly be divided into local and systemic [Reed and Clark 1985]. Local factors such as wound-infection cause impaired healing [De Haan et al. 1974]. There was no evidence of pus formation or cellulitis in, or around, the abdominal wounds in the present study although an increased incidence of wound-infections has been previously observed in burned rats [Levenson et al. 1954]. Whilst this finding may be have been due to immunosuppression, it was more likely to have resulted from contamination of the laparotomy wounds which were performed when the rats already had a burn. A distant source of infection, such as an infected burn, has also been shown to cause impaired wound-healing [De Haan et al. 1974]. A more recent study has suggested, however, that this impairment results from the associated nutritional disturbance rather than the infection itself [Greenhalgh and Gamelli 1987].

The burned rats were hypoalbuminaemic and overtly malnourished at the end of the study, both of which are associated with impaired wound-healing (see §3.6). However, Windsor et al. [1988] have recently shown that the actual food intake is better correlated with wound-healing than is the

extent of malnutrition. Although malnourished, the burned rats had only a transient fall in dietary intake compared with controls.

Following a major burn there is a fall in the platelet count [Newsome and Eurenius 1973, Heideman 1979, Simon et al. 1977, Alkjaersig et al. 1980] accompanied by abnormalities of blood coagulation [Simon et al. 1977, Alkjaersig et al. 1980]. Platelets and coagulation pathways are important in the early stages of repair not only for mechanical reasons but also for the initiation of granulation tissue formation (see §3.3).

Injury by burning is often followed by hypovolaemia and a reduced cardiac output despite fluid resuscitation [Arturson 1988]. Experimental hypovolaemia, due to acute haemorrhage, results in decreased collagen formation and wound-strength [McGinn 1976, Nasution and Taylor 1981, Taylor et al. 1987]. It is probable that this effect results from reduced wound oxygen delivery due to vasoconstriction [Brantigan et al. 1974]. Fibroblast function is very sensitive to changes in oxygen tension. Hypoxia causes a decrease in collagen accumulation in wounds [Hunt and Pai 1972, Kivisaari et al. 1975] and a reduction in wound tensile strength [Niinikoski 1969, Stephens and Hunt 1971]. These effects may be exacerbated by the general hypoxia that accompanies burns, even in the absence of a respiratory burn [Arturson 1977]. Anaemia may cause a further reduction in oxygen delivery to a wound. It has been shown that the tensile strength in laparotomy wounds performed in rats with pre-existing anaemia is significantly reduced compared with that in rats with normal haemoglobin concentrations [Bains et al. 1966].

10.4.10 Somatropin and wound-healing

The administration of somatropin resulted in a transient increase in the strength of musculofascial wounds in control rats only. Somatropin administration did not cause any increase in the strength of skin wounds. This difference between muscle and skin is difficult to account for but Irvin [1978] suggests that wounds in skin are less responsive to dietary manipulation than those in muscle. All recent studies have shown that GH increases wound-strength, independent of dose, although all have used much larger doses than the present study (see §7.6). In all studies, this response has only occurred if GH was administered both before and after wounding. The three most recent studies, as in the present one, have indicated that improvements due to somatropin are transient [Jorgensen and Andreassen 1987, 1988 and unpublished work]. In these, somatrem administration resulted in a significant increase in wound-strength after four, seven and ten days but not 16 days. This coincides almost precisely with the period of collagen deposition in a wound (see §3.3) and in one of these studies hydroxyproline deposition in subcutaneously implanted cellulose sponges was significantly increased after seven days in the treated rats [Jorgensen and Andreassen 1987].

It can be postulated that the increase in wound-strength in GH treated rats in the present and other studies is due to an increase in collagen synthesis and deposition in the wound. Following collagen deposition, the further increase in tensile strength is due to collagen remodelling rather than synthesis. This latter process appears to be unaffected by GH.

10.4.11 Fibroblasts and somatomedins

Fibroblasts can synthesise IGF-I [Clemmons et al. 1981, Adams et al. 1983, Clemmons 1984] and Clemmons and Van Wyk [1981] provided evidence that IGF-I is required for normal fibroblast replication. Van Wyk [1984] postulated that GH stimulates fibroblasts via the paracrine and/or endocrine release of somatomedins and recently, Cook et al. [1988] showed that the stimulatory effects of exogenous GH on DNA synthesis and the replication of human fibroblasts could be inhibited by antibodies to IGF-I. Although somatropin administration causes only a small increase in the concentration of serum IGF-I (see §9.5.5), Orlowski and Chernausek [1988] have recently shown that GH administration causes larger rises in the IGF-I concentration in tissues than serum. It is probable, therefore, that the improvement in wound-healing due to GH was mediated by local somatomedin release.

Somatropin administration to burned rats caused no increase in wound-strength. It may be postulated that this reflects, at a local level, the loss of somatomedin-responsiveness to somatropin observed in man (see §8.4.11) and rats (see §9.5.5) after injury by burning.

10.4.12 Conclusions

The present study confirms that somatropin administration to the rat causes an increase in the strength of a healing wound. This effect was absent in the burned rat. These findings support the view that the anabolic activity of somatropin, although limited in rats with normal pituitary function, is impaired after injury.

10.5 - PRINCIPAL FINDINGS

1. Injury by burning in the rat results in weight-loss, anaemia and hypoalbuminaemia.

2. Injury by burning in the rat causes a transient reduction in the strength of a healing laparotomy wound.

3. Somatropin administration to the rat causes no weight-gain or increase in either haemoglobin or serum albumin concentrations.

4. Somatropin administration to uninjured rats causes a transient increase in the strength of healing musculofascial wounds.

5. Somatropin administration to burned rats causes no increase in wound-strength.

11.1 - CONCLUSIONS

The three studies described in chapters eight, nine and ten investigated the actions of somatropin following injury by burning. The studies were performed in both patients and rats with normal pituitary function. In all, the maximum doses administered were comparable to those used in these species for the correction of hypopituitarism.

The administration of somatropin to the uninjured rat causes a small rise in the serum somatomedin concentration and a transient increase in the strength of a healing musculofascial wound. It causes no increase in the positivity of nitrogen balance or weight-gain. These findings indicate that clinically usable doses of somatropin are active in rats with normal pituitary function although these effects are modest compared with those seen in previous studies in hypophysectomized rats.

Injury by burning is followed by increases in REE and urinary nitrogen excretion accompanied by insulin resistance and glucose intolerance. There is a generalised fall in plasma protein levels, including somatomedins.

Somatropin administration to burned man causes no decrease in the rate of protein oxidation or increase in the positivity of nitrogen balance. It causes no rise in either serum somatomedin or plasma protein concentrations. It causes an increase in the insulin resistance already present in burned man. Somatropin administration to the burned rat causes no rise in the serum somatomedin concentration and no effect on nitrogen balance, weight-gain or wound-healing.

These studies provide evidence that injury by burning abolishes the anabolic effects of GH which have been both observed in the uninjured rat in the present work and previously described by other authors in uninjured man. It is thought that the growth-promoting and anabolic actions of GH

are mediated indirectly by somatomedins whereas its effects on glucose metabolism are exerted directly. It is apparent from the present work that its indirect actions are absent after injury by burning.

The present work is in agreement with previous studies which have shown that that the serum somatomedin concentrations fall after injury by burning and that both the extent and duration of their fall are proportional to the severity of injury. It has also shown that the normal rise in somatomedin levels in response to exogenous GH administration is absent after injury by burning. It may be postulated, therefore, that the failure of somatropin to exert any anabolic activity after injury by burning reflects the changes observed in somatomedin concentrations and responsiveness. Thus, these studies suggest that somatropin administration is least likely to be of benefit in the more severely injured patient.

It can be concluded from the present work that somatropin and related compounds, are not suitable for use as anabolic agents soon after injury by burning.

11.2 - AREAS OF FUTURE RESEARCH

11.2.1 Introduction

In view of the findings of the present work, a number of areas of research are proposed below which are worthy of investigation in the future.

11.2.2 Timing and targeting of administration

I have postulated, as a result of my study in burned patients, that the immediate post-burn period is an unsuitable time to administer somatropin because of the fall in somatomedin levels that occurs at this time (see §8.4.11). It is possible that somatropin administration may be effective later after injury when clinical improvement has occurred, nutrition is fully established and somatomedin levels have recovered.

11.2.3 Route and frequency of administration

There is evidence that the frequency of administration alters the effectiveness of a given dose of GH and that the intravenous route of administration is more effective than the subcutaneous (see §9.5.7). Jorgensen et al. [1988] have recently concluded that "subcutaneously injected GH is degraded locally to a substantial extent". Although none of these studies have been performed in the presence of normal pituitary function, it may be worthwhile to investigate the effect of both the frequency and route of administration on the anabolic activity of somatropin.

11.2.4 GH variants

A naturally occurring structural variant of GH has been discovered which has a molecular weight of approximately 20kD (see §6.1) Some studies have indicated that this variant has normal growth-promoting properties but lacks insulin-like and anti-insulin activity [Davidson 1987]. GH is a complex molecule with multiple actions. It is probable that some of its actions are promoted by different segments of the molecule. Research is in progress at a number of centres to identify and synthesise the amino-acid sequence responsible for its growth-promoting activity. It can be speculated that a purely growth-promoting preparation would be preferable and possibly more effective in an injured patient because of its greater specificity.

11.2.5 Biosynthetic IGF-I

It is thought that the anabolic actions of GH are mediated by somatomedins, particularly IGF-I (see §6.3). IGF-I directly enhances growth and therefore does not rely on the synthesis and release of an intermediary for its activity. Furthermore, IGF-I has insulin-like activity, in contrast to the insulin-antagonistic properties of GH (see §6.5). These properties potentially make IGF-I a suitable anabolic agent in hypermetabolic patients. IGF-I has, until recently, been available in only minute quantities but now biosynthetic preparations have been developed. No studies have yet examined its anabolic properties in man.

APPENDICES

APPENDIX 1. MINIMIZATION

A1.1 Introduction

The technique of "minimization" used for randomizing the patients in the clinical study was described by Taves [1974] and was developed to limit the differences between treatment groups.

Three clinical variables were selected, arranged according to their importance (TBSA>age>sex) and divided into sub-categories to allow characterization of the patients. The patients were randomized to control or somatropin-treatment groups using these variables.

A1.2 Method and example

1. There are four patients already in both the control and somatropin groups (see table below). Their characteristics are set out in the appropriate sub-category columns and the totals for each treatment-group calculated.

2. The new patient to be randomized is a 40 year old man with a 15% burn. New, theoretical, sub-category totals are then calculated for each of the treatment groups using the characteristics of the new patient.

Patients	TBSA(%) <=30	TBSA(%) >30	Age(yr) 16-33	Age(yr) 34-51	Age(yr) 52-70	Sex M	Sex F
C4		31%	19			M	
C6	12%		28			M	
C7	16%			51		M	
C9	18%			37		M	
Control totals	3	1	2	2	0	4	0
New control totals	4			3		5	

Patients	TBSA(%) <=30	TBSA(%) >30	Age(yr) 16-33	Age(yr) 34-51	Age(yr) 52-70	Sex M	Sex F
GH1	17%		28			M	
GH2	15%			44			F
GH3	17%		16			M	
GH5	25%		21			M	
Somatropin totals	4	0	3	1	0	3	1
New somatropin totals	5			2		4	

3. The new theoretical sub-category totals for each group are then compared with the existing sub-category totals in the alternative treatment group and the absolute difference (sign ignored) between the theoretical and existing sub-category totals calculated (see table below).

	TBSA(%)		Age(yr)			Sex	
	<=30	>30	16-33	34-51	52-70	M	F
Control totals	3	1	2	2	0	4	0
New somatropin totals	5			2		4	
Difference	2			0		0	=2

	TBSA(%)		Age(yr)			Sex	
	<=30	>30	16-33	34-51	52-70	M	F
Somatropin totals	4	0	3	1	0	3	1
New control totals	4			3		5	
Difference	0			2		2	=4

4. The sum of sub-category differences for each theoretical allocation is calculated for each group.

5. The total of sub-category differences is 2 when the new patient is allocated to the somatropin group compared with 4 when allocated to the control group. The two study groups are therefore better matched if the new patient is allocated to the somatropin group.

A1.3 Notes

1. The first patient to enter the trial was allocated by selection of a random number.

2. In the event of a tie in the sub-category difference totals, a patient was put into the group in which the sub-category difference for the most important variable (TBSA) was least.

3. In the event of a patient being withdrawn from the trial, the sub-category totals for the relevant treatment group were returned to their previous values (ie. the patient was also withdrawn from the minimization process). This was possible because of the slow rate of recruitment into the trial and was done to ensure as good a match as possible between treatment groups.

APPENDIX 2. CLINICAL HISTORIES

A2.1 Notes

1. #C1, #C2 and #C3 patients preceded the start of the study.
2. #C5 was excluded from entry into the study.
3. #C8 and #GH4 were withdrawn from the study.
4. PBD-7; refers to the seventh post-burn day.
5. Day-7: refers to the seventh day of the study (which, because of the timing of entry into the study, was PBD-8 or 9).
6. The distribution of the burns of the 12 patients who completed the study are illustrated in Figure 8.4 and their photographs in Figure A2.1.

A2.2 Control patients

#C4 A 19 year old, previously fit, man fell off a motorbike, splashing ignited petrol onto his trousers. He sustained 31%, predominantly full-thickness, burns on his legs and perineum (Figure A2.1).

Bilateral escharotomies were performed under general anaesthetic soon after admission. He subsequently underwent tangential excision and skin-grafting on day-2. He required transfusion with three units of blood on day-3 to correct post-operative anaemia and two units on day-12 because of a further fall in his haemoglobin concentration. He required further skin-grafting on PBD-22 and PBD-38. He was discharged from the Burns Centre on PBD-50.

#C5 A 44 year old woman sustained 20% burns, on her lower limbs and buttocks, after slipping into a hot bath from which she was unable to

get out. She had a previous history of rheumatoid arthritis, bilateral total hip replacement, dermatitis, the irritable bowel syndrome and peripheral vascular disease.

During her initial IVGTT, the blood glucose concentration five minutes after glucose injection (T_5) was 22.3mmol/l although the glucose disappearance constant (k) of 1.07 was comparable to other patients. She was therefore not entered into the study (see §8.2.3). On day-3, her basal blood glucose was 11.2mmol/l and her k-value deteriorated to 0.59. She required insulin to control her blood glucose before dying on day-4. Post-mortem examination revealed no specific cause of death.

#C6 A 28 year old, previously fit, man sustained 12% deep dermal burns on his face, neck, chest and left shoulder when boiling fat was thrown onto him (Figure A2.1). He was initially assessed to have 15% burns and therefore he received fluid resuscitation and he was entered into the study. His TBSA was recalculated at the first dressing change on day-2 of the study. He was managed conservatively and discharged on PBD-17. He subsequently required readmission on PBD-37 for skin-grafting.

#C7 A 51 year old, previously fit, electrician sustained a high-voltage electrical flash burn while mending a fuse-box which ignited his shirt. He had 16% superficial dermal burns involving his face

and anterior chest wall (Figure A2.1). In addition, both corneas were burned. He was managed conservatively and discharged on PBD-17.

#C8 A 21 year old, previously fit, man sustained 15% partial-thickness burns to face, anterior chest, abdominal wall and right arm whilst lighting a bonfire with petrol. He was managed conservatively. He was withdrawn from the study on day-8 at his own request.

#C9 A 37 year old, previously fit, man sustained an 18% mixed depth burn to his right thigh and leg whilst using methylated spirits to light a barbecue (Figure A2.1). He was initially managed conservatively and discharged on PBD-31. He subsequently required readmission for skin-grafting on PBD-45.

#C10 A 40 year old, previously fit, man was engulfed in a fireball whilst lighting a barbecue with petrol, sustaining 42% superficial dermal burns on his face, anterior chest and abdominal walls, and both arms (Figure A2.1).

He was managed conservatively. He required treatment with systemic triple-antibiotic therapy on day-2 for sepsis which was diagnosed on clinical grounds alone as no micro-organisms were detected in blood cultures. The antibiotics were stopped after five days due to rapid clinical improvement. He was discharged on PBD-22.

#C11 A 54 year old, previously fit, tea-lady spilt an urn of freshly-made tea onto herself sustaining 15% deep dermal burns on her neck, anterior chest-wall and back (Figure A2.1).

She was advised to have early surgery performed but she declined and was therefore managed conservatively. She required three units of blood on day-6 to correct anaemia. She was discharged on PBD-21 and continued to have her burns dressed as an outpatient.

A2.3 Patients treated with somatropin

#GH1 A 28 year old, previously fit, foundry-man sustained 17% superficial dermal burns on his face, anterior chest, abdominal wall and right arm when he was splashed by burning white-spirit (Figure A2.1). He was managed conservatively and discharged from the Burns Centre on PBD-25.

#GH2 A 44 year old woman with long-standing, poorly-controlled, grand-mal epilepsy had an epileptic fit while holding a saucepan of boiling water. She sustained 15% superficial dermal burns on her thighs and groin. She was managed conservatively and required two units of blood on day-4 to correct anaemia. She was discharged on PBD-29.

#GH3 A 16 year old, previously fit, gardener sustained 17% mixed-depth burns on his face, anterior chest wall, shoulders and hands while lighting a bonfire with petrol (Figure A2.1). He was initially

managed conservatively but required skin-grafting on PBD-20. He was discharged on PBD-29.

#GH4 A 22 year old, normally built, man sustained 30%, predominantly full-thickness, burns on his anterior chest-wall, face and both arms while lighting a barbecue with petrol. He had no history of ill-health but one of his grand-parents had developed diabetes at the age of 80 which had been managed by diet alone.

Early tangential excision and split-skin grafting was performed on day-3. His post-operative recovery was poor with a prolonged period of confusion. This was assumed to be due to sepsis from his central intravenous line although no micro-organisms were detected in repeated blood cultures. The line was withdrawn on day-6 and his general condition improved spontaneously over the subsequent two days.

The IVGTT in this man on day-1 was within acceptable limits for entry into the study although the k-value was low (see table below). On day-3, the IVGTT which preceded his surgery was markedly abnormal with a high basal glucose concentration and low k-value. A further IVGTT on day-6 showed a further rise in the basal glucose concentration and fall in k-value. In both, the early phase of insulin release was negligible. Somatropin treatment was withdrawn at this time according to the study protocol (see §8.2.6). On day-8 and day-11, 72 hours and 6 days after the last somatropin injection, the basal glucose concentration became more acceptable but the k-value failed to improve. His early insulin release remained poor on

#GH4	Time	Day-1	Day-3	Day-6	Day-8	Day-11
Blood glucose concentration (mmol/l)	T_0	5.8	11.2	12.2	7.1	7.3
	T_5	13.7	19.2	19.6	14.4	17.0
	T_{10}	11.9	16.7	18.2	12.8	14.9
	T_{20}	11.1	15.5	16.5	11.7	12.9
	T_{60}	7.9	12.2	14.2	9.4	11.4
Plasma insulin concentration (pmol/l)	T_0	236	631	354	426	417
	T_5	836	533	402	398	671
	T_{10}	704	527	637	593	760
	T_{20}	753	763	523	578	928
	T_{60}	530	767	511	626	800
Basal insulinogenic index (pmol/mmol)		40.7	56.6	29.0	60.4	57.5
0-5' delta-glucose (mmol/l)		7.9	8.05	7.4	7.35	9.75
Delta-glucose area (mmol/l.min)		260	212	230	243	330
Glucose disappearance constant (k)		0.85	0.6	0.38	0.55	0.31
0-5' delta-insulin (pmol/l)		600	-98	48	-28	254
0-5' insulinogenic index (pmol/mmol)		75.9	<0	6.5	<0	26.1
Delta-insulin area (pmol/l.min)		25600	7330	9730	9400	24280
Total insulinogenic index (pmol/mmol)		98.5	34.6	42.3	38.7	73.6

both these days. He declined to undergo the final IVGTT on day-15.

He was discharged on PBD-56.

#GH5 A 21 year-old, previously fit, Iranian man sustained 20%, predominantly full-thickness, burns on his back, shoulders and face when he deliberately set his bed alight (Figure A2.1).

He remained unwell after resuscitation and a clinical diagnosis of septicaemia was made on day-5 which was treated with intravenous triple antibiotic therapy, although blood cultures failed to grow any micro-organisms. He underwent tangential excision and skin-grafting on day-7. He required four units of blood on day-8 to correct post-operative anaemia. Wound swabs taken on day-8 grew ß-haemolytic streptococcus and therefore Benzyl-penicillin was added to his antibiotic regimen. All antibiotics were stopped on day-14 in view of clinical improvement and the repeated absence of ß-haemolytic streptococci from wound swabs. He underwent further grafting on PBD-20 and 34 and was discharged on PBD-49.

#GH6 A 40 year old, previously fit, man sustained 14% superficial dermal burns on his face, hands and legs due to a camping-gas explosion (Figure A2.1). He was initially assessed to have 15% burns and was therefore formally resuscitated and entered into the study. The TBSA was recalculated at the first dressing change on day-1. He was managed conservatively and was discharged on PBD-22.

#GH7 A 44 year old, previously fit, man sustained 32% superficial dermal burns on his face, upper limbs and right leg as a result of a domestic propane gas explosion (Figure A2.1). He was managed conservatively and discharged on PBD-21.

Figure A2.1a Photographs of patients soon after admission. C4.

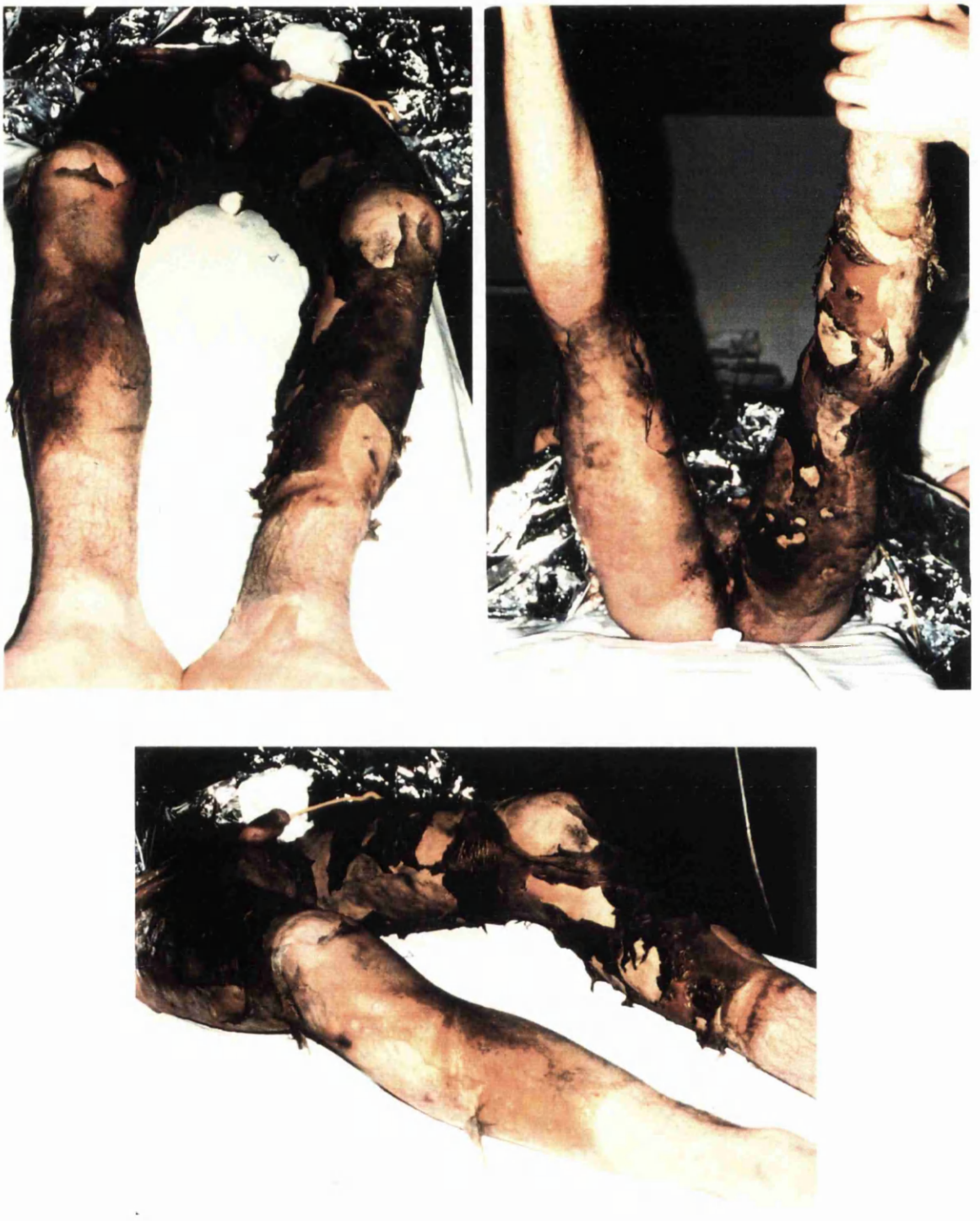


Figure A2.1b Photographs of patients soon after admission. C6.

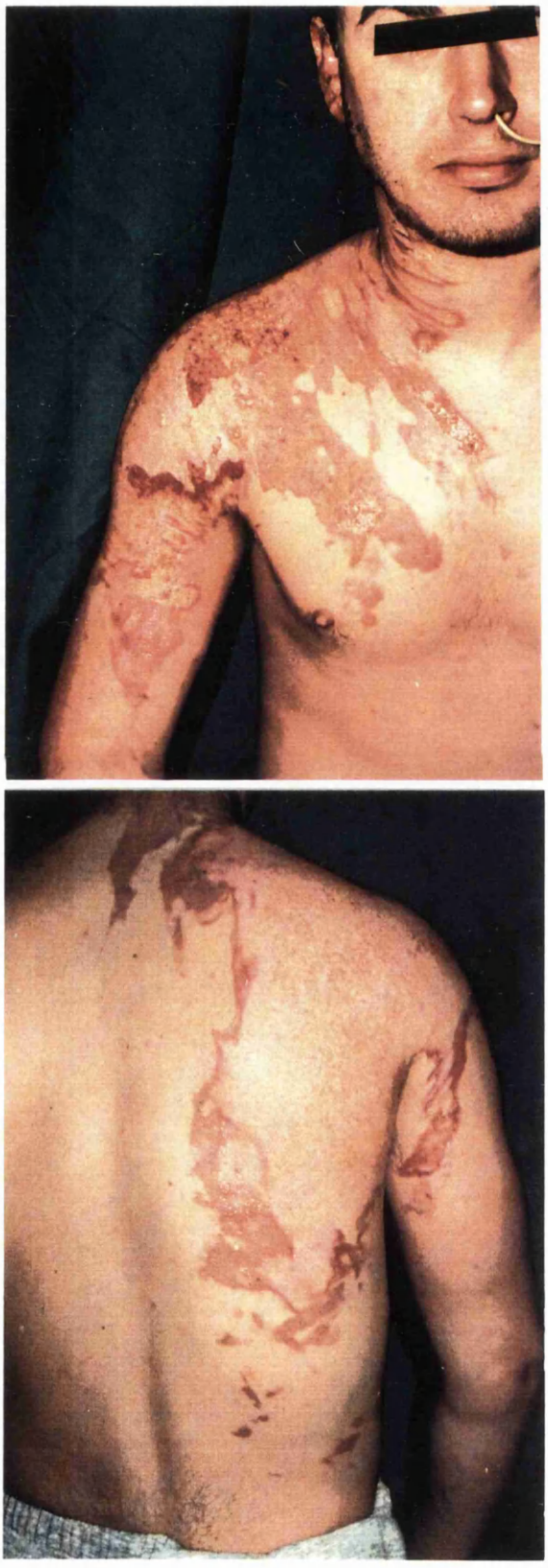


Figure A2.1c Photographs of patients soon after admission. C7.

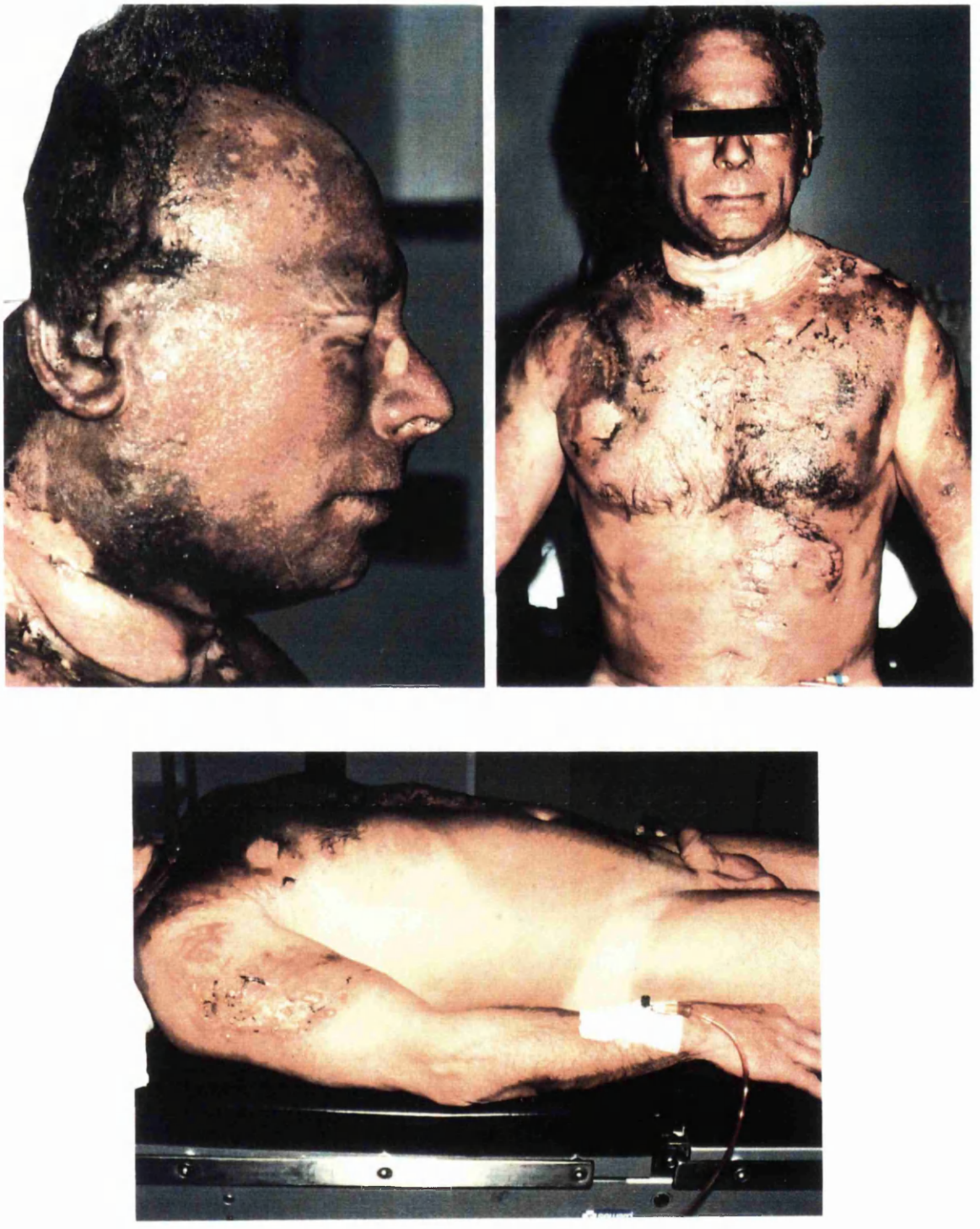


Figure A2.1d Photographs of patients soon after admission. C9.

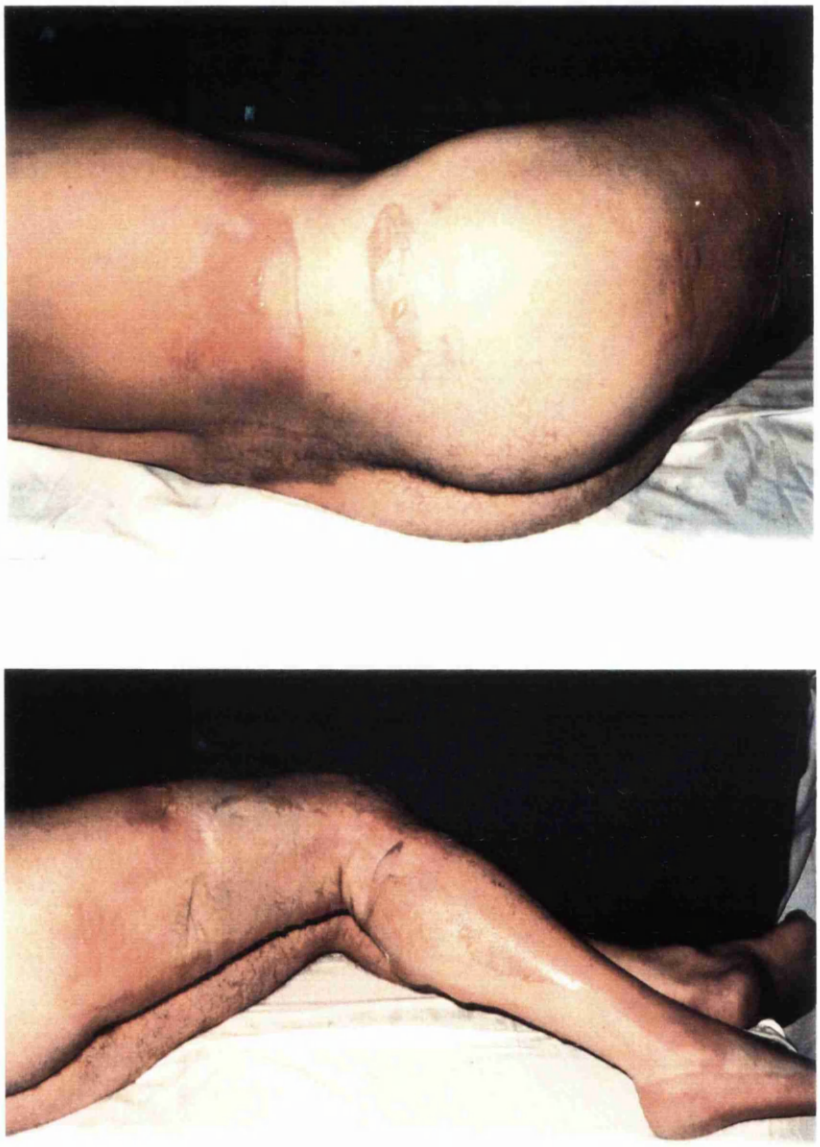


Figure A2.1e Photographs of patients soon after admission. C10.

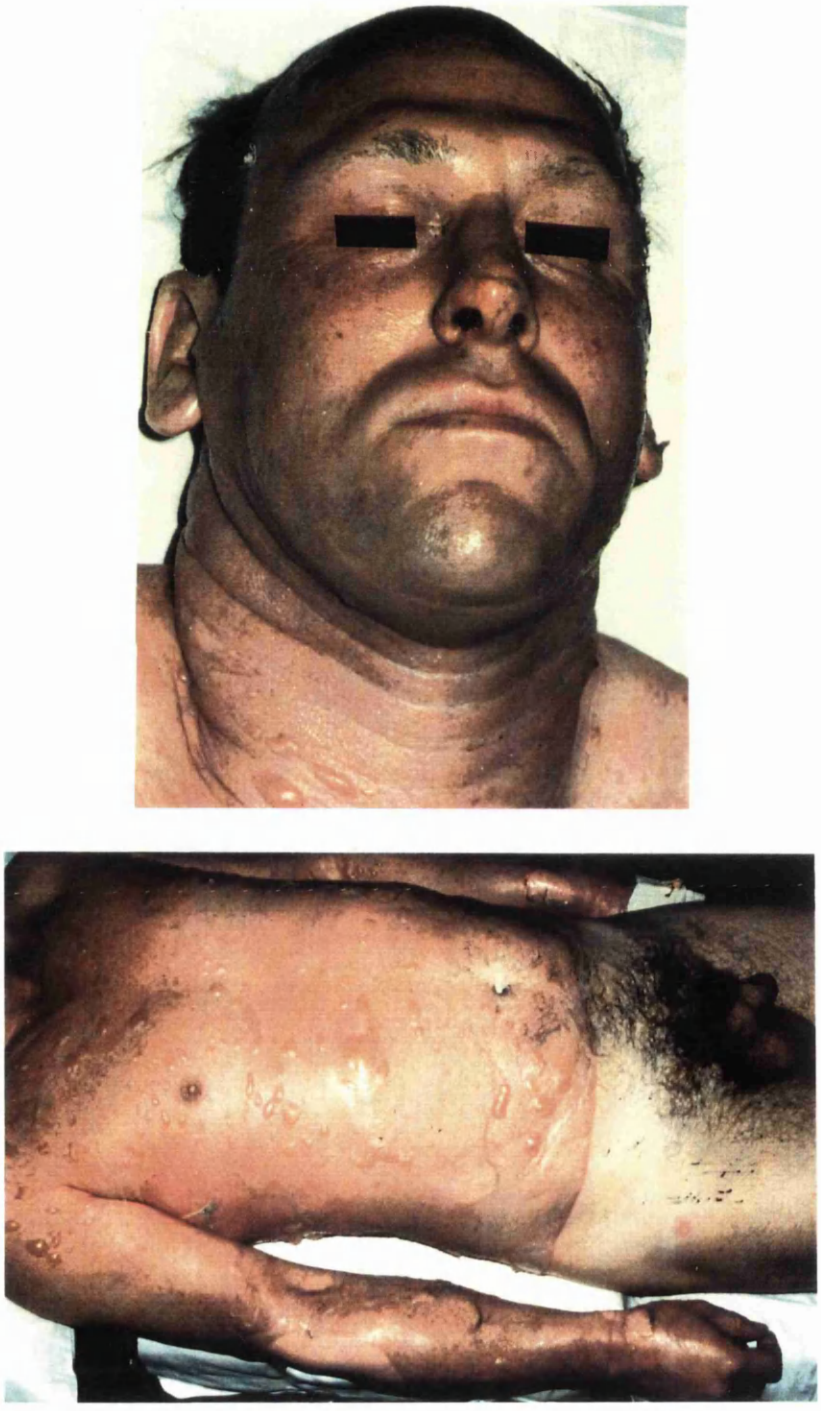


Figure A2.1f Photographs of patients soon after admission. C11.

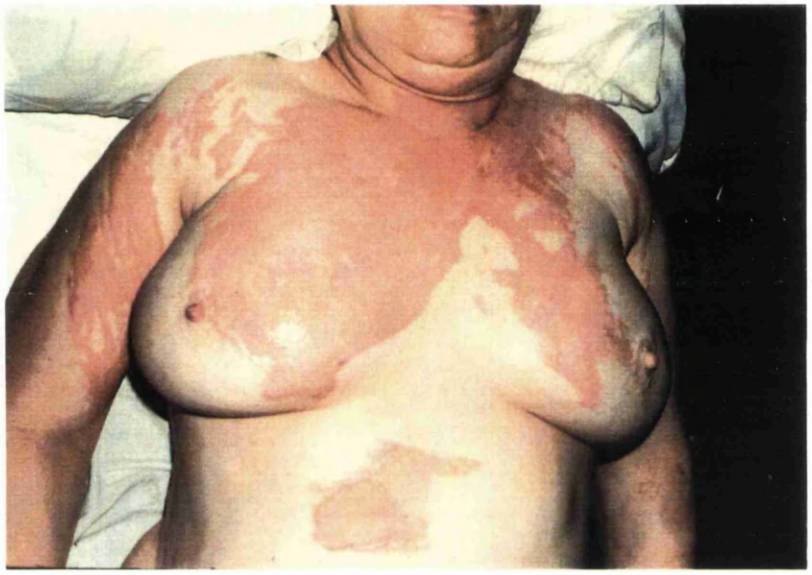


Figure A2.1g Photographs of patients soon after admission. GH1.

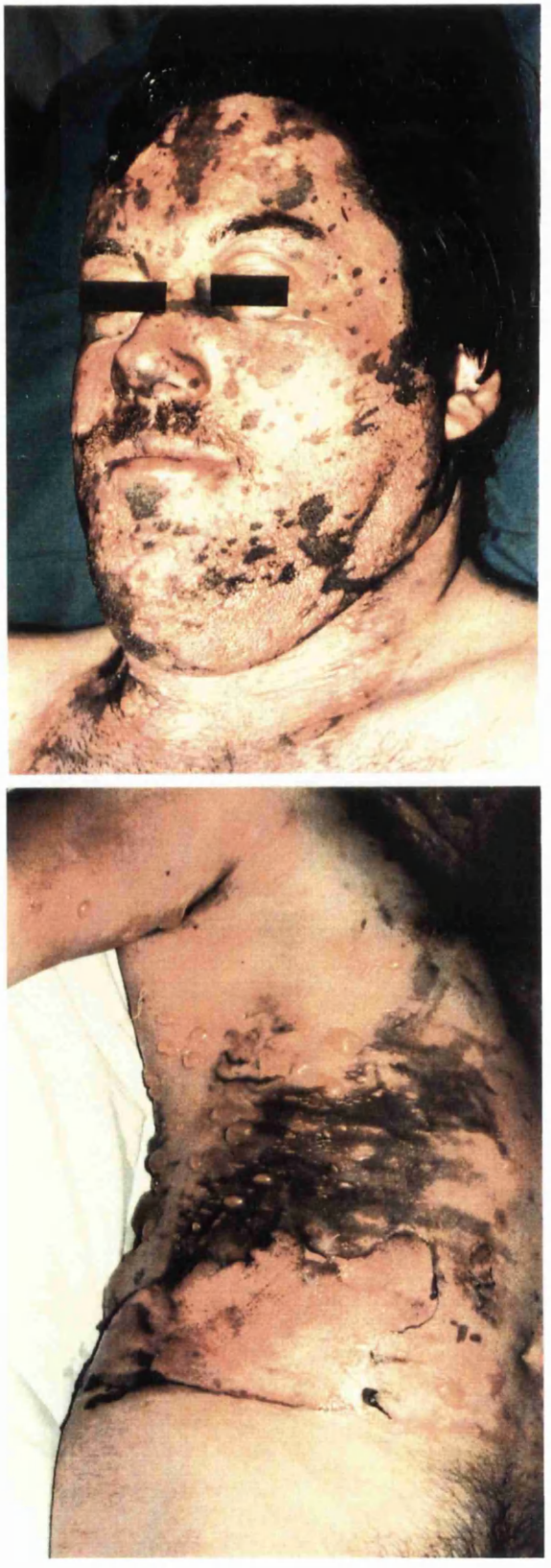


Figure A2.1h Photographs of patients soon after admission. GH3.

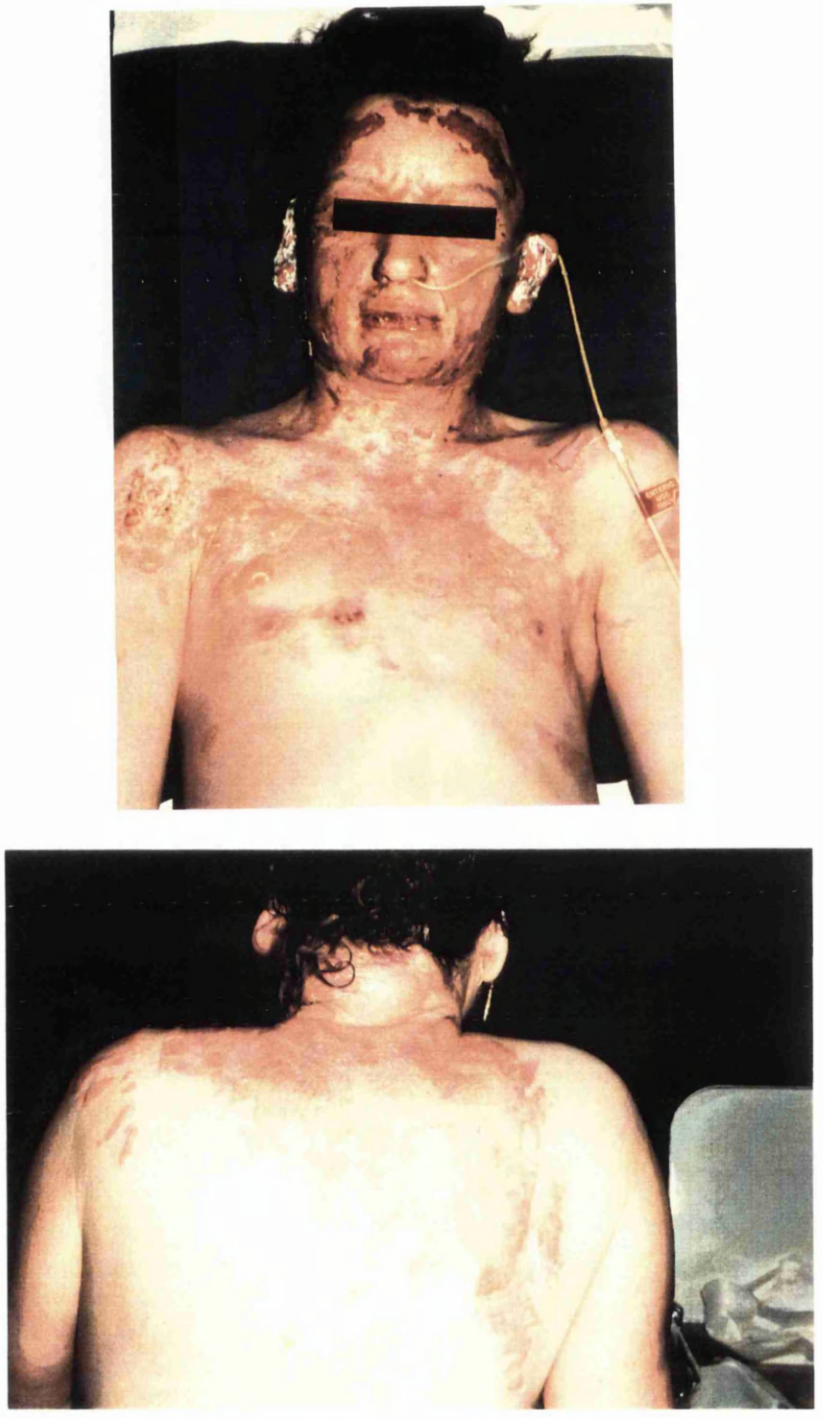


Figure A2.1i Photographs of patients soon after admission. GH5.

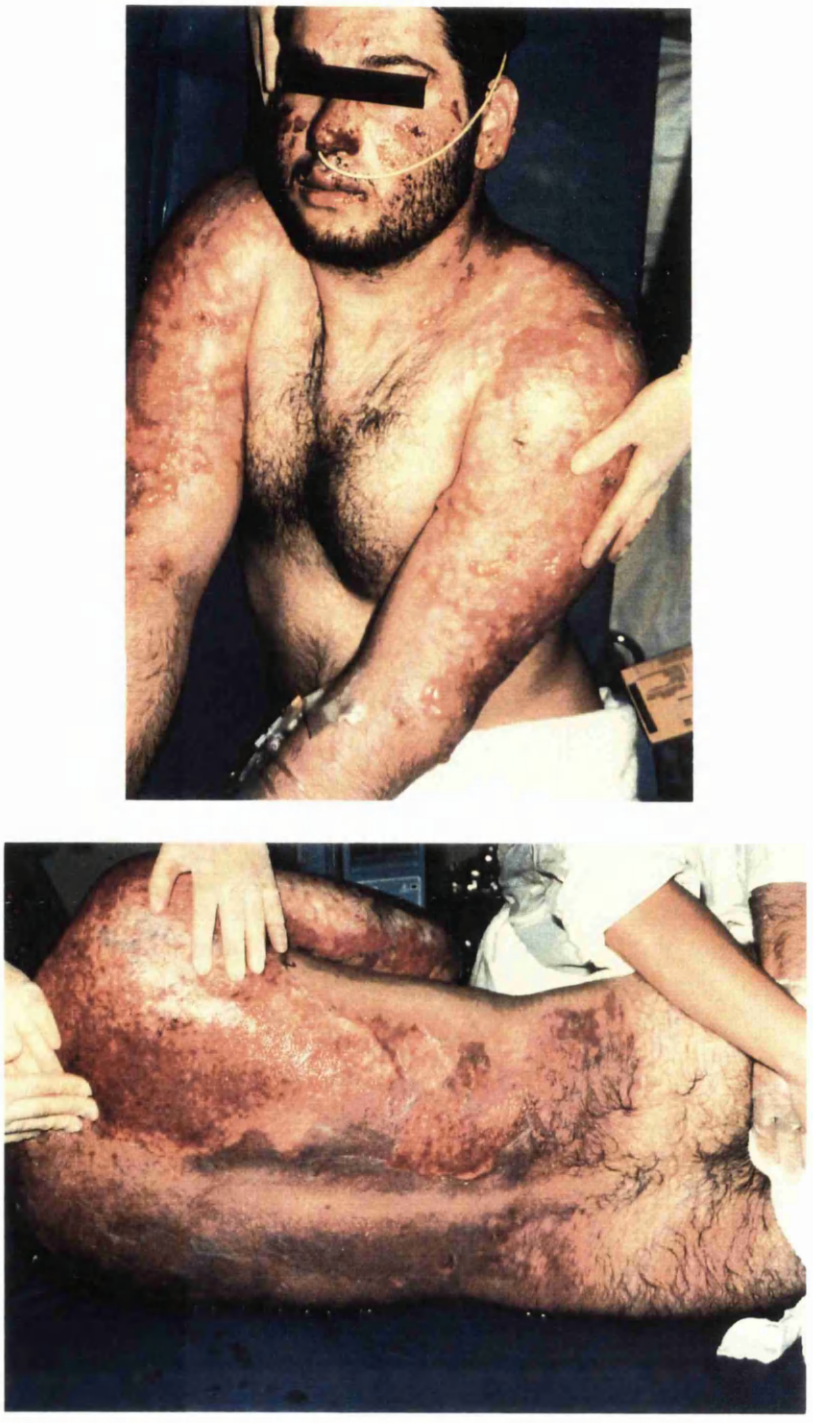


Figure A2.1j Photographs of patients soon after admission. GH6.

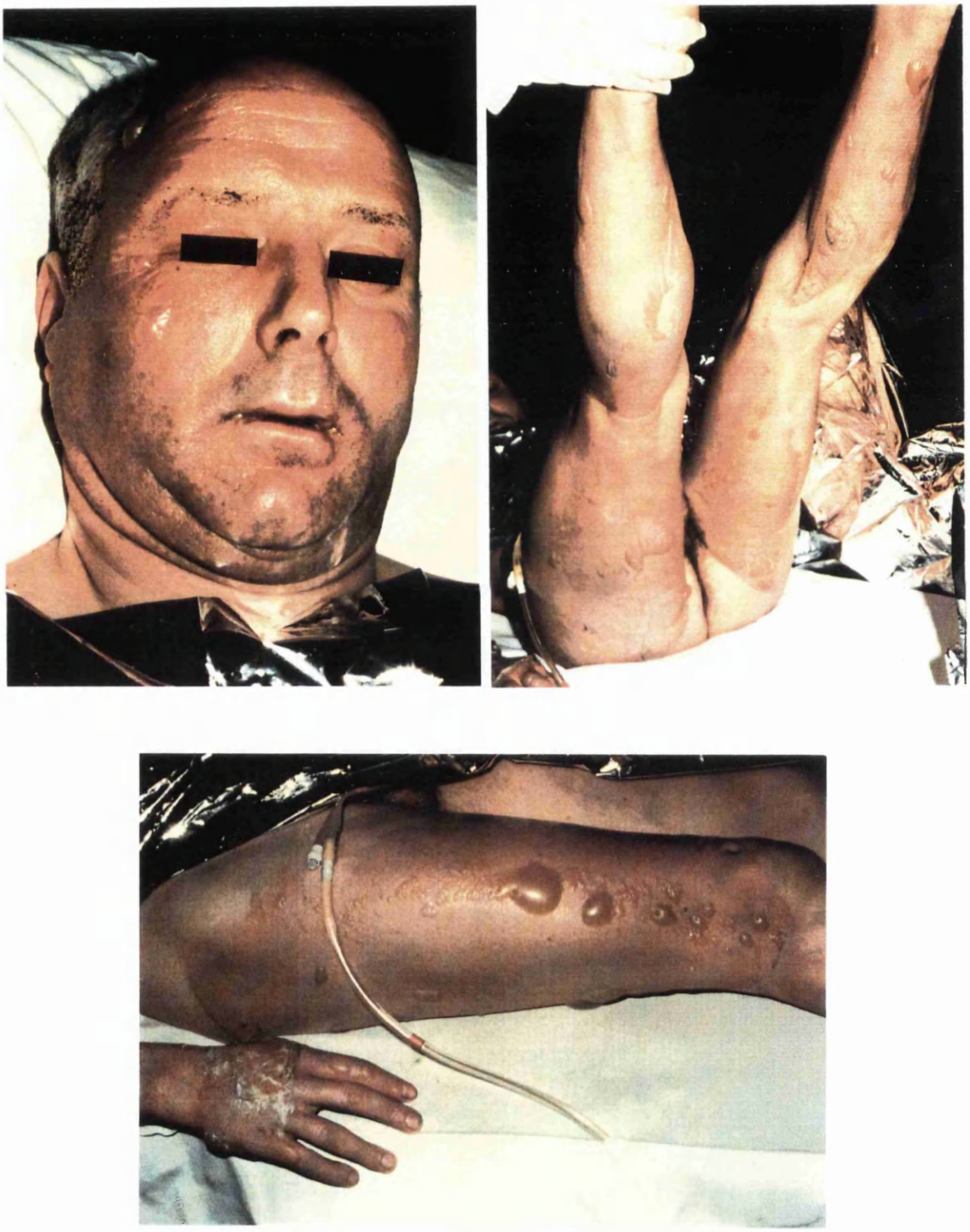
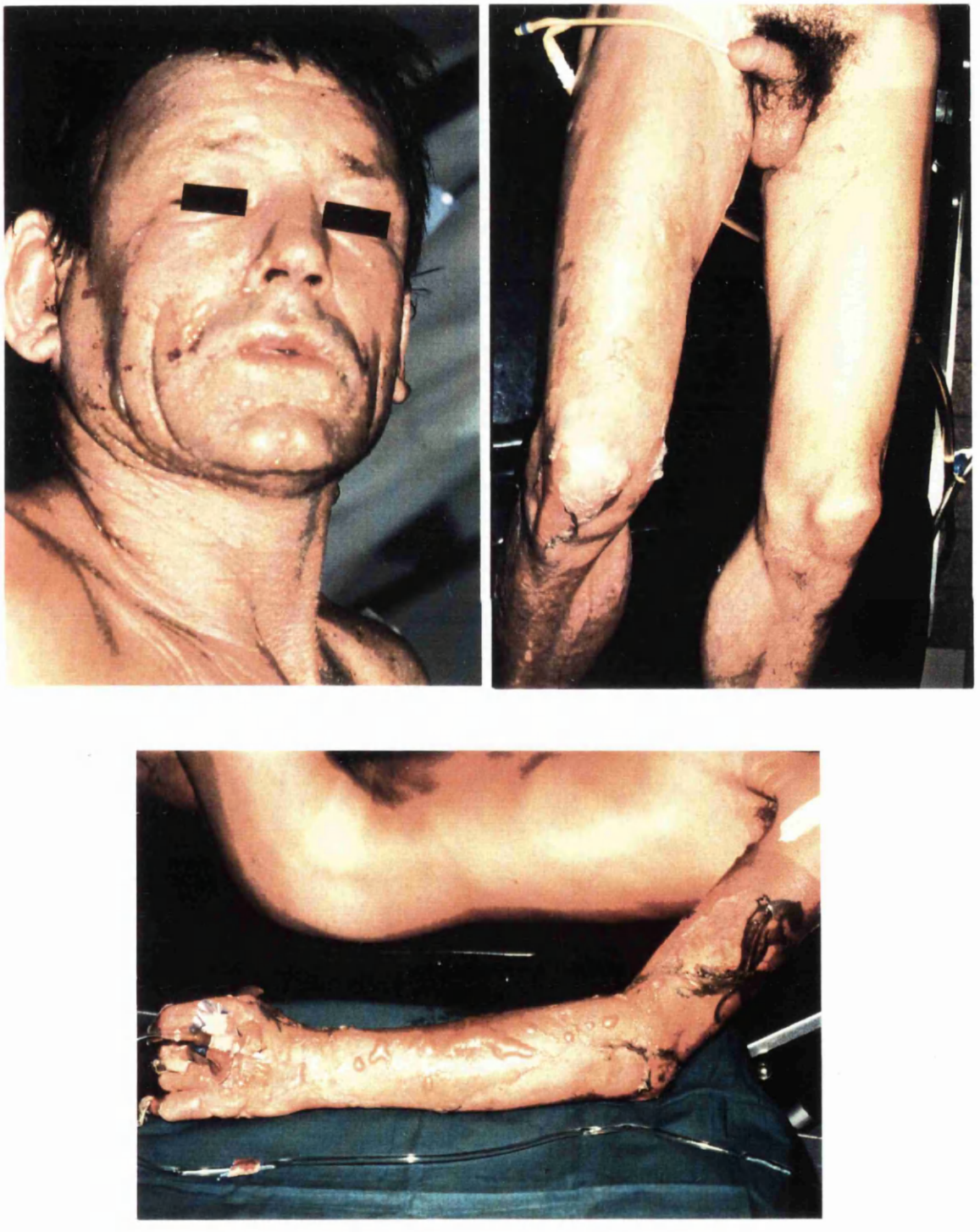


Figure A2.1k Photographs of patients soon after admission. GH7.



A3.1 Energy requirements

The energy requirements of each patient were calculated from their admission weight and TBSA(%) using the Curreri formula [Curreri et al. 1974]:

$$\text{Energy (kcal/day) (CEE)} = (25 \times \text{kg}) + (40 \times \text{TBSA})$$

It was intended that the CEE was to be administered enterally over 24 hours using Fortison Standard feeding solution.

A3.2 Enteral feed composition

	Pre-Fortison	Fortison Standard
Carbohydrate (g/l)	60.0	120.0
Lactose	<0.2	<0.25
Maltodextrin	58.0	116.0
Fat (g/l)*	20.0	40.0
Linoleic acid	8.75	17.5
Oleic acid	6.25	12.5
Palmitic acid	2.75	5.5
Lauric acid	0.75	1.5
Stearic acid	0.5	1.0
Linolenic acid	0.25	0.5
Protein (g/l)	20.0	40.0
Water (ml/l)	896	855
Osmolarity (mOsm/l)	130	260
Total energy (kcal/l)■	500	1000
NPE:N ratio (kcal/g)	131:1	131:1

* Polyunsaturated:saturated fat ratio = 0.8
■ 4186 J/l

Amino-acid group	Amino-acid	Composition*
Neutral unsubstituted	Alanine	2.9
	Glycine	1.8
	Isoleucine▪	5.0
	Leucine▪	8.8
	Valine▪	6.2
Neutral-hydroxyl	**Threonine**	4.3
	Serine	5.6
Neutral-sulphur	Cystine	0.3
	Methionine	2.6
Neutral-aromatic	**Phenylalanine**	4.9
	Tyrosine	5.4
	Tryptophan	0.8
Acidic	Aspartic acid	6.6
	Glutamic acid	20.4
Basic	**Arginine**	3.5
	Lysine	7.5
	Histidine	2.7
Imino-acid	Proline	10.7

* Percentage of all protein by weight
▪ Branch-chain amino-acids (20% of total protein)
Essential amino-acids in **bold**

Mineral (mmol/l)	Pre-Fortison	Fortison Standard
Sodium	17	35
Potassium	19	38
Calcium	6	12.5
Phosphorus	8	16
Magnesium	6	6
Iron	0.2	0.2
Zinc	0.1	0.1
Manganese	0.1	0.1
Copper	0.01	0.01

Vitamin (mg/l)	Content
Choline	450
Mesoinositol	230
Vitamin C	50
Vitamin E	32
Niacin	10
Pantothenic acid	5
Vitamin B_2	1
Vitamin B_6	1
Vitamin B_1	0.7
Vitamin A	0.7
Folic acid	0.25
Biotin	0.15
Vitamin D	0.005
Vitamin B_{12}	0.002

A3.3 Intravenous fluid contents

Fluid type	Water ml/l	Sodium mmol/l	Potassium mmol/l	Energy kcal/l	Nitrogen g/l
Plasma*	1000	145	0	180	7.2
Normal-saline	1000	150	0	0	0
Dextrose-saline	1000	30	0	160	0
5% Dextrose	1000	0	0	200	0

* These figures were used for all blood products.

A3.4 Calculation of daily dietary input

The enteral feed, oral and intravenous fluid volumes were recorded on standard fluid balance sheets by the nursing staff. All other dietary information was recorded daily on diet sheets. The latter were analysed by the Burns Centre dietician using a microcomputer and the Microdiet nutritional analysis program (Department of Mathematics and Computer Science, Salford University, Salford, Greater Manchester) which is based on published nutritional data [Paul and Southgate 1978].

The total inputs of water, sodium, potassium, energy and nitrogen were calculated each day by myself on a microcomputer from the known contents of the enteral feed solutions (see §A3.2), the known or assumed contents of intravenous fluids (see §A3.3) and the calculated dietary inputs.

APPENDIX 4. BALANCE CALCULATIONS

A4.1 Lee and Hartley correction

The use of this technique allows the estimation of the total urinary nitrogen losses from urinary urea excretion and includes a correction for variations in blood urea [Lee and Hartley 1975].

A4.2 Assumptions

1. Urea has a molecular weight (MW) of 60D (NH_2-C(=O)-NH_2) and contains two nitrogen atoms (MW=14D). Nitrogen therefore comprises 0.466 of its total molecular weight.

2. Urea comprises approximately 80% of total nitrogen excreted in the urine. This was corrected in the original publication by a factor of 1.2. This small error has not been corrected by subsequent authors [Hopkinson and Davis 1987] and has been retained in the present study.

3. Urea is distributed within the total body water which comprises 60% of body weight

4. Nitrogen comprises 0.16 of the total weight of protein (it is common practice to estimate the nitrogen content of protein by division by 6.25).

A4.3 Method

1. 24 hour urinary nitrogen excretion (UN) (g/day) calculation from urinary urea excretion (UU) (mmol/day):

$$UN = UU \times 0.001 \times 60 \times 0.466 \times 1.2$$

$$UN = UU \times 0.0336$$

2. Correction (C) (g) for blood urea change (BUC) (mmol/l):

$$C = BUC \times (0.001 \times 60 \times 0.6 \times 0.466) \times weight(kg)$$

$$C = BUC \times 0.0168 \times weight(kg)$$

NB. When blood urea results are not available for every day any changes that have occurred between sampling times have been averaged out (see §A4.4).

NB. This correction is positive for rises in the blood urea concentration.

3. Calculation of nitrogen losses due to proteinuria (PN) (g/day):

$$PN = \text{Urinary protein loss}/6.25$$

4. Calculation of corrected 24 hour nitrogen excretion (CUN) (g/day):

$$CUN = UN + C + PN$$

A4.4 Example

Patient #C10, study days 5-6:

Urea excretion (UU) = 980mmol/24hr

Proteinuria = 0g/day

Blood urea: day-4 = 4.2mmol/l, day-6 = 6.1mmol/l

Weight = 96kg

BSA = $2.23m^2$

1. $UN = 980 \times 0.0336$

   $= 32.93g/day$

2. $BUC = (6.1 - 4.2)/2$

   $= +0.95mmol/l/day$

3. $C = +0.95 \times 0.0168 \times 96$

   $= +1.53 g/day$

4. $CUN = 32.93 + 1.53$

   $= 34.46 g/day$

   $= 15.45 g/m^2/day$

A4.5 Predicted insensible water and sodium losses

1. The predicted total water loss of each patient in the present study has been calculated using the previously published formula of Harrison et al. [1964]:

   $$\text{Total water loss}(ml/m^2/hr) = 21.56 + (1.18 \times \text{TBSA}(\%))$$

2. The predicted sodium loss of each patient has been estimated as being $170 mmol/m^2$ burn/day. This approximate figure has been calculated from the data presented by Davies [1967] for losses in exudate in the first 14 days post-burn. It does not include the urinary losses in his study.

APPENDIX 5. CALORIMETRY

A5.1 Abbreviations, measurements, units and assumptions

Inspired volume (V_I) (l/min)

Expired volume (V_E) (l/min)

Expired O_2 fraction (F_EO_2)

Expired CO_2 fraction (F_ECO_2)

Oxygen consumption (VO_2) (l/min)

Carbon-dioxide production (VCO_2) (l/min)

Respiratory quotient (RQ)

Nitrogen metabolism (NM) (g/min) (calculated from nitrogen excretion)

Inspired O_2 fraction (F_IO_2) = 0.208

Inspired N_2 fraction (F_IN_2) = 0.792

Inspired CO_2 fraction = 0

Energy of carbohydrate oxidation = 4.1kcal/g

Energy of protein oxidation = 4.1kcal/g

Energy of fat oxidation = 9.3kcal/g

A5.2 Calculations

1. The inspired volume measured at ambient temperature and pressure (ATP) was corrected to standard temperature and pressure (STPD) (101kPa, 0^0C, Dry) using standard tables [Cotes 1979]. The corrected figure was used for all subsequent calculations. No correction was made for barometric pressure because this has been found to vary little in the Burns Centre which is a sealed and ventilated environment.

$$V_{I,STPD} = V_{I,ATP} \times \text{STPD conversion factor*}$$

Ambient Temp(^{0}C)	Conversion factor*
20	0.911
21	0.906
22	0.902
23	0.897
24	0.893
25	0.888
26	0.883
27	0.878
28	0.874
29	0.869
30	0.864

2. The expired volume V_E was calculated from the inspired volume and the measured F_EO_2 and F_ECO_2.

$$V_E = (V_I \times F_IN_2)/(1 - F_EO_2 + F_ECO_2)$$

3. Gas exchange was calculated using the standard equations [Westenskow et al. 1984].

$$VO_2 = V_E \times (F_IO_2 - F_EO_2 - (F_IO_2 \times F_ECO_2))/(1 - F_IO_2)$$

$$VCO_2 = V_E \times F_ECO_2$$

$$RQ = VCO_2/VO_2$$

4. The ventilation equivalent (VE) (l/100ml) is a measure of the inspired volume used in the consumption of 100ml of oxygen. High volumes indicate either leakage from the system or hyperventilation. This was calculated in the following manner.

$$VE = (V_I \times 100)/(VO_2 \times 1000)$$

5. The REE (kcal/min) and substrate oxidation rates (g/min) were calculated from the Bursztein formula [Bursztein et al. 1980] (C carbohydrate oxidation, F fat oxidation, P protein oxidation)

$$REE = (5.083 \times VO_2) - (0.138 \times VCO_2) - (0.128 \times NM)$$

$$C = (4.06 \times VCO_2) - (2.854 \times VO_2) + (0.095 \times NM)$$

$$F = (1.805 \times (VO_2 - VCO_2)) - (2.82 \times NM)$$

$$P = (6.25 \times NM)$$

NB. This section was incorrectly printed in Bursztein's paper as;

$$REE = (5.083 \times VO_2) + (0.138 \times VCO_2) - (0.128 \times NM)$$

I have received written confirmation from Professor Bursztein that the formula I have used is correct.

APPENDIX 6. GLUCOSE TOLERANCE TEST CALCULATIONS

1. Basal insulinogenic index (pmol/mmol);

   = Basal insulin/basal glucose

2. 0-5' delta-glucose (mmol/l) and 0-5' delta-insulin (pmol/l); describe the increments that occur in blood glucose and plasma insulin concentrations above their baseline values within five minutes of glucose administration [Lerner and Porte 1971]

   $= T_5$ glucose - T_0 glucose

   $= T_5$ insulin - T_0 insulin

3. 0-5' insulinogenic index (pmol/mmol) [Seltzer et al. 1967];

   = 0-5' delta-insulin/0-5' delta-glucose

4. The delta-glucose (mmol/l.min) and delta-insulin areas (pmol/l.min) describe the areas under the incremental glucose and insulin curves in the 60 minutes* after glucose administration [Seltzer et al. 1967];

   $= \int_{60}^{0}$(glucose - T_0 glucose)

   $= \int_{*}^{0}$(insulin - T_0 insulin)

   * The delta insulin area is calculated between 0 and 60 minutes or until the blood glucose has returned to its baseline value, whichever occurs first.

5. Total insulinogenic index (pmol/mmol) [Seltzer et al. 1967];

   = delta-insulin area/delta-glucose area

6. Glucose disappearance constant (k) [Marks and Marrack 1962];

   a) The blood glucose values are converted to their Log_{10} equivalents.

   b) The Log_{10} T_{20} and T_{60} values are plotted against time on a linear scale (semilog plot).

c) The half-life ($T_{\frac{1}{2}}$) of the decay in the blood glucose concentration from the T_{20} value is calculated from the slope of this line.

d) The k value is calculated by division of the natural log of 2 ($Log_e 2$) (69.31) by the $T_{\frac{1}{2}}$

$$k = 69.31/T_{\frac{1}{2}}$$

A7.1 Statistical abbreviations and symbols

d	Difference between two values	
df	Degrees of freedom	
H	Hypothesis	
k	Number of distributions	
n	The number of values	
ns	Not significant	
p	Probability that the null hypothesis (H_0) is correct	
R	Rank value	
$	Sum of	
SD	Standard deviation	$(v)^{\frac{1}{2}}$
SE	Standard error of a mean	$SD/(n)^{\frac{1}{2}}$
t	t-test statistic	
T	Signed ranks test statistic	
U	Mann-Whitney statistic	
v	Variance	$(\$(x-\tilde{x})^2)/(n-1)$
vr	Variance ratio	v_{max}/v_{min}
x	A value	
$\tilde{x}$	Mean	$(\$x)/n$
z	Statistic used for large samples in the Mann-Whitney test	

A7.2 Students t-test

This test is used to assess whether two groups of data are derived from the same or different populations. It is designed for the comparison of small samples. Its use is confined to the comparison of data which can be regarded as continuous or at least interval and which are drawn from normally or approximately normally distributed populations which have comparable variances (see §A7.6).

Method [Swinscow 1983]

1. Calculation of the difference between the two means

$$d = \tilde{x}_1 - \tilde{x}_2$$

2. Calculation of the variance of the difference between the two means:

$$v_d = (\$(x_1 - \tilde{x}_1)^2 + \$(x_2 - \tilde{x}_2)^2)/((n_1 - 1) + (n_2 - 1))$$

$$\text{NB. } \$(x - \tilde{x})^2 = \$x^2 - ((\$x)^2/n)$$

3. Calculation of the standard error of the difference between the two means:

$$SE_d = (v_d/n_1 + v_d/n_2)^{\frac{1}{2}}$$

4. Calculation of the t-value from the difference between means and the standard error of the two means:

$$t = d/SE_d$$

5. Calculation of the degrees of freedom:

$$df = (n_1 - 1) + (n_2 - 1)$$

6. The t-value is now used to test the null hypothesis (H_0) that the populations from which both groups are derived have the same mean as opposed to the alternative hypothesis (H_1) that they have different means.

The level of significance chosen to reject H_0 in the present studies was 5%. If the calculated probability of the test is equal or less than this value, H_0 is rejected in favour of the alternative H_1. It is implicit that the probability of mistakenly rejecting H_0 is therefore 5% (type-I error).

If the direction of difference between the two means is predicted, i.e., that one group will have a larger mean than the other, then the region of rejection of H_0 is one-tailed. Alternatively, if no prediction is made as to the direction of rejection, the region of rejection is two-tailed [Siegel and Castellan 1988].

The probability (p) of rejecting H_0 can be obtained from the calculated t-value by reference to the percentage points of the t-distribution for the calculated degrees of freedom (df) in standard tables [White et al. 1979].

Example

A comparison of the albumin concentrations (% of normal) between the CP and CS rats on the sixth post-operative day (table 10.12.).

CP: 115, 76, 115, 107, 86, 86, 67, 83.

CS: 107, 120, 120, 104, 115, 120, 67, 94.

Group	CP	CS
n	8	8
$\$x$	735	847
$\tilde{x}$	91.9	105.9
$\$x^2$	69845	92015
$(\$x)^2/n$	67528	89676
$\$(x - \tilde{x})^2$	2317	2339

$$d = 105.9 - 91.9 = 14$$

$$v_d = (2317 + 2339)/(7 + 7) = 333$$

$$SE_d = (333/8 + 333/8)^{\frac{1}{2}} = 9.12$$

$$t = 14/9.12 = 1.54$$

The two-tailed test is used

$$0.5 > p > 0.1$$

The difference between the two mean albumin concentrations is not significant at the 5% level

A7.3 Mann-Whitney U-test

This non-parametric test is an alternative to the parametric t-test and is preferred where there is evidence that the data is significantly non-normal or the variances are different (see §A7.6).

Method [Siegel and Castellan 1988]

1. The observations from two independent sets of data are combined and ranked in ascending order. For tied values the ranking allotted is the mean of those if the values were different. n_1 is the number of cases in the smaller of the two groups, n_2 the larger.

2. The U-value is calculated for both groups from the calculated rank sum values:

$$U_1 = (n_1 \times n_2) + (n_1(n_1 + 1)/2) - R_1$$

$$U_2 = (n_1 \times n_2) + (n_2(n_2 + 1)/2) - R_2$$

3. The probability (p) of rejecting H_0 is obtained from the smaller of the calculated U values using standard tables [White et al. 1979].

4. Where n_2 exceeds 20 these tables are not usable but because the distribution of U becomes effectively normally distributed the p-value can be derived from the normal distribution by calculation of the z-value and reference to standard tables.

$$z = (U - (n_1 \times n_2/2))/((n_1 \times n_2(n_1 + n_2 + 1)/12)^{\frac{1}{2}})$$

NB. The two-tailed p-value is obtained by doubling the one-tailed value.

Example

A comparison of the basal insulin concentrations (pmol/l) between control and somatropin-treated patients on day-1 (table 8.31).

Control	Rank	**Somatropin**	Rank
804	12	79	8
60	5.5	102	10
89	9	78	7
53	3	56	4
108	11	60	5.5
48	2	23	1
$\tilde{x}$=194	R_1=42.5	$\tilde{x}$=66	R_2=35.5

$$U = (6 \times 6) + (6(6 + 1)/2) - R$$

$$U = 57 - R$$

$$U_1 = 14.5 \quad U_2 = 21.5$$

The two-tailed test is used

$$p > 0.5$$

The difference between the two mean insulin concentrations is not significant at the 5% level

A7.4 Paired-t test

This test allows the comparison of paired data and is similar to the one-sample t-test in method. It is applicable to situations where the data from two populations are in some way inter-dependent, such as when subjects in two experimental groups have been paired together or when comparing data from a group of subjects at two different times.

Method [Swinscow 1983]

1. The results from the two populations are set out in pairs and the difference between observations (d) calculated.

2. Calculation of the mean of the differences from the number of differences (n) and their sum:

$$\tilde{d} = (\$d)/n$$

3. The variance and the standard error of the mean of differences are calculated:

$$v_d = (\$(d - \tilde{d})^2)/(n-1)$$

$$SE_d = (v_d/n)^{\frac{1}{2}}$$

4. Calculation of the t-value

$$t = \tilde{d}/SE_d$$

5. The p-value is now obtained as before from the t-distribution from standard tables [White et al. 1979].

A7.5 Wilcoxon's signed-ranks test

This test is the non-parametric equivalent of the paired t-test.

Method [Swinscow 1983]

1. The results from the two populations are set out in pairs and the difference between observations (d) calculated.
2. The differences are ranked in ascending order irrespective of arithmetic sign. A difference of zero is ignored and consequently this pair is discarded from the analysis. The sample size is therefore reduced by the number of pairs with zero differences.
3. The sign is attached to the ranking and the T-value is calculated by the sums of the "+" and "-" ranks separately.
4. The p-value is obtained from standard tables [White et al. 1979] using the smaller of the T-values and the number of pairs compared (n).
5. As with the Mann-Whitney U-test (see A7.3), these tables are not usable for large samples but the p-value can be derived from the normal distribution by calculation of the z-value.

$$z = (T^{+} - n(n + 1)/4)/(n(n + 1)(2n + 1)/24)^{\frac{1}{2}}$$

Example (Paired-t and Signed-ranks tests)

Comparison of the weight-change (g/5day) in CP compared with CS rats

(table 9.1.)

CP	CS	Differences	Ranks	Signed-ranks
7	6	-1	1.5	-1.5
-2	6	+8	6	+6
-5	-15	-10	7	-7
-7	4	+11	8	+8
4	1	-3	4	-4
10	8	-2	3	-3
0	7	+7	5	+5
-6	9	+15	9	+9
-3	14	+17	10	+10
9	10	+1	1.5	+1.5
$\tilde{x}$=0.7	$\tilde{x}$=5.0	$\tilde{d}$=4.3		T^+=39.5, T^-=15.5

Paired t-test

$$v_d = 678.1/9 = 75.3$$

$$SE_d = (75.3/10)^{\frac{1}{2}} = 2.74$$

$$t = 4.3/2.74 = 1.57$$

The two-tailed test is used

$$0.5>p>0.1$$

The difference between the two mean weight-changes is not significant at the 5% level

Signed-ranks test

$$n = 10$$

Smaller sum of ranks (T^-) = 15.5

The two-tailed test is used

$$p>0.1$$

The difference between the two mean weight-changes is not significant at the 5% level

A7.6 Heterogeneity of variances

Calculation of the variance ratio is convenient for assessing if two or more batches of independent data have variances which are similar enough to satisfy the requirements of parametric tests such as the Student's t-test.

Method and example [Pearson and Hartley 1976]

The mean urinary nitrogen excretion of groups CP, CS, BP and BS are set out in the table below (table 9.8.)

	CP	CS	BP	BS
n	10	10	12	12
g/day	0.371±0.059	0.408±0.038	0.462±0.091	0.474±0.104
g/kg/day	0.928±0.130	1.024±0.120	1.209±0.212	1.228±0.186

($\tilde{x}$±SD)

1. Find the maximum and minimum variances from each of the two sets of data each having four variances (k=4):

g/day	$v_{max} = (0.104)^2$	$v_{min} = (0.038)^2$
g/kg/day	$v_{max} = (0.212)^2$	$v_{min} = (0.120)^2$

2. Calculate the variance ratios (vr) for each of the sets:

g/day	$v_{max}/v_{min} = 7.5$
g/kg/day	$v_{max}/v_{min} = 3.1$

3. The p-value is now obtained from a table giving the percentage points of the v_{max}/v_{min} ratio for appropriate degrees of freedom and k value (Table 31, Biometrika Tables for Statisticians) [Pearson and Hartley

1976]. Where the degrees of freedom differ within sets, their average can be used, which in this case is 11.

g/day	vr = 7.5	$p<0.05$
g/kg/day	vr = 3.1	ns

NB. For the comparison of the variances of two samples (k=2) the p-value (two-tailed) can be obtained from the F-distribution.

4. This test indicates that there is a significant difference between the variances for the g/day but not the g/kg/day data. These results indicate that it would be more appropriate to use a non-parametric test to compare the g/day data.

A7.7 Notes

1. All the results in this thesis are presented as mean±SE unless otherwise stated.

2. Where there is a discrepancy between the conclusions from parametric and non-parametric tests, the rejected result has been indicated in the tables by brackets.

   For example, in table 9.8 the t-value obtained by comparison of the CS group with the BS (g/day) is 1.9 ($0.05<p<0.1$) and the U-value is 26.5 ($p<0.05$). The t-test result has been rejected and bracketed in favour of the Mann-Whitney result because the variances of the four means were significantly different.

A8.1 Contents of 41B maintenance feed

Contents by weight	
Crude Protein*	16.20%
Lysine	0.66%
Methionine	0.28%
Cystine	0.22%
Threonine	0.51%
Tryptophan	0.18%
Arginine	1.00%
Ash	9.1%
Crude Fibre	6.4%
Crude Oil	2.8%
Starches	36.1%
Sugars	3.0
Essential Fatty Acids	1.4%
Dietary Fibre	22.0%
Hemicellulose	12.1%
Cellulose	7.6%
Lignin	1.5%
Minerals	5.4%
Digestible Energy▪	2927.0kcal/kg
Digestible Protein¤	13.3%

* This value has been used for calculating the total nitrogen inputs (=2.59% nitrogen)
▪ 12246kJ/kg
¤ Protein energy = 545kcal/kg (18.6%)
Non-protein energy:nitrogen ratio = 113:1

Minerals	mmol/kg
Calcium	305
Phosphorus	300
Sodium	200
Potassium	197
Magnesium	113
Iron	0.9
Manganese	0.9
Zinc	0.2
Copper	0.08
Iodine	0.004

Vitamins	
Choline*	1000
Vitamin E*	70
Pantothenic acid*	15
Nicotinic acid*	12
Vitamin K*	10
Folic acid*	2
Vitamin B1*	5
B2*	15
B6*	7.5
B12*	0.0075
Biotin*	0.2
Vitamin A▪	5000
Vitamin D▪	2000

* mg/kg
▪ iu/kg

A8.2 A study of the normal food intake of rats

A preliminary study was performed to discover the normal daily consumption of 41B feed by rats. Ten, eleven week old, male Sprague-Dawley rats which had not been shaved were individually caged and allowed free access to 41B feed. Following a two-day acclimatisation period, their food consumption and weight were measured on alternate days for six days.

Rat	1	2	3	4	5	6	7	8	9	10
Day(s)					Weight (g)					
0	316	434	431	393	420	410	480	456	341	334
2	321	442	430	394	431	418	488	465	362	344
4	341	450	431	407	446	428	493	462	365	351
6	345	454	428	404	441	429	504	460	361	355
					Food consumption (g/2day)					
0-2	55	67	59	54	70	65	84	78	74	66
2-4	71	72	57	63	79	75	85	72	70	72
4-6	74	80	64	69	84	56	70	70	61	57
Mean	66.7	73.0	60.0	62.0	77.7	65.3	79.7	73.3	68.3	65.0
					Food consumption (g/kg/day)					
0-2	87.0	77.2	68.5	68.7	83.3	79.3	87.5	85.5	108.5	98.8
2-4	110.6	81.5	66.3	80.0	91.7	89.7	87.1	77.4	96.7	104.7
4-6	108.5	88.9	74.3	84.8	94.2	65.4	71.0	75.8	83.6	81.2
Mean	102.0	82.5	69.7	77.8	89.7	78.1	81.9	79.6	96.3	94.9

The mean food consumption for all rats over the six-day period was 69.1±2.1g/2days which was equivalent to 85.3±3.2g/kg/day. It was therefore decided to provide the rats in subsequent studies with 85g/kg/day of 41B feed (249kcal/kg/day of digestible energy and 1.81g/kg/day of digestible nitrogen) (see §9.2.11).

APPENDIX 9. FLUID RESUSCITATION AND WOUND-HEALING

A9.1 Introduction

An additional study was designed to evaluate the role of intraperitoneal fluid resuscitation on wound-healing.

A9.2 Material and methods

Twenty-six, 12 week old, male Sprague-Dawley rats (mean weight 436.4±5.4g) were submitted to a midline laparotomy (see 10.2.3) and then allocated to one of three treatment groups.

1. Control (C) rats (n=8); which were allowed to recover immediately

2. Burned rats given fluid resuscitation (BR) (Mean TBSA=18.3±0.3%) (n=10).

3. Control rats given fluid resuscitation (CR) (n=8); these rats were given an identical amount of fluid intraperitoneally as group BR (60ml/kg) before being allowed to recover.

The general management of all the rats was as previously described in §9.2. After 6 days they were sacrificed and their wounds submitted to tensiometry (see §10.2.6).

A9.3 Results

One rat in the burned group died one day after injury. The musculofascial wounds of both groups that had received fluid resuscitation (CR and BR) were significantly weaker than those that had not (C). There was no significant difference in wound-strength between the two groups that received fluid resuscitation (CR and BR).

APPENDIX 9. FLUID RESUSCITATION AND WOUND-HEALING

There was no significant difference in the strength of skin wounds between the two control groups (C and CR). Those of the burned rats were weaker than those of group-CR but not those of group-C.

Peak wound force (N/cm)

Muscle	C	BR	CR
	8.25	2.45	4.35
	6.25	3.35	3.75
	6.95	3.3	5.4
	6.0	2.95	3.95
	6.45	6.3	5.6
	7.2	3.6	5.05
	8.95	7.0	5.15
	5.7	3.1	3.65
		4.05	
		-	
Mean	7.0±0.4	4.0±0.5	4.6±0.3

C vs. BR: t=4.41, (U=8)*, p<0.001
C vs. CR: t=4.85, U=0, p<0.001
BR vs. CR: t=0.98, U=19, ns
* Variances equivalent (vr=4.04, ns)

Skin	C	BR	CR
	2.2	2.25	2.05
	1.9	1.25	2.55
	4.9	1.25	2.5
	1.8	1.2	2.3
	2.35	2.55	2.15
	2.25	1.65	2.25
	2.45	2.2	2.6
	1.35	1.25	2.65
		1.3	
		-	
Mean	2.4±0.4	1.7±0.2	2.4±0.1

C vs. BR: t=1.85, U=16, ns
C vs. CR: t=0.05, U=20.5, ns
BR vs. CR: (t=3.57)*, U=10, P<0.05
* Variances not equivalent (vr=22.9, p<0.01)

A9.4 Conclusions

This additional study shows that the use of the intraperitoneal route for fluid resuscitation contributes to the reduction in the strength of musculofascial wounds observed in burned rats (see §10.3.4). There is no evidence, however, that it contributes to the reduction observed in the strength of their cutaneous wounds.

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APPENDIX 11. ADDITIONAL INFORMATION

Preparation of this thesis

The text of the present thesis was prepared using the Locoscript-2 word-processing program (Locomotive Software Ltd., Dorking, Surrey) on an Amstrad PCW8256 microcomputer (Amstrad Consumer Electronics plc., Brentwood, Essex) and printed out on a Brother HL-8e laser-printer (Brother Office Equipment Division, Manchester, Greater Manchester). The graphics were prepared using CricketGraph, MacDraw and SuperPaint programs (P&P Principle Distribution Ltd., Haslingdon, Lancashire) on an Apple MacIntosh SE microcomputer and printed out on a Laserwriter laser-printer (Apple Computer (UK) Ltd., Uxbridge, Middlesex).

Published work arising from this thesis*

1. Belcher HJCR, Mercer D, Judkins KC, Shalaby S, Wise S, Marks V, Tanner NSB. 1989 Biosynthetic human growth hormone in burned patients: a pilot study. Burns 15: 99-107.

2. Belcher HJCR, Ellis H. 1990 An investigation of the anabolic activity of somatropin in normal and burned rats. Burns 16: 17-20.

3. Belcher HJCR, Ellis H. Somatropin and wound-healing after injury. J Clin Endocrinol Metab: in press.

* Reprints of published papers have been placed in the back-cover of this thesis

TABLES

Table 8.1 Demography of the two groups studied.

Patient	Sex	Age(yr)	Weight(kg)	Height(cm)	TBSA(%)*	PM(%)¤
C4	M	19	82	191	31	10
C6	M	28	81	183	12	0
C7	M	51	80	173	16	10
C9	M	37	77	175	18	10
C10	M	40	96	187	42	50
C11	F	54	71	164	15	10
	5:1[a]	38.2[b] ±5.4	81.2[b] ±3.4	178.8[b] ±4.1	22.3[b] ±4.8	10[c]
GH1	M	29	80	168	17	0
GH2	F	44	61	170	15	10
GH3	M	16	49	173	17	0
GH5	M	21	80	180	25	10
GH6	M	40	77	173	14	10
GH7	M	44	73	183	32	30
	5:1[a]	32.3[b] ±4.9	70.0[b] ±5.1	174.5[b] ±2.4	20.0[b] ±2.9	10[c]
t	-	0.79	1.83	0.92	0.42	-
U	-	14.5	7.5	11.5	17.5	15
p	ns	ns	ns	ns	ns	ns

* Total burn surface area (Lund and Browder 1944)
¤ Predicted mortality (Zawacki et al. 1979)
[a] Male:female ratio
[b] Mean
[c] Median
See table 8.24 for the BSA

Table 8.2 Haemoglobin concentration (g/dl) (Normal ranges; male 13.5 - 18.0, female 11.5 - 16.5).

Patient	Day-1	Day-3	Day-6	Day-11	Day-15
C4	15.5	8.2	10.9	10.1	10.1
C6	15.9	13.3	14.9	14.4	13.5
C7	-	11.8	13.2	13.0	13.1
C9	15.8	15.1	12.8	12.7	13.5
C10	15.2	-	10.8	12.1	11.0
C11	11.2	10.0	9.2	-	11.0
Mean	14.7 ±0.9	11.7 ±1.2	12.0 ±0.8	12.5 ±0.7	12.0 ±0.6
GH1	14.2	13.8	13.7	12.4	13.4
GH2	13.9	9.1	11.7▪	11.8¤	10.1
GH3	12.3	11.6	11.4*	10.0	10.6
GH5	14.3	13.2	10.4	11.5	11.6
GH6	16.7	13.7	11.7	11.5¤	11.0
GH7	11.8	11.1	10.4	10.2	10.0
Mean	13.9 ±0.7	12.1 ±0.8	11.6 ±0.5	11.2 ±0.4	11.1 ±0.5
t	0.76	0.29	0.43	1.61	1.14
U	10	14	16	6	10.5
p	ns	ns	ns	ns	ns

* Day-5
▪ Day-7
¤ Day-10

Table 8.3 Total white cell count (cells/ul) (Normal range 4.0 - 11.0).

Patient	Day-1	Day-3	Day-6	Day-11	Day-15
C4	16.6	7.7	13.6	20.7	6.9
C6	11.9	10.2	14.6	15.4	12.9
C7	-	12.9	12.5	14.7	13.1
C9	10.9	11.4	12.0	11.4	11.1
C10	9.4	-	18.5	21.1	18.5
C11	10.2	8.4	8.0	-	8.6
Mean	11.8 ±1.3	10.1 ±1.0	13.2 ±1.4	16.7 ±1.9	11.9 ±1.7
GH1	9.9	10.6	10.6	10.3	12.8
GH2	13.0	4.5	16.6▪	18.9¤	18.0
GH3	11.6	11.4	8.5*	13.0	10.4
GH5	14.0	8.7	7.2	14.7¤	13.6
GH6	11.4	9.2	10.4	14.4	11.5
GH7	9.4	7.0	20.6	16.7	11.8
Mean	11.6 ±0.7	8.6 ±1.0	12.3 ±2.1	14.7 ±1.2	13.0 ±1.1
t	0.18	1.09	0.35	0.93	0.59
U	14.5	10.5	14	9.5	15
p	ns	ns	ns	ns	ns

* Day 5
▪ Day-7
¤ Day 10

Table 8.4 Gas exchange.

Patient	VO_2[a]	VCO_2[b]	RQ[c]	VE[d]	NM[e]
C4	0.190	0.190	1.00	2.8	15.5
C6	0.174	0.165	0.95	2.7	8.4
C7	0.158	0.142	0.90	2.9	7.8
C9	0.157	0.147	0.94	2.8	10.1
C10	0.194	0.162	0.83	2.7	9.2
C11	0.132	0.128	0.98	2.9	8.5
Mean	0.168 ±0.01	0.156 ±0.009	0.93 ±0.03	2.8 ±0.04	9.9 ±1.2
GH1	0.175	0.155	0.89	2.6	8.1
GH2	0.161	0.179	1.1	3.2	9.2
GH3	0.155	0.152	0.98	3.0	12.5
GH5	0.184	0.176	0.96	3.4	4.5
GH6	0.198	0.180	0.91	2.8	8.4
GH7	0.194	0.169	0.87	2.7	3.9
Mean	0.178 ±0.007	0.169 ±0.012	0.95 ±0.03	2.95 ±0.13	7.8 ±1.3
t	0.87	1.27	0.43	1.14	1.23
U	12.5	10	17.5	14	11
p	ns	ns	ns	ns	ns

[a] O_2 consumption ($l/m^2/min$)
[b] CO_2 production ($l/m^2/min$)
[c] Respiratory quotient
[d] Ventilation equivalent ($l/100ml\ VO_2$)
[e] Nitrogen excretion on day(s) of gas exchange measurement ($g/m^2/day$)

Table 8.5 Resting energy expenditure and substrate oxidation rates.

Patient	REE*	Carbohydrate¤	Fat¤	Protein¤
C4	1351	331.4	-43.7	96.9
C6	1236	251.8	-1.4	52.8
C7	1124	179.9	20.0	49.0
C9	1119	214.7	-2.2	63.1
C10	1384	153.1	55.8	57.8
C11	942	203.7	-11.9	52.9
Mean	1193 ±68	222.4 ±25.6	2.8 ±13.6	62.1 ±7.2
GH1	1247	187.8	28.8	50.8
GH2	1142	388.1	-73.9	57.8
GH3	1100	253.9	-28.2	78.1
GH5	1309	276.2	6.5	28.1
GH6	1409	241.1	22.2	52.2
GH7	1382	193.8	52.5	24.1
Mean	1265 ±51	256.8 ±29.8	1.3 ±18.6	48.5 ±8.2
t	0.85	0.87	0.06	1.24
U	13	12	16	11
p	ns	ns	ns	ns

* Resting energy expenditure (kcal/m^2/day)
¤ Substrate oxidation (g/m^2/day)

Table 8.6 Energy inter-relationships.

Patient	BEE[a]	REE[b]	CEE[c]	Intake[d]	REE/BEE[e]	CEE/REE[e]	In/REE[e]
C4	2024	2851	3290	3878	141	115	136
C6	1905	2510	2505	4442	132	100	177
C7	1685	2183	2640	4423	130	121	203
C9	1751	2160	2645	3457	123	122	160
C10	2050	3087	4080	4688	151	132	152
C11	1384	1667	2375	3030	120	142	182
Mean	1800 ±102	2410 ±211	2923 ±265	3986 ±264	132.8 ±4.7	122.0 ±5.9	168.3 ±9.7
GH1	1815	2369	2680	4269	131	113	180
GH2	1349	1952	2125	3357	145	109	172
GH3	1495	1727	1905	3429	116	110	199
GH5	1926	2617	3000	2230	136	115	85
GH6	1718	2693	2485	3875	157	92	144
GH7	1680	2681	3105	3804	160	116	142
Mean	1664 ±86	2340 ±168	2550 ±194	3494 ±287	140.8 ±6.8	109.2 ±3.6	153.7 ±16.4
t	1.02	0.26	1.13	1.26	0.97	1.86	0.77
U	11	17	13	9	12	6.5	14
p	ns	ns	ns	ns	ns	ns	ns

[a] Basal energy expenditure (kcal/day) (Harris-Benedict formula)
[b] Resting energy expenditure (kcal/day) (Bursztein formula)
[c] Calculated energy expenditure (kcal/day) (Curreri formula)
[d] Mean energy intake for day-6 to day-10 (kcal/day)
[e] Percent

Table 8.7a Energy intake (kcal/m^2/day). Control patients.

Patient	C4	C6	C7	C9	C10	C11	Mean
Day 1	1975	1741	1972	2035	146	1544	1569
2	127	2873	2128	1945	1006	1569	1608
3	1048	2591	2754	2445	666	1634	1856
4	1444	1762	2637	1764	1253	662	1587
5	2001	1925	1955	1698	1333	1729	1774
Mean	1319	2178	2289	1977	881	1428	**1679±227**
Day 6	1499	1832	2041	1503	2313	1495	1780
7	753	1964	2315	1567	1795	1395	1632
8	1822	2211	2358	1897	2186	2092	2094
9	2024	2832	2401	1808	2009	1762	2139
10	3091	2099	2287	2181	2205	1815	2279
Mean	1838	2188	2280	1791	2102	1712	**1984±96**
Day 11	2363	2024	2521	1554	2221	2255	2156
12	2655	1990	2531	1846	2536	2288	2308
13	2612	2161	2088	1740	2576	2391	2261
14	2208	1940	2230	1795	2875	2288	2223
15	1998	1729	2399	1981	2407	2206	2120
Mean	2367	1969	2354	1783	2523	2286	**2214±114**
Grand mean	1841	2112	2308	1851	1835	1808	**1959±84**

Table 8.7b Energy intake (kcal/m^2/day). Somatropin-treated patients.

Patient	GH1	GH2	GH3	GH5	GH6	GH7	Mean
Day 1	1800	419	2187	354	1250	1869	1313
2	1597	484	1847	332	1323	1295	1146
3	2225	719	2611	367	1149	1082	1359
4	2276	1004	2180	956	1610	2068	1682
5	2049	1926	1991	802	1778	2011	1759
Mean	1989	910	2163	562	1422	1665	**1452±253**
Day 6	2559	2171	2067	1235	2225	1711	1995
7	2212	1589	2103	831	1737	1792	1711
8	2099	2116	2041	1194	1892	1762	1851
9	1888	2057	2691	990	2280	2273	2030
10	2476	1883	2018	1325	2010	2264	1996
Mean	2247	1963	2184	1115	2029	1961	**1916±167**
Day 11	2373	2123	2677	1175	2450	2335	2189
12	2305	2220	2716	1060	2138	2594	2172
13	2498	1734	2838	875	2156	2625	2121
14	2698	2089	2613	900	2514	1740	2092
15	2151	1232	2252	1218	2286	2495	1939
Mean	2405	1880	2619	1046	2309	2358	**2103±233**
Grand mean	2214	1584	2322	908	1920	1995	**1824±211**

C vs. GH	t	U	p
Days 1-5	0.67	14	ns
Days 6-10	0.36	18	ns
Days 11-15	0.43	17	ns
All days	0.60	17	ns

Table 8.8a Energy intake (% of CEE). Control patients.

Patient	C4	C6	C7	C9	C10	C11	Mean
Day 1	126	141	145	148	8	115	114
2	8	233	156	142	55	117	119
3	67	210	202	178	36	122	136
4	92	143	194	129	68	49	113
5	128	156	144	124	73	129	126
Mean	84	177	168	144	48	106	**121.3±20.7**
Day 6	96	148	150	110	126	111	124
7	48	159	170	114	98	104	116
8	116	179	173	138	119	156	147
9	129	229	176	132	110	131	151
10	198	170	168	159	121	135	158
Mean	117	177	168	131	115	128	**139.1±10.8**
Day 11	151	164	185	113	121	168	151
12	170	161	186	135	139	171	160
13	167	175	153	127	141	178	157
14	141	157	164	131	157	171	153
15	128	140	176	145	132	164	147
Mean	**151**	**160**	**173**	**130**	**138**	**170**	**153.7±7.1**
Grand mean	118	171	170	135	100	135	**138.0±11.5**

Table 8.8b Energy intake (% of CEE). Somatropin-treated patients.

Patient	GH1	GH2	GH3	GH5	GH6	GH7	Mean
Day 1	128	34	180	25	96	117	97
2	113	39	152	24	102	81	85
3	158	58	215	26	88	68	102
4	161	81	180	68	124	130	124
5	145	155	164	57	137	126	131
Mean	141	73	178	40	109	104	**107.7±19.9**
Day 6	181	174	170	88	171	107	149
7	157	128	173	59	134	112	127
8	149	170	168	85	145	111	138
9	134	165	222	71	175	143	152
10	176	151	166	95	155	142	147
Mean	159	158	180	80	156	123	**142.6±14.6**
Day 11	168	170	221	84	188	146	163
12	163	178	224	76	164	163	161
13	177	139	234	63	166	165	157
14	191	168	215	64	193	109	157
15	152	99	186	87	176	157	143
Mean	171	151	216	75	177	148	**156.2±19.1**
Grand mean	157	127	191	65	148	125	**135.5±17.2**

C vs. GH	t	U	p
Days 1-5	0.48	15	ns
Days 6-10	0.18	16	ns
Days 11-15	0.13	15	ns
All days	0.13	18	ns

Table 8.9 Serum urea concentration (mmol/l) (Normal range 2.5 - 6.5).

Patient	Day-1	Day-3	Day-6	Day-11	Day-15
C4	6.4	7.6	5.5	7.6	5.5
C6	3.8	4.3	4.2	6.0	5.9
C7	5.0	4.9	5.3	7.3	7.3
C9	2.9	3.9	4.3	5.0	6.2
C10	6.9	6.2	6.1	8.4	7.1
C11	4.2	4.7	7.7	9.2	9.0
Mean	4.87 ±0.63	5.27 ±0.56	5.52 ±0.53	7.25 ±0.63	6.83[a] ±0.52
GH1	1.5	2.9	3.6	3.5	6.4
GH2	1.9	3.0	2.6	4.6	3.2
GH3	3.2	3.6	5.4	5.5	7.3
GH5	3.2	5.4	4.6	4.4	11.6
GH6	4.1	2.9	4.2	4.1	7.1
GH7	3.4	3.0	4.6	4.8	8.5
Mean	2.88 ±0.40	3.47 ±0.40	4.17 ±0.39	4.48 ±0.28	7.35[b] ±1.11
t	2.65	2.6	2.05	4.03	0.42
U	5	4	7.5	1	14
p	<0.05	<0.05	ns	<0.01	ns

[a] Day-11 vs. 15: t=0.89, T=3, ns
[b] Day-11 vs. 15: (t=2.52, vr=16.6, p<0.01), T=1, p<0.05

Table 8.10a Nitrogen intake ($g/m^2/day$). Control patients.

Patient	C4	C6	C7	C9	C10	C11	Mean
Day 1	11.9	10.6	11.0	16.1	0.9	9.4	10.0
2	4.1	20.1	15.1	11.9	6.6	9.2	11.2
3	4.4	17.7	19.0	16.9	2.8	12.4	12.2
4	8.7	10.5	18.3	9.4	7.1	4.1	9.7
5	12.8	12.1	12.0	9.6	7.9	11.3	11.0
Mean	8.4	14.2	15.1	12.8	5.1	9.3	**10.8±1.6**
Day 6	9.5	12.0	12.9	8.6	14.0	12.5	11.6
7	4.5	13.9	15.3	8.7	10.4	8.8	10.2
8	11.1	15.3	16.3	10.6	13.4	15.1	13.6
9	12.2	22.0	17.3	11.0	11.8	9.9	14.0
10	18.8	14.4	13.9	12.3	11.8	10.7	13.7
Mean	11.2	15.5	15.1	10.2	12.3	11.4	**12.6±0.9**
Day 11	16.9	13.6	17.0	11.4	13.6	15.7	14.7
12	18.8	12.6	18.1	12.1	16.2	13.2	15.2
13	19.2	17.0	16.2	10.4	15.3	14.6	15.5
14	12.6	9.8	15.4	10.8	17.0	14.4	13.3
15	10.7	10.1	16.0	13.1	16.2	13.3	13.2
Mean	15.6	12.6	16.6	11.5	15.7	14.2	**14.4±0.8**
Grand mean	11.7	14.1	15.6	11.5	11.0	11.6	**12.6±0.7**

Table 8.10b Nitrogen intake ($g/m^2/day$). Somatropin-treated patients.

Patient	GH1	GH2	GH3	GH5	GH6	GH7	Mean
Day 1	12.4	1.4	15.5	1.8	4.2	11.5	7.8
2	9.9	8.0	12.9	2.1	8.9	7.4	8.2
3	14.3	4.3	23.6	3.2	11.8	5.9	10.5
4	16.4	9.8	15.3	5.2	10.3	11.3	11.4
5	14.8	11.2	13.6	5.1	10.7	11.9	11.2
Mean	13.5	6.9	16.2	3.4	9.2	9.6	**9.8±1.9**
Day 6	17.1	13.4	15.2	7.8	15.0	10.6	13.2
7	14.5	9.5	12.9	4.8	12.0	10.6	10.7
8	13.3	16.9	13.6	7.5	11.4	11.2	12.3
9	10.9	13.2	19.9	6.3	14.9	13.7	13.1
10	15.0	12.6	13.4	8.4	13.7	14.6	12.9
Mean	14.2	13.1	15.0	6.9	13.4	12.1	**12.5±1.2**
Day 11	12.8	13.6	17.3	7.4	14.5	15.9	13.6
12	15.2	14.0	20.6	6.8	12.5	17.5	14.4
13	14.0	10.5	22.4	5.5	12.9	15.6	13.5
14	14.4	14.9	18.3	5.7	15.8	12.0	13.5
15	13.7	7.5	15.1	8.2	11.1	15.3	11.8
Mean	14.0	12.1	18.7	6.7	13.3	15.2	**13.4±1.6**
Grand mean	13.9	10.7	16.6	5.7	12.0	12.3	**11.9±1.5**

C vs. GH	t	U	p
Days 1-5	0.42	16	ns
Days 6-10	0.11	17	ns
Days 11-15	0.57	14	ns
All days	0.43	18	ns

Table 8.11a Urinary nitrogen excretion ($g/m^2/day$). Control patients.

Patient	C4	C6	C7	C9	C10	C11	Mean
Day 1	12.4	7.6	8.2	7.0	1.4	6.3	7.2
2	15.2	7.2	8.8	10.2	7.4	6.3	9.2
3	15.6	6.0	8.2	6.7	9.8	7.7	9.0
4	20.0	6.5	6.6	6.7	12.1	5.0	9.5
5	18.3	6.7	7.7	6.8	15.5	13.3	11.4
Mean	16.3	6.8	7.9	7.5	9.2	7.7	**9.2±1.4**
Day 6	18.4	7.0	10.4	10.5	10.3	6.0	10.4
7	15.5	12.2	10.1	10.1	9.2	6.0	10.5
8	26.0	12.3	9.1	11.6	9.6	8.5	12.9
9	14.7	10.3	7.8	8.2	15.0	8.5	10.8
10	19.7	14.5	10.5	8.6	14.9	9.2	12.9
Mean	18.8	11.3	9.6	9.8	11.8	7.6	**11.5±1.6**
Day 11	16.0	9.9	6.7	8.9	14.7	8.9	10.9
12	22.6	8.0	10.0	15.2	11.7	8.9	12.7
13	30.5	5.5	7.9	13.7	9.1	8.0	12.5
14	20.0	5.1	7.2	12.8	14.7	11.0	11.8
15	14.6	11.8	10.7	11.5	8.9	9.7	11.2
Mean	20.8	8.1	8.5	12.4	11.8	9.3	**11.8±1.9**
Grand mean	18.6	8.7	8.7	9.9	11.0	8.2	**10.8±1.6**

Table 8.11b Urinary nitrogen excretion ($g/m^2/day$). Somatropin-treated patients.

Patient	GH1	GH2	GH3	GH5	GH6	GH7	Mean
Day 1	8.4	2.1	10.1	4.7	4.9	4.5	5.8
2	5.6	4.4	7.7	7.2	2.8	4.9	5.4
3	9.7	4.9	11.5	6.3	6.4	3.6	7.1
4	7.6	8.2	15.2	6.2	10.5	7.0	9.1
5	7.1	5.4	10.3	7.9	9.2	8.2	8.0
Mean	7.7	5.0	10.9	6.5	6.8	5.6	**7.1±0.9**
Day 6	10.4	9.2	17.6	10.4	7.6	5.8	10.2
7	12.2	7.1	9.8	6.9	5.5	3.9	7.6
8	8.3	7.6	9.9	4.5	9.1	5.4	7.5
9	5.1	8.6	15.9	9.5	9.1	6.1	9.1
10	7.3	12.8	8.4	14.0	7.8	6.9	9.5
Mean	8.6	9.1	12.3	9.0	7.8	5.6	**8.8±0.9**
Day 11	10.7	9.4	13.2	13.9	9.8	10.9	11.3
12	5.0	8.3	11.7	13.6	8.4	12.5	9.9
13	10.4	8.1	17.4	14.1	10.4	8.8	11.5
14	11.1	6.8	11.7	15.0	16.4	8.5	11.6
15	16.3	5.9	9.3	18.0	12.3	5.6	11.2
Mean	10.7	7.7	12.7	14.9	11.5	9.3	**11.1±1.0**
Grand mean	9.0	7.3	12.0	10.1	8.7	6.8	**9.0±0.8**

C vs. GH	t	U	p
Days 1-5	1.28	8	ns
Days 6-10	1.52	9	ns
Days 11-15	0.31	18	ns
All days	1.05	14	ns

Table 8.12a Nitrogen balance ($g/m^2/day$). Control patients.

Patient	C4	C6	C7	C9	C10	C11	Mean
Day 1	-0.4	3.0	2.8	9.1	-0.5	3.1	2.9
2	-11.1	12.9	6.3	1.7	-0.8	2.8	2.0
3	-11.2	11.7	10.9	10.3	-7.0	4.7	3.2
4	-11.3	4.0	11.7	2.6	-5.0	-1.0	0.2
5	-5.6	5.4	4.3	2.8	-7.5	-2.0	-0.4
Mean	-7.9	7.4	7.2	5.3	-4.2	1.5	**1.6±2.6**
Day 6	-8.9	5.0	2.5	-1.9	3.7	6.5	1.2
7	-11.0	1.7	5.2	-1.5	1.1	2.8	-0.3
8	-14.8	3.0	7.2	-0.9	3.8	6.6	0.8
9	-2.5	11.6	9.5	2.8	-3.3	1.4	3.3
10	-0.9	-0.1	3.4	3.7	-3.1	1.6	0.8
Mean	-7.6	4.3	5.6	0.5	0.5	3.8	**1.1±2.0**
Day 11	0.9	3.7	10.3	2.5	-1.1	6.8	3.9
12	-3.8	4.6	8.1	-3.2	4.5	4.3	2.4
13	-11.3	11.5	8.3	-3.3	6.2	6.6	3.0
14	-7.4	4.7	8.3	-2.0	2.3	3.4	1.5
15	-3.9	-1.7	5.4	1.6	7.3	3.6	2.0
Mean	-5.1	4.6	8.1	-0.9	3.8	4.9	**2.6±1.9**
Grand mean	-6.9	5.4	6.9	1.6	0.1	3.4	**1.8±2.0**

Table 8.12b Nitrogen balance ($g/m^2/day$). Somatropin-treated patients.

Patient	GH1	GH2	GH3	GH5	GH6	GH7	Mean
Day 1	4.0	-0.6	5.5	-3.0	-0.7	6.9	2.0
2	4.3	3.6	5.2	-5.2	6.1	2.4	2.7
3	4.6	-0.5	12.1	-3.1	5.3	2.3	3.4
4	8.7	1.6	0.1	-1.0	-0.3	4.3	2.2
5	7.7	5.7	3.3	-2.8	1.6	3.7	3.2
Mean	5.9	1.9	5.2	-3.0	2.4	3.9	**2.7±1.3**
Day 6	6.7	4.2	-2.4	-2.6	7.4	4.7	3.0
7	2.4	2.3	3.1	-2.1	6.5	6.6	3.1
8	5.1	9.3	3.6	3.0	2.3	5.8	4.8
9	5.8	4.6	4.0	-3.3	5.8	7.6	4.1
10	7.6	-0.2	5.0	-5.6	5.9	7.7	3.4
Mean	5.5	4.1	2.7	-2.1	5.6	6.5	**3.7±1.3**
Day 11	2.1	4.2	4.1	-6.5	4.7	5.0	2.3
12	10.2	5.7	9.0	-6.8	4.0	5.0	4.5
13	3.6	2.3	5.0	-8.6	2.5	6.8	1.9
14	3.3	8.0	6.6	-9.4	-0.6	3.5	1.9
15	-2.6	1.7	5.8	-9.8	-1.3	9.7	0.6
Mean	3.3	4.4	6.1	-8.2	1.9	6.0	**2.2±2.2**
Grand mean	4.9	3.5	4.7	-4.4	3.3	5.5	**2.9±1.5**

C vs. GH	t	U	p
Days 1-5	0.4	18	ns
Days 6-10	1.08	12	ns
Days 11-15	0.11	17	ns
All days	0.47	15	ns

Table 8.13 Serum insulin-like growth factor-I concentration (unit/ml) (Normal range 0.4 - 2.0).

Patient	Day-1	Day-3	Day-6	Day-11	Day-15
C4	0.64	0.44	0.62	0.52	0.68
C6	1.36	0.97	0.94	1.15	1.75
C7	0.98	1.07	1.36	1.15	1.21
C9	0.74	0.75	0.64	1.03	1.26
C10	0.37	0.20	0.22	0.71	0.95
C11	0.63	0.52	0.72	0.84	1.10
Mean	0.79 ±0.14	0.66 ±0.14	0.75 ±0.15	0.90 ±0.10	1.16[a c] ±0.15
GH1	1.12	1.56	2.13	4.1	1.95
GH2	0.36	0.24	0.73	1.39	0.36
GH3	1.90	4.32	0.99	4.23	2.88
GH5	0.18	0.18	0.26	0.78	0.24
GH6	0.77	0.85	0.99	2.18	0.93
GH7	0.21	0.58	0.33	1.00	0.80
Mean	0.76 ±0.27	1.29 ±0.64	0.91 ±0.28	2.28 ±0.63	1.19[b d] ±0.42
t	0.1	0.96	0.49	2.17	0.08
U	15	16	14	7	14
p	ns	ns	ns	ns	ns

[a] Day-1 vs. 15: t=4.49, (T=0, vr=1.08, ns), $p<0.01$
[b] Day-1 vs. 15: t=2.55, T=0, ns
[c] Day-11 vs. 15: t=3.46, T=0, $p<0.05$
[d] Day-11 vs. 15: t=3.91, T=0, $p<0.05$

Table 8.14 Serum albumin concentration (g/l) (Normal range 27 - 42).

Patient	Day-1	Day-3	Day-6	Day-11	Day-15
C4	35	30	18	15	15
C6	32	29	30	31	31
C7	40	35	31	33	33
C9	43	34	29	33	34
C10	41	36	29	29	28
C11	35	32	29	30	31
Mean	37.7 ±1.7	32.7 ±1.1	27.7[a] ±2.0	28.5 ±2.8	28.7[c] ±2.9
GH1	42	34	29	27	31
GH2	34	23	18	21	24
GH3	34	30	26	28	29
GH5	41	26	24	22	25
GH6	25	23	22	22	25
GH7	36	27	21	20	23
Mean	35.3 ±2.5	27.2 ±1.7	23.3[b] ±1.6	23.3 ±1.4	26.2[d] ±1.3
t	0.77	2.64	1.72	1.67	0.8
U	14.5	5	7	6	9
p	ns	<0.05	ns	ns	ns

[a] Day-1 vs. 6: t=4.47, (T=0, vr=1.51, ns), p<0.01
[b] Day-1 vs. 6: t=5.4, (T=0, vr=2.49, ns), p<0.01
[c] Day-6 vs. 15: t=0.89, T=6.5, ns
[d] Day 6 vs. 15: t=4.03, T=0, p<0.05

Table 8.15 Serum thyroxine-binding pre-albumin concentration (mg/l) (Normal range 170 - 420).

Patient	Day-1	Day-3	Day-6	Day-11	Day-15
C4	120	100	70	130	190
C6	250	150	170	310	380
C7	160	140	160	310	420
C9	320	190	150	340	460
C10	160	160	80	280	350
C11	170	110	190	350	400
Mean	196.7 ±30.2	141.7 ±13.5	136.7 ±20.3	286.7 ±32.9	366.7[a] ±38.5
GH1	160	150	170	270	380
GH2	130	70	70	150	200
GH3	290	210	220	300	370
GH5	110	210	120	160	340
GH6	130	100	120	200	220
GH7	170	90	80	230	390
Mean	165.0 ±26.6	138.3 ±25.1	130.0 ±23.1	218.3 ±24.4	316.7[b] ±34.5
t	0.79	0.12	0.22	1.67	0.97
U	12.5	16	16.5	7	10.5
p	ns	ns	ns	ns	ns

[a] Day-1 vs. 15: t=5.94, (T=0, vr=1.62, ns), $p<0.01$
[b] Day-1 vs. 15: t=4.71, (T=0, vr=1.69, ns), $p<0.01$

Table 8.16 Serum retinol-binding protein concentration (mg/l) (Normal range 31 - 75).

Patient	Day-1	Day-3	Day-6	Day-11	Day-15
C4	16	20	22	35	37
C6	45	33	37	69	78
C7	27	31	40	70	82
C9	50	30	33	77	106
C10	14	14	17	73	85
C11	23	15	48	86	101
Mean	29.2 ±6.1	23.8 ±3.5	32.8 ±4.7	68.3 ±7.1	81.5[a] ±10.0
GH1	27	25	34	58	75
GH2	17	10	13	33	43
GH3	41	27	32	51	70
GH5	9	7	12	30	88
GH6	32	18	30	56	64
GH7	24	18	18	51	94
Mean	25.0 ±4.6	17.5 ±3.2	23.2 ±4.1	46.5 ±4.9	72.3[b] ±7.4
t	0.54	1.33	1.55	2.53	0.74
U	16.5	10	8	4	12
p	ns	ns	ns	<0.05	ns

[a] Day-1 vs. 15: t=5.87, (T=0, vr=2.78, ns), p<0.01
[b] Day-1 vs. 15: t=5.14, (T=0, vr=2.63, ns), p<0.01

Table 8.17 Serum total immunoglobulin-G concentration (g/l) (Normal range 5.4 - 16.5).

Patient	Day-1	Day-3	Day-6	Day-11	Day-15
C4	2.3	4.7	7.6	11.7	11.6
C6	8.0	7.1	8.7	12.2	11.9
C7	6.4	7.6	8.4	11.3	12.8
C9	4.7	4.6	5.9	10.3	10.4
C10	2.4	3.5	6.6	11.8	11.8
C11	7.1	7.2	8.1	10.1	10.2
Mean	5.2 ±1.0	5.8 ±0.7	7.6 ±0.5	11.2 ±0.4	11.5[a] ±0.4
GH1	5.1	6.0	7.2	10.2	11.4
GH2	4.2	2.4	3.8	6.8	8.8
GH3	10.9	9.6	9.3	12.0	12.4
GH5	2.7	3.4	5.3	10.8	13.0
GH6	7.8	7.1	7.7	9.9	10.9
GH7	3.0	3.8	6.3	12.2	13.3
Mean	5.6 ±1.3	5.4 ±1.1	6.6 ±0.8	10.3 ±0.8	11.6[b] ±0.7
t	0.29	0.31	1.05	1.05	0.23
U	16	13.5	12	13.5	15
p	ns	ns	ns	ns	ns

[a] Day-1 vs. 15: t=5.83, (T=0, vr=6.06, ns), $p<0.01$
[b] Day-1 vs. 15: t=4.01, T=0, $p<0.05$

Table 8.18a Sodium intake (mmol/m^2/day). Control patients.

Patient	C4	C6	C7	C9	C10	C11	Mean
Day 1	74	47	78	75	183	63	86.6
2	87	136	111	103	145	88	111.6
3	46	109	144	75	57	66	82.6
4	113	54	125	58	64	22	72.7
5	126	71	80	59	56	84	79.0
Mean	89.1	83.5	107.5	73.7	100.9	64.3	**86.6±6.6**
Day 6	117	78	114	49	63	169	98.4
7	26	86	110	47	63	83	69.1
8	43	75	126	86	82	107	86.5
9	57	104	142	70	57	97	87.9
10	109	93	120	93	64	86	94.0
Mean	70.1	87.2	122.7	68.8	65.7	108.4	**87.2±9.7**
Day 11	68	69	167	88	80	102	95.7
12	86	73	166	81	71	116	98.9
13	64	90	114	68	94	108	89.7
14	50	71	103	73	89	99	80.7
15	58	59	114	64	84	105	80.7
Mean	65.1	72.3	132.8	74.7	83.6	106.1	**89.1±10.5**
Grand mean	74.8	81.0	121.0	72.4	83.4	92.9	**87.7±7.3**

Table 8.18b Sodium intake (mmol/m^2/day). Somatropin-treated patients.

Patient	GH1	GH2	GH3	GH5	GH6	GH7	Mean
Day 1	108	178	80	74	28	71	89.8
2	65	215	65	259	49	59	118.7
3	74	160	162	317	53	31	132.8
4	88	198	95	43	79	95	99.8
5	92	132	65	29	75	79	78.6
Mean	85.3	176.4	93.5	144.3	57.1	67.1	**103.9±19.0**
Day 6	112	116	81	17	87	61	78.9
7	91	66	99	40	72	77	74.3
8	67	134	78	20	99	52	75.0
9	66	68	125	35	130	100	87.2
10	130	64	74	46	74	91	79.8
Mean	93.0	89.7	91.3	31.6	92.5	76.1	**79.1±9.8**
Day 11	67	64	162	41	138	85	92.7
12	68	104	140	37	143	102	98.9
13	87	57	139	31	130	119	94.0
14	88	74	109	31	120	62	80.8
15	78	43	101	43	125	108	83.1
Mean	77.8	68.3	130.2	36.6	131.4	95.1	**89.9±15.1**
Grand mean	85.3	111.5	105.0	70.8	93.7	79.4	**91.0±6.3**

C vs. GH	t	U	p
Days 1-5	0.87	16	ns
Days 6-10	0.59	17	ns
Days 11-15	0.04	18	ns
All days	0.35	15	ns

Table 8.19a Sodium balance (mmol/m^2/day). Control patients.

Patient	C4	C6	C7	C9	C10	C11	Mean
Day 1	72	7	46	18	115	20	46.5
2	62	99	48	73	87	-72	49.5
3	41	67	74	58	-4	-12	37.2
4	108	20	61	26	-74	-46	16.0
5	120	35	45	12	-92	24	24.2
Mean	80.6	45.5	55.1	37.4	6.6	-17.2	**34.6±14.3**
Day 6	110	78	-2	18	-83	118	40.0
7	25	-39	35	-29	-64	14	-10.0
8	41	1	45	17	-4	33	22.1
9	54	38	93	-13	-36	33	28.1
10	103	-55	45	11	3	19	21.1
Mean	66.5	4.6	43.3	0.8	-36.9	43.5	**20.3±15.4**
Day 11	63	-19	128	9	34	32	41.1
12	77	13	107	-38	35	42	39.2
13	48	27	78	39	43	46	46.8
14	39	18	58	5	48	-2	27.5
15	51	-36	30	16	43	7	18.7
Mean	55.7	0.6	80.1	6.1	40.4	25.1	**34.7±12.4**
Grand mean	67.6	16.9	59.5	14.8	3.4	17.1	**29.9±10.9**

Table 8.19b Sodium balance (mmol/m^2/day). Somatropin-treated patients.

Patient	GH1	GH2	GH3	GH5	GH6	GH7	**Mean**
Day 1	26	178	52	71	-81	51	49.2
2	35	215	27	258	37	30	100.2
3	34	158	108	313	43	-47	101.6
4	47	196	22	36	69	67	73.0
5	67	115	31	-16	65	53	52.4
Mean	41.9	172.4	47.9	132.2	26.6	30.6	**75.3±25.1**
Day 6	76	99	-120	-122	24	49	1.3
7	50	58	34	-25	-21	73	28.2
8	-4	130	57	-13	-82	15	17.3
9	30	27	83	15	53	54	43.6
10	99	37	15	16	35	35	39.6
Mean	50.2	70.3	14.0	-25.6	1.8	45.3	**26.0±14.5**
Day 11	37	-17	71	15	70	37	35.4
12	52	4	55	10	75	35	38.4
13	13	-48	57	12	61	55	25.0
14	-17	-45	70	20	-7	-8	2.3
15	-39	-36	47	20	4	41	6.1
Mean	9.3	-28.3	59.8	15.2	40.6	32.1	**21.4±12.4**
Grand mean	33.8	71.5	40.6	40.6	23.0	36.0	**40.9±6.7**

C vs. GH	t	U	p
Days 1-5	1.41	13	ns
Days 6-10	0.27	14	ns
Days 11-15	0.75	16	ns
All days	0.86	10	ns

Table 8.20a Fluid intake (ml/m^2/day). Control patients.

Patient	C4	C6	C7	C9	C10	C11	Mean
Day 1	2872	2293	2543	3210	2454	2362	2622
2	2183	2424	2376	2529	3856	2514	2647
3	3012	2916	2797	2761	1808	2503	2633
4	2068	2555	2743	1140	2462	1130	2016
5	3492	2400	2606	2949	1758	1633	2473
Mean	2725	2518	2613	2518	2468	2028	**2478±98**
Day 6	2786	2667	2670	2818	1870	2503	2552
7	3734	2005	2918	2585	1637	1949	2471
8	3631	1950	2268	3054	1816	2486	2534
9	5697	2587	2072	3215	1928	2209	2951
10	3298	2291	2959	3127	2152	2401	2705
Mean	3829	2300	2577	2960	1881	2310	**2643±278**
Day 11	3019	2475	2160	2274	1888	2225	2340
12	5157	2424	2650	3637	1973	2537	3063
13	4705	2566	2897	3114	1503	1994	2796
14	2706	2374	2237	2032	2148	2034	2255
15	3064	2621	2959	2096	1690	1920	2393
Mean	3730	2492	2581	2631	1840	2142	**2569±263**
Grand mean	3428	2437	2590	2703	2063	2160	**2563±200**

Table 8.20b Fluid intake (ml/m^2/day). Somatropin-treated patients.

Patient	GH1	GH2	GH3	GH5	GH6	GH7	Mean
Day 1	2655	3158	2705	2030	775	2876	2367
2	1895	2710	2943	2865	2429	2096	2490
3	2797	2439	3767	3625	2367	1773	2795
4	2777	2848	2570	2540	2016	2346	2516
5	3641	3328	2771	1536	2377	2346	2666
Mean	2753	2896	2951	2519	1993	2287	**2566±153**
Day 6	2762	2392	2280	1651	3036	1804	2321
7	2825	2468	2822	1805	2456	1944	2387
8	2888	3172	1720	1866	2775	2140	2427
9	2468	3159	2675	2640	2246	2812	2667
10	2757	1883	2369	2450	2657	2681	2466
Mean	2740	2615	2373	2082	2634	2276	**2453±103**
Day 11	3561	3105	3121	2475	2854	3160	3046
12	2383	2339	2370	2761	2356	2845	2509
13	2730	3684	1669	1650	2762	3113	2601
14	3113	3024	3134	2000	2869	2283	2737
15	3362	2576	3261	2726	3184	2673	2963
Mean	3030	2946	2711	2322	2805	2815	**2771±101**
Grand mean	2841	2819	2678	2308	2477	2459	**2597±88**

C vs. GH	t	U	p
Days 1-5	0.49	13	ns
Days 6-10	0.64	17	ns
Days 11-15	0.72	9	ns
All days	0.15	14	ns

Table 8.21a Fluid balance (ml/m^2/day). Control patients.

Patient	C4	C6	C7	C9	C10	C11	**Mean**
Day 1	1991	1268	1223	1422	1956	1514	1562
2	864	946	1229	690	3239	1497	1411
3	2108	1704	1946	1699	937	1048	1574
4	855	1126	1325	26	1397	579	885
5	2291	1131	1652	1796	368	362	1267
Mean	1622	1235	1475	1127	1579	1000	**1339±104**
Day 6	1428	1534	1273	1264	726	1927	1359
7	2656	-901	1835	829	713	1031	1027
8	1972	103	1224	1023	1031	1285	1107
9	2024	1626	1093	1324	1020	910	1333
10	739	591	1335	744	1345	1130	981
Mean	1763	591	1352	1037	967	1257	**1161±162**
Day 11	1645	1170	1335	720	1036	706	1102
12	2242	1195	1567	930	1188	1181	1384
13	2466	1138	2088	1006	460	638	1299
14	133	995	1381	940	1094	311	809
15	863	256	1438	104	928	621	702
Mean	1469	951	1562	740	941	692	**1059±151**
Grand mean	1618	925	1463	968	1163	983	**1187±118**

Table 8.21b Fluid balance (ml/m^2/day). Somatropin-treated patients.

Patient	GH1	GH2	GH3	GH5	GH6	GH7	**Mean**
Day 1	942	2837	1106	1729	94	2197	1484
2	1132	1580	1236	2309	932	941	1355
3	718	1210	2111	3209	1372	732	1559
4	1685	2316	882	1505	727	1057	1362
5	2746	1173	1064	833	636	1160	1267
Mean	1445	1823	1280	1917	752	1217	**1406±175**
Day 6	762	269	3	643	942	1005	604
7	957	1225	1102	943	1359	1088	1112
8	1191	1535	1038	1243	869	851	1121
9	1678	1419	1153	1669	322	943	1197
10	1336	626	1127	1385	1097	1160	1122
Mean	1185	1015	885	1176	918	1009	**1031±52**
Day 11	890	582	1306	1569	995	531	979
12	1304	497	682	1395	890	335	850
13	1046	-82	188	989	825	1299	711
14	1245	245	1637	1369	304	737	923
15	415	-231	1828	1688	1024	585	885
Mean	980	202	1128	1402	807	697	**869±168**
Grand mean	1203	1013	1097	1498	826	975	**1102±94**

C vs. GH	t	U	p
Days 1-5	0.32	16	ns
Days 6-10	0.76	12	ns
Days 11-15	0.84	15	ns
All days	0.56	17	ns

Table 8.22a Potassium intake (mmol/m^2/day). Control patients.

Patient	C4	C6	C7	C9	C10	C11	**Mean**
Day 1	50	63	63	85	5	55	53.5
2	0	111	86	72	27	54	58.3
3	33	100	102	89	17	64	67.6
4	37	63	103	59	43	23	54.4
5	39	76	72	61	36	63	57.9
Mean	31.9	82.7	85.3	73.1	25.5	51.8	**58.3±10.6**
Day 6	48	80	78	52	79	46	63.9
7	18	76	83	70	58	53	59.8
8	50	83	87	60	64	81	70.9
9	61	104	92	62	59	62	73.1
10	94	73	77	80	61	59	74.4
Mean	54.2	83.4	83.5	65.0	64.4	60.1	**68.4±5.0**
Day 11	106	74	93	72	69	84	83.0
12	115	69	93	74	89	77	86.0
13	85	78	97	64	69	89	80.1
14	58	78	86	73	83	81	76.4
15	59	60	91	69	65	67	68.4
Mean	84.6	72.0	91.8	70.2	74.8	79.4	**78.8±3.4**
Grand mean	56.9	79.3	86.8	69.4	54.9	63.8	**68.5±5.1**

Table 8.22b Potassium intake (mmol/m^2/day). Somatropin-treated patients.

Patient	GH1	GH2	GH3	GH5	GH6	GH7	Mean
Day 1	57	9	83	11	28	71	43.2
2	53	8	81	13	47	47	41.4
3	85	64	124	13	64	49	66.4
4	91	60	90	31	61	65	66.2
5	81	70	76	30	65	73	65.9
Mean	73.2	42.2	90.6	19.6	53.0	61.2	**56.6±10.1**
Day 6	98	90	78	47	97	63	78.9
7	85	55	75	29	69	60	62.0
8	77	87	84	45	77	64	72.3
9	66	83	113	38	96	76	78.6
10	87	79	94	50	85	86	80.1
Mean	82.5	78.7	88.7	41.8	84.7	70.0	**74.4±7.0**
Day 11	92	93	111	45	95	88	87.2
12	96	93	101	40	80	104	85.7
13	92	63	114	34	102	99	84.0
14	103	82	106	34	121	65	85.4
15	90	49	89	49	92	90	76.5
Mean	94.5	76.0	104.2	40.3	98.1	89.4	**83.8±9.5**
Grand mean	83.4	65.7	94.5	33.9	78.6	73.5	**71.6±8.5**

C vs. GH	t	U	p
Days 1-5	0.12	18	ns
Days 6-10	0.69	12	ns
Days 11-15	0.49	10	ns
All days	0.31	14	ns

Table 8.23a Potassium balance (mmol/m^2/day). Control patients.

Patient	C4	C6	C7	C9	C10	C11	**Mean**
Day 1	7	6	38	41	-22	32	16.9
2	-95	45	55	12	-7	15	4.4
3	-39	56	79	51	-23	41	27.3
4	-6	28	78	10	12	7	21.6
5	-7	32	57	3	1	32	19.7
Mean	-28.1	33.4	61.4	23.4	-7.8	25.4	**18.0±12.9**
Day 6	1	26	27	-7	65	27	23.1
7	-46	-5	18	0	45	25	6.3
8	41	-13	31	5	50	34	24.7
9	-1	53	43	30	30	12	27.8
10	-1	-6	4	41	23	7	11.4
Mean	-1.1	10.9	24.5	13.8	42.7	21.1	**18.6±6.0**
Day 11	24	-2	34	25	39	23	23.9
12	-14	17	35	-24	52	10	12.7
13	-106	34	44	30	32	40	12.4
14	-60	30	42	3	31	14	9.9
15	17	-6	12	4	33	11	11.8
Mean	-27.8	14.6	33.3	7.6	37.5	19.6	**14.2±9.6**
Grand mean	-19.0	19.6	39.8	14.9	24.1	22.0	**16.9±8.0**

Table 8.23b Potassium balance (mmol/m^2/day). Somatropin-treated patients.

Patient	GH1	GH2	GH3	GH5	GH6	GH7	Mean
Day 1	44	4	41	-7	8	51	23.7
2	34	-7	50	-6	33	24	21.5
3	43	36	66	-5	26	5	28.3
4	39	39	2	14	0	14	18.1
5	35	4	1	10	13	34	16.1
Mean	39.0	15.2	32.0	1.3	16.1	25.7	**21.5±5.5**
Day 6	32	42	-66	32	39	35	18.9
7	4	12	11	3	38	42	18.2
8	17	42	43	12	14	27	26.0
9	32	35	45	-20	27	33	25.1
10	43	13	57	-8	24	38	27.9
Mean	25.6	28.7	18.0	3.8	28.3	35.0	**23.2±4.5**
Day 11	-2	37	29	-10	19	14	14.7
12	74	49	21	-29	17	34	27.8
13	38	22	17	-13	30	41	22.5
14	33	40	45	-30	21	14	20.6
15	-7	15	29	-90	17	27	-1.7
Mean	27.2	32.8	28.2	-34.4	20.7	26.2	**16.8±10.4**
Grand mean	30.6	25.5	26.0	-9.8	21.7	28.9	**20.5±6.2**

C vs. GH	t	U	p
Days 1-5	0.26	17	ns
Days 6-10	0.61	12	ns
Days 11-15	0.19	16	ns
All days	0.36	12	ns

Table 8.24 Predicted insensible losses.

Patient	BSA[a]	TBSA[b]	Sodium loss[c]	Fluid loss[d]
C4	2.11	31	52.7	1395
C6	2.03	12	20.4	857
C7	1.94	16	27.2	971
C9	1.93	18	30.6	1027
C10	2.23	42	71.4	1707
C11	1.77	15	25.5	942
Mean	2.00 ±0.07	22.3 ±4.8	38.0 ±8.1	1150 ±135
GH1	1.90	17	28.9	999
GH2	1.71	15	25.5	942
GH3	1.57	17	28.9	999
GH5	2.00	25	42.5	1225
GH6	1.91	14	23.8	914
GH7	1.94	32	54.4	1424
Mean	1.84 ±0.07	20.0 ±2.9	34.0 ±4.9	1084 ±81
t	1.75	0.42	0.42	0.42
U	7.5	17.5	17.5	17.5
p	ns	ns	ns	ns

[a] Body surface area (Du Bois and Du Bois 1916) (m^2)
[b] Total burn surface area (%)
[c] Predicted sodium losses (derived from Davies 1967) (mmol/m^2/day)
[d] Predicted fluid losses (Harrison et al. 1964) (ml/m^2/day)

Table 8.25 Serum sodium concentration (mmol/l) (Normal range 136 - 148).

Patient	Day-1	Day-3	Day-6	Day-11	Day-15
C4	133	132	131	132	134
C6	136	137	139	140	139
C7	137	139	139	139	138
C9	141	137	136	141	142
C10	137	135	136	134	136
C11	135	137	139	142	141
Mean	136.5 ±1.1	136.2 ±1.0	136.7 ±1.3	138.0 ±1.7	138.3 ±1.2
GH1	139	137	138	140	140
GH2	125	124	131	136	139
GH3	139	140	138	139	139
GH5	135	132	134	131	134
GH6	135	134	136	135	137
GH7	133	139	136	139	132
Mean	134.3 ±2.1	134.3 ±2.4	135.5 ±1.1	136.7 ±1.4	136.8 ±1.3
t	0.91	0.71	0.69	0.62	0.84
U	13.5	16.5	11.5	12.5	13.5
p	ns	ns	ns	ns	ns

Table 8.26 Serum potassium concentration (mmol/l) (Normal range 3.8 - 5.0).

Patient	Day-1	Day-3	Day-6	Day-11	Day-15
C4	4.5	4.5	4.1	4.6	4.5
C6	4.4	4.3	4.6	4.9	4.7
C7	4.4	3.7	4.3	4.4	4.0
C9	4.0	4.0	4.5	4.8	4.5
C10	3.8	3.4	3.0	4.1	4.4
C11	3.4	3.5	4.1	4.8	4.6
Mean	4.08 ±0.18	3.90 ±0.18	4.10 ±0.24	4.60 ±0.12	4.45 ±0.10
GH1	3.7	3.9	4.5	4.8	4.5
GH2	3.7	3.2	3.6	4.0	4.0
GH3	4.0	4.3	4.0	4.4	4.5
GH5	3.7	3.5	3.4	4.2	4.7
GH6	3.7	3.7	4.0	4.7	4.8
GH7	3.9	3.7	4.2	5.1	4.6
Mean	3.78 ±0.05	3.72 ±0.15	3.95 ±0.16	4.53 ±0.17	4.52 ±0.11
t	1.63	0.78	0.52	0.32	0.44
U	8.5	14	11.5	15.5	13.5
p	ns	ns	ns	ns	ns

Table 8.27 Enteral feed administration rate during the IVGTT (ml/m^2/hr).

Patient	Day-1	Day-3	Day-6	Day-11	Day-15
C4	0	47	57	57	57
C6	0	52	52	52	52
C7	0	57	57	57	57
C9	0	57	57	57	57
C10	0	76	76	76	76
C11	0	54	54	54	54
Mean	0	57.2 ±4.1	58.8 ±3.5	58.8 ±3.5	58.8 ±3.5
GH1	0	59	59	59	59
GH2	0	23	46	53	53
GH3	0	51	51	51	51
GH5	0	30	60	60	60
GH6	0	42	52	52	52
GH7	0	52	57	57	70
Mean	0	42.8 ±5.7	54.2 ±2.2	55.3 ±1.6	57.5 ±2.9
t	-	2.05	1.12	0.91	0.29
U	-	7.5	14	15	16.5
p	-	ns	ns	ns	ns

Table 8.28a Blood glucose concentration before and during the IVGTT (mmol/l) (Normal fasting range 3.0 - 5.5). Day-1.

Patient	T_0	T_5	T_{10}	T_{20}	T_{60}
C4	7.4	15.7	13.5	11.5	7.5
C6	4.55	12.8	11.1	9.6	5.5
C7	5.15	13.0	11.1	9.2	6.2
C9	5.05	13.9	12.8	10.9	6.7
C10	5.7	12.8	11.5	9.7	6.5
C11	5.8	15.1	13.2	11.6	7.7
Mean	5.61 ±0.40	13.88 ±0.51	12.2 ±0.45	10.42 ±0.43	6.68 ±0.34
GH1	4.95	13.3	12.7	10.2	7.3
GH2	4.9	16.1	14.0	11.8	8.0
GH3	5.6	18.6	16.9	13.6	8.8
GH5	5.05	14.6	12.0	10.4	7.1
GH6	4.85	16.0	12.9	9.9	4.9
GH7	4.95	13.3	11.4	9.9	7.0
Mean	5.05 ±0.11	15.32 ±0.83	13.32 ±0.80	10.97 ±0.60	7.18 ±0.53
t	1.33	1.47	1.22	0.75	0.79
U	8.5	8	12	12	12
p	ns	ns	ns	ns	ns

Table 8.28b Blood glucose concentration before and during the IVGTT (mmol/l). Day-3.

Patient	T_0	T_5	T_{10}	T_{20}	T_{60}
C4	6.85	13.1	11.8	10.6	8.4
C6	5.05	12.4	10.4	7.2	4.4
C7	4.6	12.6	10.5	8.9	6.0
C9	6.9	17.5	14.7	13.3	8.8
C10	5.25	12.0	10.7	9.2	6.6
C11	5.7	14.0	13.4	12.1	8.8
Mean	5.73 ±0.39	13.60 ±0.83	11.92 ±0.72	10.22 ±0.91	7.17 ±0.73
GH1	5.7	12.5	11.4	9.6	6.0
GH2	6.35	15.8	14.3	12.5	8.7
GH3	4.85	15.1	12.8	9.9	6.2
GH5	6.3	13.1	12.4	10.9	8.3
GH6	7.3	16.6	14.1	11.9	7.6
GH7	5.85	14.1	12.9	11.0	6.7
Mean	6.06 ±0.33	14.53 ±0.65	12.98 ±0.44	10.97 ±0.46	7.25 ±0.46
t	0.65	0.89	1.26	0.73	0.1
U	13.5	10.5	11	13	16.5
p	ns	ns	ns	ns	ns

Table 8.28c Blood glucose concentration before and during the IVGTT (mmol/l). Day-6.

Patient	T_0	T_5	T_{10}	T_{20}	T_{60}
C4	6.3	11.7	10.4	8.8	5.8
C6	4.2	13.5	11.1	8.7	5.0
C7	5.0	13.0	11.5	9.6	6.4
C9	8.15	16.1	14.7	13.0	10.9
C10	7.2	13.8	12.2	11.4	9.3
C11	5.75	13.7	13.2	11.2	6.7
Mean	6.10 ±0.59	13.63 ±0.59	12.18 ±0.64	10.45 ±0.69	7.35 ±0.92
GH1	6.7	15.6	13.0	10.9	8.6
GH2	4.9	14.8	11.4	9.5	6.9
GH3	6.4	17.3	14.4	11.6	5.8
GH5	7.0	14.1	12.4	11.0	8.7
GH6	9.15	21.8	15.6	14.2	12.1
GH7	5.75	12.7	11.7	10.5	7.6
Mean	6.65 ±0.59	16.05 ±1.31	13.08 ±0.67	11.28 ±0.65	8.28 ±0.88
t	0.66	1.69	0.98	0.88	0.73
U	14.5	8	12	14	12.5
p	ns	ns	ns	ns	ns

Table 8.28d Blood glucose concentration before and during the IVGTT (mmol/l). Day-11.

Patient	T_0	T_5	T_{10}	T_{20}	T_{60}
C4	5.9	11.8	10.6	9.1	6.1
C6	4.25	11.1	10.4	8.3	4.2
C7	5.3	13.8	11.6	8.9	4.5
C9	5.3	12.6	11.6	9.8	7.2
C10	5.2	13.3	10.8	8.7	6.7
C11	5.05	14.1	12.2	9.6	5.5
Mean	5.17 ±0.22	12.78 ±0.48	11.20 ±0.29	9.07 ±0.23	5.70 ±0.49
GH1	4.8	12.9	10.9	9.7	7.9
GH2	5.0	13.9	12.2	9.9	6.0
GH3	4.35	16.1	13.1	11.0	5.2
GH5	7.1	14.5	13.0	11.6	9.4
GH6	7.1	15.9	14.7	13.1	10.7
GH7	5.15	12.8	11.2	8.7	4.4
Mean	5.58 ±0.49	14.35 ±0.58	12.52 ±0.57	10.67 ±0.64	7.27 ±1.01
t	0.77	2.08	2.06	(2.35)*	1.39
U	17	7	6.5	5.5	12
p	ns	ns	ns	ns	ns

* Variances not equivalent (vr=7.9, $p<0.05$)

Table 8.28e Blood glucose concentration before and during the IVGTT (mmol/l). Day-15.

Patient	T_0	T_5	T_{10}	T_{20}	T_{60}
C4	4.45	11.7	9.8	8.3	5.8
C6	3.8	12.3	9.9	6.7	4.5
C7	4.45	13.9	11.3	7.1	4.4
C9	4.6	13.9	12.6	10.1	7.0
C10	4.95	12.7	11.0	9.1	5.7
C11	4.65	13.1	11.7	9.1	4.9
Mean	4.48 ±0.16	12.93 ±0.36	11.05 ±0.44	8.40 ±0.53	5.38 ±0.40
GH1	5.65	13.8	12.1	10.1	6.4
GH2	4.1	18.0	13.9	8.7	4.3
GH3	4.55	14.7	12.9	10	3.3
GH5	6.7	14.6	13.3	10.8	7.2
GH6	5.4	12.4	10.8	7.4	4.4
GH7	5.9	20.8	12.4	11.0	6.8
Mean	5.38 ±0.38	15.72 ±1.27	12.57 ±0.44	9.67 ±0.56	5.40 ±0.65
t	2.17	2.12	2.44	1.64	0.02
U	8	6	(6)*	8.5	16.5
p	ns	ns	<0.05	ns	ns

* Variances equivalent (vr=0, ns)

Table 8.29 Mean basal blood glucose concentration before the IVGTT during the study (mmol/l) (see table 8.28).

Group	Day-1	Day-3	Day-6	Day-11	Day-15
C	5.61±0.40	5.73±0.39[a]	6.10±0.59	5.17±0.22	4.48±0.16[c]
GH	5.05±0.11	6.06±0.33[b]	6.65±0.59	5.58±0.49	5.38±0.38[d]
t	1.33	0.65	0.66	0.77	2.17
U	8.5	13.5	14.5	17	8
p	ns	ns	ns	ns	ns

[a] Day-1 vs. 3: t=0.3, T=9, ns
[b] Day-1 vs. 3: t=2.35, T=1.5, ns
[c] Day-11 vs. 15: t=3.86, T=0, $p<0.05$
[d] Day-11 vs. 15: t=0.49, T=8, ns

Table 8.30a Plasma insulin concentration before and during the IVGTT (pmol/l). Day-1.

	T_0	T_5	T_{10}	T_{20}	T_{60}
C4	804	1681	1732	1522	814
C6	60	467	341	300	115
C7	89	403	334	277	142
C9	53	254	246	171	149
C10	108	401	469	432	241
C11	48	315	249	225	143
Mean	194 ±122	587 ±221	562 ±236	488 ±210	267 ±111
GH1	79	263	288	257	203
GH2	102	533	601	642	310
GH3	78	704	618	483	243
GH5	56	443	342	313	161
GH6	60	1158	882	622	174
GH7	23	121	41	37	42
Mean	66 ±11	537 ±150	462 ±121	392 ±96	189 ±37
t	1.04	0.19	0.38	0.41	0.67
U	14.5	16	15	15	14
p	ns	ns	ns	ns	ns

Table 8.30b Plasma insulin concentration before and during the IVGTT (pmol/l). Day-3.

Patient	T_0	T_5	T_{10}	T_{20}	T_{60}
C4	172	989	931	1072	538
C6	220	911	1035	775	91
C7	122	615	514	425	296
C9	152	704	942	1080	663
C10	138	689	447	530	379
C11	158	494	575	589	512
Mean	160 ±34	734 ±76	741 ±105	745 ±114	413 ±83
GH1	348	1058	1144	894	364
GH2	177	1052	954	1035	1110
GH3	454	1753	1801	1428	1500
GH5	198	729	690	577	278
GH6	1046	1700	1589	1500	934
GH7	79	167	281	376	151
Mean	384 ±143	1077 ±245	1077 ±230	968 ±183	723 ±220
t	1.55	1.34	1.33	1.03	1.32
U	8	8	10	14	14
p	ns	ns	ns	ns	ns

Table 8.30c Plasma insulin concentration before and during the IVGTT (pmol/l). Day-6.

Patient	T_0	T_5	T_{10}	T_{20}	T_{60}
C4	418	986	1195	975	323
C6	77	691	509	282	82
C7	158	668	560	519	434
C9	337	594	554	575	353
C10	357	752	787	789	593
C11	168	632	590	612	292
Mean	253 ±55	721 ±57	699 ±107	625 ±97	346 ±69
GH1	645	1476	1488	1201	1154
GH2	170	734	584	697	662
GH3	1112	1546	1371	1475	483
GH5	62	351	241	448	305
GH6	595	596	775	523	569
GH7	93	220	217	189	336
Mean	446 ±169	821 ±231	779 ±223	756 ±199	585 ±127
t	1.09	0.42	0.32	0.59	1.66
U	14.5	16.5	17	17	9
p	ns	ns	ns	ns	ns

Table 8.30d Plasma insulin concentration before and during the IVGTT (pmol/l). Day-11.

Patient	T_0	T_5	T_{10}	T_{20}	T_{60}
C4	222	999	919	760	312
C6	56	494	386	285	64
C7	131	636	418	277	99
C9	162	722	450	321	346
C10	297	856	931	747	712
C11	94	615	274	360	151
Mean	160 ±36	720 ±74	563 ±117	458 ±94	281 ±98
GH1	342	529	567	716	671
GH2	315	1619	1147	836	547
GH3	306	1671	1107	1080	230
GH5	348	433	381	304	349
GH6	689	824	1089	1239	891
GH7	238	1167	901	735	197
Mean	373 ±65	1041 ±218	865 ±130	818 ±133	481 ±111
t	2.86	1.39	1.72	2.21	1.35
U	(1)*	13	9	8	9
p	<0.05	ns	ns	ns	ns

* Variances equivalent (vr=3.3, ns)

Table 8.30e Plasma insulin concentration before and during the IVGTT (pmol/l). Day-15.

Patient	T_0	T_5	T_{10}	T_{20}	T_{60}
C4	38	442	207	138	67
C6	165	1188	873	451	84
C7	256	934	804	384	104
C9	154	488	330	204	241
C10	229	989	772	735	758
C11	225	617	474	309	114
Mean	178 ±32	776 ±124	577 ±113	370 ±87	228 ±109
GH1	424	1346	1129	756	561
GH2	86	741	351	386	122
GH3	343	1541	1211	901	266
GH5	274	131	212	332	150
GH6	189	875	593	361	88
GH7	66	204	184	251	138
Mean	230 ±58	806 ±235	613 ±186	497 ±108	221 ±72
t	0.79	0.11	0.17	0.92	0.05
U	13	18	18	12.5	12
p	ns	ns	ns	ns	ns

Table 8.31 Mean basal plasma insulin concentration before the IVGTT during the study (pmol/l) (see table 8.30).

Group	Day-1	Day-3	Day-6	Day-11	Day-15
C	194±122	160±34[a]	253±55	160±36	178±32[c]
GH	66±11	384±143[b]	446±169	373±65	230±58[d]
t	1.04	1.55	1.09	2.86	0.79
U	14.5	8	14.5	(1)*	13
p	ns	ns	ns	<0.05	ns

* Variances equivalent (vr=3.3, ns)
[a] Day-1 vs. 3: t=0.27, T=6, ns
[b] Day-1 vs. 3: (t=2.22, vr=171, p<0.01), T=0, p<0.05
[c] Day-11 vs. 15: t=0.34, T=9, ns
[d] Day-11 vs. 15: t=1.65, T=4, ns

Table 8.32 Basal insulinogenic index (pmol/mmol).

Patient	Day-1	Day-3	Day-6	Day-11	Day-15
C4	108.7	25.1	66.4	37.6	8.6
C6	13.2	43.6	18.3	13.2	43.4
C7	17.3	26.5	31.6	24.7	57.5
C9	10.5	22.0	41.4	30.6	33.5
C10	19.0	26.3	49.6	57.1	46.3
C11	8.3	27.7	29.2	18.6	48.4
Mean	29.5 ±15.9	28.5[a] ±3.1	39.4 ±6.9	30.3 ±6.4	39.6[c] ±7.0
GH1	16.0	61.1	96.3	71.3	75.0
GH2	20.8	27.9	34.7	63.0	20.1
GH3	13.9	93.6	173.8	70.3	75.4
GH5	11.1	31.4	8.9	49.0	40.9
GH6	12.4	143.3	65.0	97.0	35.0
GH7	4.7	13.5	16.2	46.7	11.2
Mean	13.2 ±2.2	61.8[b] ±20.1	65.8 ±25.3	66.2 ±7.5	42.9[d] ±11.1
t	1.02	1.64	1.0	3.65	0.25
U	15	8.5	16	2	18
p	ns	ns	ns	<0.01	ns

[a] Day-1 vs. 3: t=0.06, T=6, ns
[b] Day-1 vs. 3: (t=2.45, vr=84.0, p<0.01), T=0, p<0.05
[c] Day-11 vs. 15: t=0.89, T=5, ns
[d] Day-11 vs. 15: t=2.07, T=3, ns

Table 8.33 0-5' delta-glucose (mmol/l).

Patient	Day-1	Day-3	Day-6	Day-11	Day-15
C4	8.3	6.25	5.4	5.9	7.25
C6	8.25	7.35	9.3	6.85	8.5
C7	7.85	8.0	8.0	8.5	9.45
C9	8.85	10.6	7.95	7.3	9.3
C10	7.1	6.75	6.6	8.1	7.75
C11	9.3	8.3	7.95	9.05	8.45
Mean	8.28 ±0.31	7.88 ±0.63	7.53 ±0.55	7.62 ±0.47	8.45 ±0.35
GH1	8.35	6.8	8.9	8.1	8.15
GH2	11.2	9.45	9.9	8.9	13.9
GH3	13.0	10.25	10.9	11.75	10.15
GH5	9.55	6.8	7.1	7.4	7.9
GH6	11.15	9.3	12.65	8.8	7.0
GH7	8.35	8.25	6.95	7.7	14.9
Mean	10.27 ±0.75	8.48 ±0.59	9.40 ±0.91	8.78 ±0.64	10.33 ±1.36
t	2.44	0.7	1.76	1.45	1.34
U	4	13	9	10.5	14
p	<0.05	ns	ns	ns	ns

Table 8.34 Delta-glucose area (mmol/l.min).

Patient	Day-1	Day-3	Day-6	Day-11	Day-15
C4	192	193	112	149	200
C6	236	120	227	182	175
C7	206	220	232	174	164
C9	282	310	265	234	292
C10	195	200	218	200	203
C11	285	321	251	222	211
Mean	233 ±17	227 ±31	218 ±22	194 ±13	208 ±18
GH1	278	180	235	271	215
GH2	359	308	253	241	262
GH3	414	264	231	308	231
GH5	275	235	210	240	202
GH6	243	219	297	323	112
GH7	255	240	235	160	269
Mean	304 ±28	241 ±18	244 ±12	257 ±24	215 ±23
t	2.19	0.38	1.03	2.35	0.26
U	8	16	13	5	13
p	ns	ns	ns	<0.05	ns

Table 8.35 Glucose disappearance constant (k) (Normal range 1.47 - 1.97).

Patient	Day-1	Day-3	Day-6	Day-11	Day-15
C4	1.07	0.58	1.04	1.00	0.90
C6	1.39	1.23	1.38	1.70	1.00
C7	0.99	0.99	1.01	1.70	1.20
C9	1.22	1.03	0.44	0.77	0.92
C10	1.00	0.83	0.51	0.65	1.17
C11	1.02	0.80	1.28	1.39	1.55
Mean	1.12 ±0.06	0.91[a] ±0.09	0.94 ±0.16	1.20 ±0.19	1.12[c] ±0.10
GH1	0.84	1.17	0.59	0.51	1.14
GH2	0.97	0.91	0.80	1.25	1.76
GH3	1.09	1.17	1.73	1.87	2.77
GH5	0.95	0.68	0.59	0.53	1.01
GH6	1.76	1.12	0.40	0.51	1.30
GH7	0.87	1.24	0.81	1.70	1.20
Mean	1.08 ±0.14	1.05[b] ±0.09	0.82 ±0.19	1.06 ±0.26	1.53[d] ±0.27
t	0.23	1.1	0.49	0.44	1.42
U	10	11	14	14	8.5
p	ns	ns	ns	ns	ns

[a] Day-1 vs. 3: t=3.15, (T=0, vr=2.0, ns), $p<0.05$
[b] Day-1 vs. 3: t=0.2, T=10, ns
[c] Day-11 vs. 15: t=0.42, T=10, ns
[d] Day-11 vs. 15: t=2.29, T=2, ns

Table 8.36 0-5' delta-insulin (pmol/l).

Patient	Day-1	Day-3	Day-6	Day-11	Day-15
C4	877	817	568	777	404
C6	407	691	614	438	1023
C7	314	493	510	505	678
C9	201	552	257	560	334
C10	293	551	395	559	760
C11	267	336	464	521	392
Mean	393 ±101	573 ±68	468 ±53	560 ±47	599 ±110
GH1	184	710	831	187	922
GH2	431	875	564	1304	655
GH3	626	1299	434	1365	1198
GH5	387	531	289	85	-143
GH6	1098	654	1	135	686
GH7	98	88	127	929	138
Mean	471 ±147	693 ±163	374 ±123	668 ±246	576 ±203
t	0.44	0.68	0.7	0.43	0.1
U	16	13	13	18	18
p	ns	ns	ns	ns	ns

Table 8.37 0-5' Insulinogenic index (pmol/mmol).

Patient	Day-1	Day-3	Day-6	Day-11	Day-15
C4	105.6	130.7	105.2	132.7	55.7
C6	49.3	94.0	66.0	63.9	120.4
C7	40.0	61.6	63.8	59.4	71.8
C9	22.7	52.1	32.3	76.7	35.9
C10	41.3	81.6	59.9	69.0	98.1
C11	28.7	40.5	58.4	57.6	46.4
Mean	48.0 ±12.2	76.8[a] ±13.4	64.3 ±9.6	76.6 ±11.6	71.4[c] ±13.2
GH1	22.0	104.4	93.4	23.1	113.1
GH2	38.5	92.6	57.0	146.5	47.1
GH3	48.2	126.7	39.8	116.2	118.0
GH5	40.5	78.1	40.7	11.5	-18.1
GH6	98.5	70.3	0.1	15.3	98.0
GH7	11.7	10.7	18.3	120.6	9.3
Mean	43.2 ±12.3	80.5[b] ±16.2	41.5 ±13.2	72.2 ±25.3	61.2d ±23.4
t	0.27	0.18	1.4	0.16	0.38
U	14	16	8	16	16.5
p	ns	ns	ns	ns	ns

[a] Day-1 vs. 3: t=5.8, (T=0, vr=1.2, ns), p<0.01
[b] Day-1 vs. 3: t=2.06, T=3, ns
[c] Day-11 vs. 15: t=0.26, T=10, ns
[d] Day-11 vs. 15: t=0.31, T=8, ns

Table 8.38 Delta-insulin area (pmol/l.min).

Patient	Day-1	Day-3	Day-6	Day-11	Day-15
C4	29500	39600	20920	24360	6370
C6	11240	21500	11540	10550	16290
C7	9170	16460	20110	7890	9290
C9	7320	42110	9180	12620	5980
C10	14930	19690	20720	27100	31100
C11	9170	22660	19070	11750	4960
Mean	13560 ±3360	27000 ±4470	16920 ±2110	15710 ±3250	12330 ±4110
GH1	9420	23490	34560	18550	20940
GH2	23560	50310	28940	30430	13480
GH3	20610	61870	8650	31020	25330
GH5	12610	17420	17330	570	490
GH6	27990	16880	1090	21470	9470
GH7	1360	10820	8830	21300	7640
Mean	15930 ±4050	30130 ±8500	16570 ±5290	20560 ±4520	12890 ±3710
t	0.45	0.33	0.06	0.87	0.1
U	14	18	14	12	15
p	ns	ns	ns	ns	ns

Table 8.39 Total insulinogenic index (pmol/mmol).

Patient	Day-1	Day-3	Day-6	Day-11	Day-15
C4	153.7	205.2	186.8	163.5	31.9
C6	47.6	179.2	50.8	58.0	93.1
C7	44.5	74.8	86.7	45.3	56.7
C9	26.0	135.8	34.6	53.9	20.5
C10	76.6	98.5	95.1	135.5	153.2
C11	32.2	70.6	76.0	52.9	23.5
Mean	63.4 ±19.4	127.4[a] ±22.8	88.3 ±21.8	84.9 ±20.8	63.2[c] ±21.1
GH1	33.9	130.5	147.1	68.5	97.4
GH2	65.6	163.3	114.4	126.3	51.5
GH3	49.8	234.4	37.5	100.7	109.7
GH5	45.9	74.1	82.4	2.4	2.4
GH6	115.2	77.1	3.7	66.5	84.6
GH7	5.3	45.1	37.6	133.1	28.4
Mean	52.6 ±15.0	120.8[b] ±38.7	70.5 ±22.0	82.9 ±19.7	62.3[d] ±17.2
t	0.44	0.18	0.58	0.07	0.03
U	18	16	15	16	17
p	ns	ns	ns	ns	ns

[a] Day-1 vs. 3: t=3.44, T=0, $p<0.05$
[b] Day-1 vs. 3: t=2.19, T=2, ns
[c] Day-11 vs. 15: t=0.88, T=8, ns
[d] Day-11 vs. 15: t=0.91, T=6, ns

Table 9.1a Animal weights in the four experimental groups at study entry and during each drug administration period (g). Group CPS.

Rat		1	2	3	4	5
Entry		390	407	409	426	418
Period I	Day-1	369	392	397	407	423
	Day-5	376	390	392	400	427
	Change	+7	-2	-5	-7	+4
Period II	Day-1	377	400	395	403	431
	Day-5	385	407	404	417	441
	Change	+8	+7	+9	+14	+10

Table 9.1b Animal weights in the four experimental groups at study entry and during each drug administration period (g). Group CSP.

Rat		1	2	3	4	5
Entry		397	398	418	460	423
Period I	Day-1	380	373	401	451	390
	Day-5	386	379	386	455	391
	Change	+6	+6	-15	+4	+1
Period II	Day-1	374	380	389	459	396
	Day-5	384	380	383	456	405
	Change	+10	0	-6	-3	9

Table 9.1c Animal weights in the four experimental groups at study entry and during each drug administration period (g). Group BPS.

Rat		1	2	3	4	5	6
Entry		428	406	386	415	383	401
Period I	Day-1	409	418	390	412	386	386
	Day-5	382	377	362	395	351	361
	Change	-27	-41	-28	-17	-35	-25
Period II	Day-1	373	381	345	389	325	342
	Day-5	343	349	325	359	279	301
	Change	-30	-32	-20	-30	-46	-41

Table 9.1d Animal weights in the four experimental groups at study entry and during each drug administration period (g). Group BSP.

Rat		1	2	3	4	5	6
Entry		450	415	402	430	394	406
Period I	Day-1	447	405	401	425	367	409
	Day-5	423	345	377	402	363	394
	Change	-24	-60	-24	-23	-4	-15
Period II	Day-1	395	313	358	389	340	389
	Day-5	356	279	334	364	286	353
	Change	-39	-34	-24	-25	-54	-36

Table 9.2a Total burn surface areas in the two burned groups. Group BPS.

Rat	1	2	3	4	5	6
BSA[a]	605	584	565	593	562	579
Burn[b]	12x11½	12x12½	12x11	13x13½	12x11½	12x12
TBSA[c]	17.9	20.2	18.4	23.3	19.2	19.5

Table 9.2b Total burn surface areas in the two burned groups. Group BSP.

Rat	1	2	3	4	5	6
BSA[a]	625	593	580	607	572	584
Burn[b]	14x13½	12x12½	12x13½	13½x13	12x11	13x13
TBSA[c]	23.7	19.9	22.1	22.7	18.2	22.8

[a] Body surface area (cm^2)
[b] Burn-size (cm)
[c] Total burn surface area (%)

Table 9.3 Mean weight change during the drug administration periods in the four treatment groups (g/5day).

Group	CP	CS	BP	BS
n	10	10	12	12
Change	+0.7±2.0	+5.0±2.5	-32.1±2.8	-29.1±4.3

CP vs. CS			BP vs. BS		
t	T	p	t	T	p
1.57	15.5	ns	0.86	29	ns
CP vs. BP			**CS vs. BS**		
t	U	p	t	U	p
9.04	0	<0.0001	6.56	1.5	<0.0001

Table 9.4a Food consumption during each drug administration period in the four experimental groups (g/day). Group CPS.

Rat		1	2	3	4	5
Period I	Day-1	29	31	31	30	34
	Day-2	29	30	32	33	35
	Day-3	29	30	32	32	35
	Day-4	27	27	33	29	32
	Day-5	28	31	31	28	30
	Mean	28.4	29.8	31.8	30.4	33.2
	g/kg/day	77.0	76.0	80.1	74.7	78.5
Period II	Day-1	30	33	32	33	33
	Day-2	30	33	32	33	31
	Day-3	31	31	31	33	33
	Day-4	30	31	31	33	33
	Day-5	31	31	32	32	33
	Mean	30.4	31.8	31.6	32.8	32.6
	g/kg/day	80.6	79.5	80.0	81.4	75.6

Table 9.4b Food consumption during each drug administration period in the four experimental groups (g/day). Group CSP.

Rat		1	2	3	4	5
Period I	Day-1	31	30	28	26	20
	Day-2	31	30	28	35	27
	Day-3	31	30	28	34	27
	Day-4	30	24	28	29	27
	Day-5	31	28	27	35	27
	Mean	30.8	28.4	27.8	31.8	25.6
	g/kg/day	81.1	76.1	69.3	70.5	65.6
Period II	Day-1	31	28	28	30	33
	Day-2	31	30	26	30	32
	Day-3	31	31	30	35	32
	Day-4	31	31	30	32	31
	Day-5	31	31	31	35	32
	Mean	31.0	30.2	29.0	32.4	32.0
	g/kg/day	82.9	79.5	74.6	70.6	80.8

Table 9.4c Food consumption during each drug administration period in the four experimental groups (g/day). Group BPS.

Rat		1	2	3	4	5	6
Period I	Day-1	25	24	27	33	25	23
	Day-2	31	31	26	32	25	26
	Day-3	32	23	21	29	25	25
	Day-4	25	30	24	27	29	21
	Day-5	28	27	28	34	30	26
	Mean	28.2	27.0	25.2	31.0	26.8	24.2
	g/kg/day	68.9	64.6	64.6	75.2	69.4	62.7
Period II	Day-1	30	30	27	32	26	28
	Day-2	31	31	27	32	25	28
	Day-3	30	30	25	32	25	28
	Day-4	30	29	27	31	25	28
	Day-5	30	29	27	31	25	28
	Mean	30.2	29.8	26.6	31.6	25.2	28.0
	g/kg/day	81.0	78.2	77.1	81.2	77.5	81.9

Table 9.4d Food consumption during each drug administration period in the four experimental groups (g/day). Group BSP.

Rat		1	2	3	4	5	6
Period I	Day-1	21	25	28	29	22	30
	Day-2	26	28	30	25	28	28
	Day-3	35	29	30	31	27	5
	Day-4	31	30	27	25	29	27
	Day-5	31	30	27	30	27	31
	Mean	28.8	28.4	28.4	28.0	26.6	24.2
	g/kg/day	64.4	70.1	70.8	65.9	72.5	59.2
Period II	Day-1	32	26	28	31	27	30
	Day-2	33	27	29	32	27	30
	Day-3	32	26	29	32	27	31
	Day-4	33	26	29	31	28	29
	Day-5	33	25	28	24	28	28
	Mean	32.6	26.0	28.6	30.0	27.4	29.6
	g/kg/day	82.5	83.1	79.9	77.1	80.6	76.1

Table 9.5 Mean food consumption during the drug administration periods in the four treatment groups (g/day and g/kg/day).

Group	CP	CS	BP	BS
n	10	10	12	12
g/day	30.8±0.5	30.4±0.7	28.1±0.7	28.0±0.6
g/kg/day	77.5±1.1	76.0±1.8	73.7±2.1	73.3±2.1

	CP vs. CS			BP vs. BS		
	t	T	p	t	T	p
g/day	0.46	26	ns	0.1	30.5	ns
g/kg/day	0.65	17.5	ns	0.28	38.5	ns
	CP vs. BP			**CS vs. BS**		
	t	U	p	t	U	p
g/day	3.09	20.5	<0.01	2.51	25.5	<0.05
g/kg/day	1.46	46	ns	0.93	51	ns

Table 9.6a Nitrogen balance data during the last three days of each drug administration period in the four experimental groups (g/day). Group CPS.

Rat		1	2	3	4	5
			Dietary intake			
Period I	Day-3	0.752	0.778	0.829	0.829	0.907
	Day-4	0.700	0.700	0.855	0.752	0.829
	Day-5	0.726	0.804	0.804	0.726	0.778
	Mean	0.726	0.761	0.829	0.769	0.838
	g/kg/day	1.967	1.94	2.089	1.889	1.981
Period II	Day-3	0.804	0.804	0.804	0.855	0.855
	Day-4	0.778	0.804	0.804	0.855	0.855
	Day-5	0.804	0.804	0.829	0.829	0.855
	Mean	0.795	0.804	0.812	0.846	0.855
	g/kg/day	2.108	2.009	2.056	2.101	1.985
			Urinary excretion			
Period I	Day-3	0.412	0.249	0.485	0.337	0.413
	Day-4	0.253	0.289	0.359	0.300	0.634
	Day-5	0.315	0.265	0.296	0.475	0.272
	Mean	0.327	0.268	0.380	0.371	0.440
	g/kg/day	0.886	0.683	0.957	0.911	1.039
Period II	Day-3	0.274	0.436	0.449	0.391	0.314
	Day-4	0.375	0.374	0.523	0.349	0.511
	Day-5	0.400	0.294	0.405	0.345	0.367
	Mean	0.350	0.368	0.459	0.362	0.397
	g/kg/day	0.928	0.920	1.162	0.897	0.922
			Faecal excretion			
Period I	Day-3	0.062	0.147	0.135	0.081	0.199
	Day-4	0.203	0.233	0.321	0.221	0.096
	Day-5	0.203	0.148	0.220	1.377	0.303
	Mean	0.156	0.176	0.225	0.560	0.199
	g/kg/day	0.423	0.449	0.568	1.375	0.471
Period II	Day-3	0.398	0.198	0.311	0.432	0.158
	Day-4	0.311	0.134	0.206	0.319	0.301
	Day-5	0.281	0.401	0.301	0.229	0.269
	Mean	0.330	0.244	0.273	0.327	0.243
	g/kg/day	0.875	0.611	0.690	0.811	0.563

Table 9.6a Nitrogen balance data during the last three days of each drug administration period in the four experimental groups (g/day). Group CPS.

Rat		1	2	3	4	5
			Balance			
Period I	Day-3	0.278	0.382	0.209	0.411	0.295
	Day-4	0.244	0.178	0.175	0.231	0.099
	Day-5	0.208	0.391	0.288	-1.126	0.203
	Mean	0.243	0.317	0.224	-0.161	0.199
	g/kg/day	0.659	0.808	0.565	-0.396	0.471
Period II	Day-3	0.132	0.170	0.044	0.032	0.383
	Day-4	0.092	0.296	0.075	0.187	0.043
	Day-5	0.123	0.109	0.123	0.255	0.219
	Mean	0.116	0.192	0.081	0.158	0.215
	g/kg/day	0.306	0.478	0.204	0.393	0.500

Table 9.6b Nitrogen balance data during the last three days of each drug administration period in the four experimental groups (g/day). Group CSP.

Rat		1	2	3	4	5
			Dietary intake			
Period I	Day-3	0.804	0.778	0.726	0.881	0.700
	Day-4	0.778	0.622	0.726	0.752	0.700
	Day-5	0.804	0.726	0.700	0.907	0.700
	Mean	0.795	0.709	0.717	0.847	0.700
	g/kg/day	2.092	1.900	1.788	1.878	1.794
Period II	Day-3	0.804	0.804	0.778	0.907	0.829
	Day-4	0.804	0.804	0.778	0.829	0.804
	Day-5	0.804	0.804	0.804	(0.907)	0.829
	Mean	0.804	0.804	0.787	0.868	0.821
	g/kg/day	2.148	2.115	2.021	1.892	2.073
			Urinary excretion			
Period I	Day-3	0.412	0.437	0.414	0.443	0.463
	Day-4	0.491	0.468	0.430	0.387	0.458
	Day-5	0.423	0.445	0.419	0.420	0.323
	Mean	0.442	0.450	0.421	0.417	0.415
	g/kg/day	1.163	1.207	1.050	0.924	1.063
Period II	Day-3	0.484	0.450	0.404	0.497	0.317
	Day-4	0.396	0.124	0.219	0.396	0.451
	Day-5	0.443	0.425	0.350	-	0.354
	Mean	0.441	0.333	0.324	0.447	0.374
	g/kg/day	1.179	0.876	0.834	0.973	0.945
			Faecal excretion			
Period I	Day-3	0.128	0.211	0.090	0.356	0.187
	Day-4	0.299	0.198	0.183	0.246	0.260
	Day-5	0.159	0.149	0.204	0.271	0.186
	Mean	0.195	0.186	0.159	0.291	0.211
	g/kg/day	0.514	0.499	0.396	0.645	0.541
Period II	Day-3	0.093	0.194	0.368	0.463	0.243
	Day-4	0.385	0.272	0.306	0.431	0.215
	Day-5	0.207	0.602	0.291	-	0.299
	Mean	0.228	0.356	0.322	0.477	0.252
	g/kg/day	0.610	0.937	0.827	0.974	0.637

Table 9.6b Nitrogen balance data during the last three days of each drug administration period in the four experimental groups (g/day). Group CSP.

Rat		1	2	3	4	5
			Balance			
Period I	Day-3	0.264	0.130	0.222	0.082	0.050
	Day-4	-0.012	-0.044	0.113	0.119	-0.018
	Day-5	0.222	0.132	0.077	0.216	0.191
	Mean	0.158	0.073	0.137	0.139	0.074
	g/kg/day	0.414	0.194	0.342	0.308	0.190
Period II	Day-3	0.227	0.160	0.006	-0.053	0.269
	Day-4	0.023	0.408	0.253	0.002	0.138
	Day-5	0.154	-0.223	0.163	-	0.176
	Mean	0.135	0.115	0.141	-0.026	0.194
	g/kg/day	0.359	0.301	0.360	-0.055	0.491

Table 9.6c Nitrogen balance data during the last three days of each drug administration period in the four experimental groups (g/day). Group BPS.

Rat		1	2	3	4	5	6
				Dietary intake			
Period I	Day-3	0.829	0.596	0.544	0.752	0.648	0.648
	Day-4	0.648	0.778	0.622	0.700	0.752	0.544
	Day-5	0.726	0.700	0.726	0.881	0.778	0.674
	Mean	0.734	0.691	0.631	0.778	0.726	0.622
	g/kg/day	1.795	1.653	1.617	1.887	1.880	1.612
Period II	Day-3	0.778	0.778	0.648	0.829	0.648	0.726
	Day-4	0.778	0.752	0.700	0.804	0.648	0.726
	Day-5	0.778	0.752	0.700	0.804	0.648	0.726
	Mean	0.778	0.761	0.683	0.812	0.648	0.726
	g/kg/day	2.085	1.996	1.979	2.088	1.994	2.122
				Urinary excretion			
Period I	Day-3	0.554	0.538	0.563	0.562	0.531	0.536
	Day-4	1.034	0.337	0.483	0.507	0.498	0.396
	Day-5	0.488	0.359	0.433	0.559	0.436	0.495
	Mean	0.692	0.411	0.493	0.543	0.488	0.476
	g/kg/day	1.692	0.984	1.264	1.317	1.265	1.232
Period II	Day-3	0.422	0.483	0.318	0.375	0.441	0.438
	Day-4	0.399	0.472	0.327	0.394	0.406	0.451
	Day-5	0.347	0.396	0.373	0.659	0.497	0.457
	Mean	0.389	0.450	0.339	0.476	0.448	0.449
	g/kg/day	1.044	1.182	0.984	1.224	1.378	1.312
				Faecal excretion			
Period I	Day-3	0.186	0.151	0.071	0.184	0.101	0.053
	Day-4	0.094	0.327	0.053	0.160	0.138	0.090
	Day-5	0.216	0.203	0.097	0.362	0.233	0.097
	Mean	0.165	0.227	0.074	0.235	0.157	0.080
	g/kg/day	0.404	0.543	0.189	0.571	0.408	0.207
Period II	Day-3	0.284	0.076	0.540	0.360	0.366	0.410
	Day-4	0.251	0.167	0.170	0.206	0.404	0.273
	Day-5	0.316	0.459	0.175	0.291	0.115	0.208
	Mean	0.284	0.234	0.295	0.286	0.295	0.297
	g/kg/day	0.760	0.614	0.855	0.734	0.908	0.868

Table 9.6c Nitrogen balance data during the last three days of each drug administration period in the four experimental groups (g/day). Group BPS.

Rat		1	2	3	4	5	6
				Balance			
Period I	Day-3	0.089	-0.093	-0.090	0.006	0.016	0.059
	Day-4	-0.480	0.114	0.086	0.033	0.116	0.058
	Day-5	0.022	0.138	0.196	-0.040	0.109	0.082
	Mean	-0.123	0.053	0.064	0	0.080	0.066
	g/kg/day	-0.301	0.127	0.164	-0.001	0.207	0.172
Period II	Day-3	0.072	0.219	-0.210	0.094	-0.159	-0.122
	Day-4	0.128	0.113	0.203	0.204	-0.162	0.002
	Day-5	0.115	-0.103	0.152	-0.146	0.036	0.061
	Mean	0.105	0.076	0.048	0.051	-0.095	-0.020
	g/kg/day	0.280	0.200	0.140	0.130	-0.292	-0.058

Table 9.6d Nitrogen balance data during the last three days of each drug administration period in the four experimental groups (g/day). Group BSP.

Rat		1	2	3	4	5	6
				Dietary intake			
Period I	Day-3	0.907	0.752	0.778	0.804	0.700	0.130
	Day-4	0.804	0.778	0.700	0.648	0.752	0.700
	Day-5	0.804	0.778	0.700	0.778	0.700	(0.804)
	Mean	0.838	0.769	0.726	0.743	0.717	0.415
	g/kg/day	1.875	1.899	1.810	1.749	1.954	1.014
Period II	Day-3	0.829	0.674	0.752	0.829	0.700	0.804
	Day-4	0.855	0.674	0.752	0.804	0.726	0.752
	Day-5	0.855	0.648	0.726	0.622	0.726	0.726
	Mean	0.846	0.665	0.743	0.752	0.717	0.761
	g/kg/day	2.143	2.125	2.076	1.932	2.109	1.955
				Urinary excretion			
Period I	Day-3	0.756	0.439	0.574	0.524	0.423	0.408
	Day-4	1.107	0.425	0.383	0.509	0.418	0.607
	Day-5	0.437	0.543	0.494	0.442	0.413	-
	Mean	0.767	0.469	0.484	0.492	0.418	0.508
	g/kg/day	1.715	1.158	1.206	1.157	1.139	1.241
Period II	Day-3	0.573	0.425	0.380	0.523	0.467	0.457
	Day-4	0.139	0.385	0.286	0.301	0.085	0.265
	Day-5	0.449	0.534	0.538	0.572	0.512	0.419
	Mean	0.387	0.448	0.401	0.465	0.355	0.380
	g/kg/day	0.980	1.431	1.121	1.196	1.043	0.978
				Faecal excretion			
Period I	Day-3	0.071	0.148	0.108	0.174	0.112	0.012
	Day-4	0.165	0.101	0.318	0.183	0.103	0.209
	Day-5	0.190	0.170	0.320	0.117	0.435	-
	Mean	0.142	0.140	0.249	0.158	0.217	0.111
	g/kg/day	0.318	0.345	0.620	0.372	0.590	0.270
Period II	Day-3	0.294	0.297	0.436	0.228	0.297	0.608
	Day-4	0.230	0.167	0.298	0.206	0.368	0.240
	Day-5	0.290	0.157	0.251	0.630	0.167	0.199
	Mean	0.271	0.207	0.328	0.355	0.277	0.349
	g/kg/day	0.687	0.662	0.917	0.912	0.816	0.897

Table 9.6d Nitrogen balance data during the last three days of each drug administration period in the four experimental groups (g/day). Group BSP.

Rat		1	2	3	4	5	6
				Balance			
Period I	Day-3	0.08	0.165	0.096	0.106	0.165	-0.290
	Day-4	-0.468	0.252	-0.001	-0.044	0.231	-0.116
	Day-5	0.177	0.065	-0.114	0.219	-0.148	-
	Mean	-0.070	0.161	-0.006	0.094	0.083	-0.203
	g/kg/day	-0.158	0.396	-0.017	0.219	0.225	-0.497
Period II	Day-3	-0.038	-0.048	-0.064	0.078	-0.064	-0.261
	Day-4	0.486	0.122	0.168	0.297	0.273	0.247
	Day-5	0.116	-0.043	-0.063	-0.580	0.047	0.108
	Mean	0.188	0.010	0.014	-0.068	0.085	0.031
	g/kg/day	0.477	0.033	0.037	-0.176	0.250	0.080

Table 9.7 Mean nitrogen intake during the last three days of the drug administration periods in the four treatment groups (g/day and g/kg/day).

Group	CP	CS	BP	BS
n	10	10	12	12
g/day	0.801±0.013	0.788±0.019	0.722±0.018	0.718±0.031
g/kg/day	2.011±0.029	1.971±0.039	1.899±0.057	1.88±0.085

	CP vs. CS			BP vs. BS		
	t	T	p	t	T	p
g/day	0.6	23.5	ns	0.29	32.5	ns
g/kg/day	0.98	17	ns	0.15	35	ns
	CP vs. BP			CS vs. BS		
	t	U	p	t	U	p
g/day	3.34	17	<0.01	1.99	34	ns
g/kg/day	1.71	38	ns	0.84	49.5	ns

Table 9.8 Mean urinary nitrogen excretion during the last three days of the drug administration periods in the four treatment groups (g/day and g/kg/day).

Group	CP	CS	BP	BS
n	10	10	12	12
g/day	0.371±0.019	0.408±0.012	0.462±0.026	0.474±0.03
g/kg/day	0.928±0.041	1.024±0.038	1.209±0.061	1.228±0.054

	CP vs. CS			BP vs. BS		
	t	T	p	t	T	p
g/day	1.33	15	ns	0.79	27	ns
g/kg/day	1.35	14	ns	0.37	35	ns
	CP vs. BP			CS vs. BS		
	t	U	p	t	U	p
g/day	(2.71)*	18.5	<0.01	(1.9)*	26.5	<0.05
g/kg/day	3.65	(8)¤	<0.01	2.99	(21)¤	<0.01

* Variances not equivalent (vr=7.5, p<0.05)
¤ Variances equivalent (vr=2.7, ns)

Table 9.9 Mean faecal nitrogen excretion during the last three days of the drug administration periods in the four treatment groups (g/day and g/kg/day).

Group	CP	CS	BP	BS
n	10	10	12	12
g/day	0.292±0.041	0.246±0.019	0.227±0.028	0.226±0.020
g/kg/day	0.727±0.095	0.615±0.046	0.601±0.075	0.605±0.066

	CP vs. CS			BP vs. BS		
	t	T	p	t	T	p
g/day	1.41	16	ns	0.07	32	ns
g/kg/day	1.3	15	ns	0.07	37	ns
	CP vs. BP			CS vs. BS		
	t	U	p	t	U	p
g/day	1.35	47	ns	0.72	52	ns
g/kg/day	1.05	46	ns	0.12	59	ns

Table 9.10 Mean nitrogen balance during the last three days of the drug administration periods in the four treatment groups (g/day and g/kg/day).

Group	CP	CS	BP	BS
n	10	10	12	12
g/day	0.138±0.044	0.134±0.015	0.033±0.023	0.019±0.029
g/kg/day	0.356±0.111	0.333±0.036	0.089±0.058	0.047±0.075

	CP vs. CS			BP vs. BS		
	t	T	p	t	T	p
g/day	0.08	24	ns	0.42	39	ns
g/kg/day	0.19	23	ns	0.46	39	ns
	CP vs. BP			CS vs. BS		
	t	U	p	t	U	p
g/day	2.22	25	<0.05	3.27	19	<0.01
g/kg/day	2.24	26	<0.05	3.21	18	<0.01

Table 9.11 Mean serum insulin-like growth factor-I concentration at the time of sacrifice in the four treatment groups (U/ml) (Normal value 1U/ml).

Group\Rat	1	2	3	4	5	6	**Mean**
CP	0.68	0.65	0.67	0.64	0.60	-	**0.65±0.01**
CS	0.79	1.00	0.67	0.74	0.71	-	**0.78±0.06**
BP	0.58	0.46	0.55	0.63	0.52	0.45	**0.53±0.03**
BS	0.57	0.72	0.46	0.57	0.39	0.76	**0.58±0.06**

CP vs. CS			BP vs. BS		
t	U	p	t	U	p
(2.25)*	1.5	<0.05	0.72	14.5	ns
CP vs. BP			**CS vs. BS**		
t	U	p	t	U	p
3.43	1	<0.01	2.45	5	<0.05

* Variances not equivalent (vr=21.3, p<0.05)

Table 10.1a Body-weight and food consumption during the study in the eight experimental groups. Group CP6.

Rat	1	2	3	4	5	6	7	8
Day(s)				Weight (g)				
0	425	446	435	431	411	461	369	352
2	409	417	411	410	388	424	341	326
4	417	406	421	408	377	433	342	327
6	412	404	424	407	382	433	342	318
				Food consumption (g/2day)				
0-2	42	35	41	45	25	35	40	24
2-4	64	47	64	66	46	68	58	55
4-6	71	60	71	63	59	67	58	55
				Food consumption (g/kg/day)				
0-2	49	39	47	52	30	38	54	34
2-4	78	56	78	81	59	80	85	84
4-6	85	74	84	77	78	77	85	84

Table 10.1b Body-weight and food consumption during the study in the eight experimental groups. Group CS6.

Rat	1	2	3	4	5	6	7	8
Day(s)				Weight (g)				
0	385	446	435	428	379	348	417	442
2	365	415	416	396	356	317	391	403
4	368	418	418	404	365	320	392	415
6	366	415	419	397	358	322	398	411
				Food consumption (g/2day)				
0-2	40	37	44	35	27	28	46	35
2-4	54	59	64	64	58	46	66	65
4-6	55	64	66	65	60	54	66	71
				Food consumption (g/kg/day)				
0-2	52	42	51	41	36	40	55	40
2-4	74	71	77	81	82	73	84	81
4-6	75	77	79	80	82	84	84	86

Table 10.1c Body-weight and food consumption during the study in the eight experimental groups. Group BP6.

Rat	1	2	3	4	5	6	7	8	9	10	11	12
Day(s)					Weight (g)							
0	408	452	476	445	378	469	352	471	402	393	403	351
2	376	436	439	434	359	452	334	442	387	383	395	339
4	384	427	436	435	356	450	326	440	386	387	398	349
6	386	407	422	426	351	455	315	444	364	371	386	328
					Food consumption (g/2day)							
0-2	2	17	12	17	4	18	11	7	25	24	15	16
2-4	43	55	56	53	40	64	37	52	65	65	59	58
4-6	60	53	74	68	60	77	47	73	65	65	68	60
					Food consumption (g/kg/day)							
0-2	3	19	13	19	5	19	16	7	31	31	19	23
2-4	57	63	64	61	56	71	55	59	84	85	75	86
4-6	78	62	85	78	84	86	72	83	84	84	85	86

Table 10.1d Body-weight and food consumption during the study in the eight experimental groups. Group BS6.

Rat	1	2	3	4	5	6	7	8	9	10	11	12
Day(s)					Weight (g)							
0	420	460	432	449	459	422	397	444	409	395	387	357
2	406	430	393	426	428	410	377	427	400	385	374	340
4	407	441	410	433	457	410	373	418	396	370	379	351
6	397	424	391	419	443	383	366	393	386	348	362	323
					Food consumption (g/2day)							
0-2	25	23	6	12	12	19	3	27	29	23	18	10
2-4	54	61	44	48	60	60	34	45	68	56	64	58
4-6	60	55	49	62	69	37	50	47	67	63	65	60
					Food consumption (g/kg/day)							
0-2	30	25	7	13	13	23	4	30	36	29	23	14
2-4	67	71	56	56	70	73	45	53	85	73	86	85
4-6	74	62	60	72	76	45	67	56	85	85	86	86

Table 10.1e Body-weight and food consumption during the study in the eight experimental groups. Group CP14.

Rat	1	2	3	4	5	6	7	8
Day(s)				Weight (g)				
0	419	441	387	408	388	391	380	385
2	388	413	363	400	367	394	370	356
4	393	425	371	399	377	400	377	366
6	393	421	358	403	371	404	383	374
8	391	405	364	404	369	412	393	377
10	394	409	357	406	374	405	396	379
12	386	430	360	422	373	415	399	383
14*	392	430	352	423	367	413	404	379
				Food consumption (g/2day)				
0-2	15.	35	38	46	33	50	32	29
2-4	51	64	62	62	62	67	60	60
4-6	57	66	63	59	64	68	64	62
6-8	49	71	61	63	63	69	65	64
8-10	61	69	62	69	63	60	67	64
10-12	54	70	60	69	64	69	67	65
12-14	49	73	61	68	64	71	68	65
				Food consumption (g/kg/day)				
0-2	18	40	49	56	43	64	42	38
2-4	66	78	85	78	85	85	81	83
4-6	73	78	85	74	85	85	85	85
6-8	62	84	85	78	85	85	85	86
8-10	78	85	85	85	85	73	85	85
10-12	69	86	84	85	86	85	85	86
12-14	64	85	85	81	86	86	85	85

* Day-2 vs. 14 (381±7 vs. 395±10): $t=2.62$, $T=2$, $p<0.05$

Table 10.1f Body-weight and food consumption during the study in the eight experimental groups. Group CS14.

Rat	1	2	3	4	5	6	7	8
Day(s)				Weight (g)				
0	429	406	388	420	375	400	401	406
2	425	380	355	376	352	385	372	384
4	418	385	357	390	369	403	378	389
6	423	383	360	393	376	405	386	393
8	423	381	370	385	372	402	395	393
10	423	395	370	393	373	399	396	393
12	431	397	378	406	372	406	405	406
14*	432	390	382	416	384	408	404	419
				Food consumption (g/2day)				
0-2	38	27	32	23	36	42	30	40
2-4	50	54	49	64	60	65	52	62
4-6	60	58	60	66	58	69	58	60
6-8	54	56	61	67	64	69	65	55
8-10	59	65	63	65	63	68	65	67
10-12	60	67	63	67	64	68	67	67
12-14	60	64	65	69	63	69	69	69
				Food consumption (g/kg/day)				
0-2	44	33	41	27	48	53	37	49
2-4	59	71	69	85	85	84	70	81
4-6	72	75	84	85	79	86	77	77
6-8	64	73	85	85	85	85	84	70
8-10	70	85	85	84	85	85	82	85
10-12	71	85	85	85	86	85	85	85
12-14	70	81	86	85	85	85	85	85

* Day-2 vs. 14 (379±8 vs. 404±6): t=6.17, T=0, p<0.001

Table 10.1g Body-weight and food consumption during the study in the eight experimental groups. Group BP14.

Rat	1	2	3	4	5	6	7	8	9	10	11	12
Day(s)						Weight (g)						
0	420	383	422	393	380	445	419	336	436	393	394	410
2	416	368	401	385	375	446	420	333	421	380	371	396
4	423	370	405	394	368	455	429	Died	424	391	378	397
6	403	366	395	384	354	436	415	-	404	375	371	388
8	384	374	392	380	331	437	388	-	397	348	355	380
10	355	381	386	367	304	427	349	-	382	330	322	369
12	345	373	362	352	267	414	300	-	355	302	283	342
14*	312	365	329	326	236	397	265	-	329	262	263	310
						Food consumption (g/2day)						
0-2	18	15	12	14	28	21	25	0	18	18	13	25
2-4	57	40	54	62	57	65	63	-	68	65	59	67
4-6	57	58	68	67	63	72	66	-	72	66	62	67
6-8	69	62	67	65	60	74	57	-	69	64	63	64
8-10	65	64	66	65	56	74	66	-	67	60	60	65
10-12	60	65	65	62	52	72	60	-	65	56	54	63
12-14	59	64	61	60	45	71	51	-	60	51	48	58
						Food consumption (g/kg/day)						
0-2	21	20	14	18	37	24	30	0	21	23	17	31
2-4	69	54	67	81	76	73	75	-	81	86	80	85
4-6	67	78	84	85	86	79	77	-	85	84	82	84
6-8	86	85	85	85	85	85	69	-	85	85	85	83
8-10	85	86	84	86	85	85	85	-	84	86	85	86
10-12	85	85	84	85	86	84	86	-	85	85	84	85
12-14	86	86	84	85	84	86	85	-	85	84	85	85

* Day-2 vs. 14 (393±9 vs. 309±15): t=6.91, T=0, $p<0.001$

Table 10.1h Body-weight and food consumption during the study in the eight experimental groups. Group BS14.

Rat	1	2	3	4	5	6	7	8	9	10	11	12
Day(s)					Weight (g)							
0	358	412	406	389	369	442	419	370	439	396	373	375
2	333	374	387	379	Died	441	415	358	435	376	359	365
4	339	386	395	375	-	438	425	330	446	389	367	376
6	342	385	371	364	-	424	412	347	434	376	347	357
8	351	386	357	352	-	413	395	312	425	369	333	349
10	349	389	336	339	-	400	356	289	423	336	319	315
12	341	376	304	304	-	379	329	269	406	301	291	281
14*	334	355	260	271	-	330	302	247	385	253	250	265
					Food consumption (g/2day)							
0-2	5	13	13	14	-	27	23	0	30	17	9	16
2-4	35	51	51	55	-	60	67	8	68	61	45	62
4-6	58	65	67	64	-	59	72	43	76	66	62	64
6-8	58	65	63	62	-	68	70	59	70	64	59	61
8-10	60	61	60	60	-	71	67	53	72	63	57	60
10-12	60	66	57	58	-	68	60	49	72	57	54	54
12-14	58	64	52	52	-	65	56	46	69	51	49	48
					Food consumption (g/kg/day)							
0-2	7	16	16	18	-	31	27	0	34	21	12	21
2-4	53	68	66	73	-	68	81	11	78	81	63	85
4-6	86	84	85	85	-	67	85	65	85	85	85	85
6-8	85	84	85	85	-	80	85	85	81	85	85	85
8-10	86	79	84	85	-	86	85	85	85	85	86	86
10-12	86	85	85	86	-	85	84	85	85	85	85	86
12-14	85	85	86	86	-	86	85	86	85	85	84	85

* Day-2 vs. 14 (384±10 vs. 296±15): t=6.62, T=1, $p<0.001$

Table 10.2 Mean weight in the four treatment groups during the study (g).

Day/Group	CP	n	CS	n	BP	n	BS	n
0	408±8	16	407±7	16	410±8	24	407±7	24
2*	386±7	16	381±7	16	395±7	24	392±6	23
4	390±7	16	387±7	16	400±7	23	396±7	23
6	389±8	16	388±7	16	389±7	23	382±7	23
8	389±6	8	390±6	8	379±8	11	367±10	11
10	390±7	8	393±6	8	361±10	11	350±12	11
12	396±9	8	400±7	8	336±13	11	326±14	11
14	395±10	8	404±6	8	309±15	11	296±15	11

* Day-0 vs. 2: CP t=8.79, T=1, p<0.001
CS t=10.94, T=0, p<0.001
BP t=7.3, T=3, p<0.001
BS t=7.99, T=0, p<0.001

Day-2 vs. 14: See Table 10.1

Day	t	U	p	t	U	p
	CP vs. CS			BP vs. BS		
0	0.15	123	ns	0.22	278.5	ns
2	0.55	111.5	ns	0.33	254.5	ns
4	0.31	116	ns	0.42	246	ns
6	0.15	122	ns	0.66	227	ns
8	0.09	31.5	ns	0.85	49	ns
10	0.32	29.5	ns	0.69	49.5	ns
12	0.38	29	ns	0.55	53	ns
14	0.81	25.5	ns	0.63	52.5	ns
	CP vs. BP			CS vs. BS		
0	0.14	185	ns	0.09	191.5	ns
2	0.86	163	ns	1.2	143	ns
4	0.99	151	ns	0.9	155	ns
6	0.03	179	ns	0.56	158	ns
8	0.94	31	ns	1.71	24.5	ns
10	2.15	19	<0.05	2.85	16.5	<0.05
12	3.5	7.5	<0.01	4.42	(8.5)*	<0.001
14	(4.51)¤	5	<0.01	6.04	2	<0.001

* Variances equivalent (vr=5.9, ns)
¤ Variances not equivalent (vr=7.4, p<0.05)

Table 10.3 Total burn surface areas in the four burned groups.

Group\Rat	1	2	3	4	5	6	7	8	9	10	11	12
BP6												
BSA[a]	588	625	648	621	559	644	529	644	578	573	583	529
Burn[b]	12x10	12x12	12x12½	12x12	12x11	13x12	11x9	12½x11½	12½x11	12x12	12½x11	12x11
TBSA[c]	16.4	18.0	18.2	18.2	18.7	19.1	14.9	17.5	18.8	19.8	18.8	19.6
Mean[c]	**18.2±0.4**											
BS6												
BSA[a]	597	635	607	625	635	597	573	621	588	573	564	534
Burn[b]	12x11	12x11	12x12	13x12½	12x12	12x11	12½x12½	11½x11½	11½x11	12x11	11½x11	12x10½
TBSA[c]	17.4	16.4	18.6	20.5	17.8	17.4	21.4	16.8	16.9	18.1	17.5	18.7
Mean[c]	**18.1±0.4**											
BP14												
BSA[a]	597	564	597	573	559	621	597	514	611	573	573	588
Burn[b]	13x10½	11x10½	12½x11½	12x12	11x11	12x11	13x11	12x11	12½x11½	12x11	12½x11½	12x11
TBSA[c]	18.4	16.2	18.9	19.8	17.0	16.8	19.1	20.2	18.5	18.2	19.7	17.7
Mean[c]	18.4±0.4											
BS14												
BSA[a]	539	588	583	568	549	616	597	549	616	573	554	554
Burn[b]	11x11	11x11½	12x12	13x12	12x10	12x12½	11½x12½	12½x11½	12½x12	12x11	12½x11½	11x11
TBSA[c]	17.7	16.8	19.4	21.7	17.5	19.1	18.9	20.6	19.2	18.1	20.5	17.2
Mean[c]	**18.9±0.4**											

[a] Body surface area (cm^2)
[b] Burn size (cm)
[c] Total burn surface area (%)

Table 10.4 Mean food consumption in the four treatment groups during the study (g/2day).

Days/Group	CP	n	CS	n	BP	n	BS	n
0-2	35.3±2.3	16	35.0±1.7	16	15.6±1.5	24	16.3±1.8	23
2-4	59.8±1.7	16	58.3±1.6	16	56.7±1.9	23	52.8±2.9	23
4-6	62.9±1.2	16	61.9±1.2	16	64.7±1.5	23	60.0±2.0	23
6-8	63.1±2.3	8	61.4±2.0	8	64.9±1.4	11	63.6±1.3	11
8-10	64.4±1.3	8	64.4±1.0	8	64.4±1.4	11	62.2±1.7	11
10-12	64.8±1.9	8	65.4±1.0	8	61.3±1.7	11	59.6±2.0	11
12-14	64.9±2.6	8	66.0±1.2	8	57.1±2.3	11	55.5±2.3	11

Day	t	U	p	t	U	p
	CP vs. CS			BP vs. BS		
0-2	0.11	122	ns	0.27	269	ns
2-4	0.64	111.5	ns	1.13	225.5	ns
4-6	0.62	114.5	ns	1.91	183.5	ns
6-8	0.56	27	ns	0.71	50.5	ns
8-10	0	30.5	ns	0.98	44.5	ns
10-12	0.29	29	ns	0.65	50.5	ns
12-14	0.34	31	ns	0.5	53.5	ns
	CP vs. BP			CS vs. BS		
0-2	7.56	21	<0.001	7.3	15	<0.001
2-4	1.14	151	ns	1.47	143	ns
4-6	0.87	142.5	ns	0.73	178	ns
6-8	0.69	39.5	ns	0.94	34.5	ns
8-10	0.01	44	ns	0.99	29.5	ns
10-12	1.33	27	ns	2.29	19.5	<0.05
12-14	2.24	16.5	<0.05	3.61	12	<0.01

Table 10.5 Mean food consumption in the four treatment groups during the study (g/kg/day).

Days/Group	CP	n	CS	n	BP	n	BS	n
0-2	43.4±2.8	16	43.1±1.9	16	19.1±1.9	24	19.6±2.1	23
2-4	77.7±2.3	16	76.6±1.9	16	71.3±2.3	23	67.2±3.5	23
4-6	80.8±1.2	16	80.0±1.1	16	80.9±1.3	23	76.0±2.5	23
6-8	81.3±2.8	8	78.9±3.0	8	83.3±1.5	11	84.1±0.6	11
8-10	82.8±1.7	8	82.7±1.9	8	85.0±0.2	11	84.6±0.6	11
10-12	83.0±2.1	8	83.4±1.8	8	84.9±0.2	11	85.0±0.2	11
12-14	81.9±2.7	8	82.6±1.9	8	84.9±0.2	11	85.2±0.1	11

Day	t	U	p	t	U	p
	CP vs. CS			BP vs. BS		
0-2	0.07	125	ns	0.13	275	ns
2-4	0.32	110.5	ns	0.99	232	ns
4-6	0.46	114.5	ns	1.64	252.5	ns
6-8	0.57	25.5	ns	0.41	46	ns
8-10	0	29	ns	0.70	49	ns
10-12	0.05	28.5	ns	0.98	47.5	ns
12-14	0.19	29.5	ns	0.90	48.5	ns
	CP vs. BP			CS vs. BS		
0-2	7.45	21	<0.001	7.9	9	<0.001
2-4	1.84	127	ns	(2.09)*	116	ns
4-6	0.05	179	ns	1.26	182.5	ns
6-8	0.74	37.5	ns	1.98	30.5	ns
8-10	1.83	27	ns	1.23	25	ns
10-12	0.95	38.5	ns	1.2	36.5	ns
12-14	1.28	41	ns	1.56	29	ns

* Variances not equivalent (vr=5.0, $p<0.05$)

Table 10.6 Haemoglobin concentration at the time of sacrifice in the eight experimental groups (g/dl).

Group\Rat	1	2	3	4	5	6	7	8	9	10	11	12
CP6	13.5	14.9	13.4	13.4	15.0	13.8	12.7	13.5				
CS6	13.7	14.0	12.9	13.2	12.2	14.5	13.5	14.1				
BP6	11.3	11.1	12.9	11.9	11.3	12.1	12.1	12.0	12.8	12.9	11.9	13.0
BS6	13.6	11.1	11.5	12.3	11.9	10.6	13.9	12.7	12.0	11.1	13.3	12.9
CP14	14.1	13.9	14.8	14.6	15.2	14.8	13.9	14.5				
CS14	13.8	15.0	14.8	13.1	12.8	13.9	12.3	13.5				
BP14	12.1	12.8	11.7	13.8	14.7	13.2	10.7	-	13.2	15.8	7.1	12.2
BS14	13.7	12.3	14.8	10.3	-	14.3	14.0	*	12.2	14.9	12.9	6.8

* Clotted sample

Table 10.7 Mean haemoglobin concentration at the time of sacrifice in the four treatment groups (g/dl).

Group	Day-6	n	Day-14	n
CP	13.8±0.3	8	14.5±0.2	8
CS	13.5±0.3	8	13.7±0.3	8
BP	12.1±0.2	12	12.5±0.7	11
BS	12.2±0.3	12	12.6±0.8	10

Comparison	t	U	p
CP vs CS, Day-6	0.69	29	ns
CP vs CS, Day-14	2.23	14	ns
BP vs BS, Day-6	0.37	68.5	ns
BP vs BS, Day-14	0.13	47.5	ns
CP vs BP, Day-6	5.08	4	<0.001
CP vs BP, Day-14	2.42	13	<0.05
CS vs BS, Day-6	(2.94)*	15.5	<0.05
CS vs BS, Day-14	1.11	32	ns
CP, Day-6 vs Day-14	2.15	14	ns
CS, Day-6 vs Day-14	0.33	30.5	ns
BP, Day-6 vs Day-14	0.54	45.5	ns
BS, Day-6 vs Day-14	0.48	40	ns

* Variances not equivalent (vr=12.8, p<0.05)

Table 10.8 Serum albumin concentration at the time of sacrifice in the eight experimental groups (% of normal).

Group\Rat	1	2	3	4	5	6	7	8	9	10	11	12
CP6	115	76	115	107	86	86	67	83				
CS6	107	120	120	104	115	120	67	94				
BP6	30	11	73	73	56	62	40	45	67	24	62	70
BS6	76	11	11	83	45	10	83	30	48	9	73	75
CP14	120	118	94	85	98	92	98	89				
CS14	104	98	94	89	92	94	92	92				
BP14	56	36	30	62	53	60	51	-	51	56	53	44
BS14	62	48	40	18	-	75	50	60	51	50	60	40

Table 10.9 Mean serum albumin concentration at the time of sacrifice in the four treatment groups (% of normal).

Group	Day-6	n	Day-14	n
CP	91.9±6.4	8	99.3±4.6	8
CS	105.9±6.5	8	94.4±1.6	8
BP	51.1±6.0	12	50.2±3.0	11
BS	46.2±9.0	12	50.4±4.5	11

Comparison	t	U	p
CP vs CS, Day-6	1.54	17	ns
CP vs CS, Day-14	1.0	27	ns
BP vs BS, Day-6	0.46	72	ns
BP vs BS, Day-14	0.03	57.5	ns
CP vs BP, Day-6	4.5	3.5	<0.001
CP vs BP, Day-14	9.41	0	<0.001
CS vs BS, Day-6	4.88	5	<0.001
CS vs BS, Day-14	8.06	0	<0.001
CP, Day-6 vs Day-14	0.93	20	ns
CS, Day-6 vs Day-14	(1.72)*	11.5	<0.05
BP, Day-6 vs Day-14	0.13	53.5	ns
BS, Day-6 vs Day-14	0.41	61	ns

* Variances not equivalent (vr=43.5, p<0.01)

Table 10.10 Peak forces applied to the musculofascial wounds in the eight experimental groups and the mean for each animal (N/cm).

Group\Rat	1	2	3	4	5	6	7	8	9	10	11	12
CP6	4.7	3.5	7.3	5.0	1.8	7.9	2.9	5.2				
	5.2	5.6	2.6	10.6	5.2	2.8	6.0	5.3				
Mean	4.95	4.55	4.95	7.8	3.5	5.35	4.45	5.25				
CS6	6.1	10.0	4.6	12.9	6.9	7.3	5.2	11.0				
	9.9	3.0	10.3	3.9	5.5	2.6	8.7	4.5				
Mean	8.0	6.5	7.45	8.4	6.2	4.95	6.95	7.75				
BP6	1.9	1.8	3.4	2.9	1.7	1.9	3.8	3.1	2.7	3.2	4.9	2.1
	5.4	5.5	4.4	5.2	2.2	4.3	1.5	4.1	2.2	5.1	4.1	1.4
Mean	3.65	3.65	3.9	4.05	1.95	3.1	2.65	3.6	2.45	4.15	4.5	1.75
BS6	5.7	2.9	1.3	2.0	5.8	8.2	2.3	2.9	3.9	3.8	3.8	0.9
	4.0	6.2	4.2	6.2	2.4	2.4	2.3	6.1	6.7	4.5	2.7	2.6
Mean	4.85	4.55	2.75	4.1	4.1	5.3	2.3	4.5	5.3	4.15	3.25	1.75
CP14	4.3	20.1	7.3	5.5	8.0	9.2	12.3	6.9				
	6.3	6.4	10.5	10.6	9.8	3.7	6.7	15.6				
Mean	5.3	13.25	8.9	8.05	8.9	6.45	9.5	11.25				
CS14	10.3	9.3	10.3	5.9	15.3	9.4	6.0	5.0				
	6.7	13.1	13.0	8.6	9.4	5.2	14.1	13.6				
Mean	8.5	11.2	11.65	7.25	12.35	7.3	10.05	9.3				
BP14	8.3	7.9	7.6	10.3	11.1	13.8	7.2	-	10.7	14.5	5.3	6.7
	15.5	7.2	3.7	3.4	7.2	8.8	17.0	-	5.8	3.0	2.2	6.0
Mean	11.9	7.55	5.65	6.85	9.15	11.3	12.1	-	8.25	8.75	3.75	6.35
BS14	14.4	12.3	8.2	3.6	-	5.7	6.7	1.4	9.1	9.9	8.2	5.7
	10.8	9.7	6.0	11.8	-	10.7	11.7	5.0	8.0	13.1	5.1	12.9
Mean	12.6	11.0	7.1	7.7	-	8.2	9.2	3.2	8.55	11.5	6.65	9.3

Table 10.11 Mean peak forces applied to the muscle wounds in the four treatment groups (N/cm).

Group	Day-6	n	Day-14	n
CP	5.1±0.4	8	9.0±0.9	8
CS	7.0±0.4	8	9.7±0.7	8
BP	3.3±0.3	12	8.3±0.8	11
BS	3.9±0.3	12	8.6±0.8	11

Comparison	t	U	p
CP vs CS, Day-6	(3.26)*	9	<0.05
CP vs CS, Day-14	0.67	26	ns
BP vs BS, Day-6	1.48	42.5	ns
BP vs BS, Day-14	0.28	54	ns
CP vs BP, Day-6	3.82	8	<0.01
CP vs BP, Day-14	0.51	38	ns
CS vs BS, Day-6	5.98	2	<0.001
CS vs BS, Day-14	0.97	32.5	ns
CP, Day-6 vs Day-14	3.88	3	<0.01
CS, Day-6 vs Day-14	3.37	8	<0.01
BP, Day-6 vs Day-14	6.18	4	<0.001
BS, Day-6 vs Day-14	5.74	9	<0.001

* Variances not equivalent (vr=9, $p<0.05$)

Table 10.12 Peak forces applied to the skin wounds in the eight experimental groups and the mean for each animal (N/cm).

Group\Rat	1	2	3	4	5	6	7	8	9	10	11	12
CP6	3.5	2.7	3.5	1.8	2.4	1.9	2.6	1.9				
	2.9	1.6	2.2	1.9	1.7	2.0	2.7	3.1				
Mean	3.2	2.15	2.85	1.85	2.05	1.95	2.65	2.5				
CS6	0.9	3.2	3.3	3.4	2.3	1.9	2.8	2.7				
	2.6	1.8	2.7	3.3	1.4	2.2	3.4	2.2				
Mean	1.75	2.5	3.0	3.35	1.85	2.05	3.1	2.45				
BP6	2.2	0.8	1.6	2.8	1.3	1.9	2.0	2.0	1.2	2.2	3.3	1.9
	1.7	1.3	1.2	1.4	1.6	1.4	1.3	2.4	1.9	1.7	3.0	1.7
Mean	1.95	1.05	1.4	2.1	1.45	1.65	1.65	2.2	1.55	1.95	3.15	1.8
BS6	2.3	0.9	1.4	2.5	1.6	1.2	3.4	1.1	2.4	1.2	2.1	2.0
	2.4	1.5	1.4	2.4	1.3	0.6	1.0	2.0	3.0	1.4	2.8	2.5
Mean	2.35	1.2	1.4	2.45	1.45	0.9	2.2	1.55	2.7	1.3	2.45	2.25
CP14	3.7	10.3	8.9	5.5	4.4	6.1	5.4	12.1				
	3.7	8.6	6.1	9.4	7.6	10.1	7.8	9.2				
Mean	3.7	9.45	7.5	7.45	6.0	8.1	6.6	10.65				
CS14	5.9	3.0	6.8	5.6	11.1	6.7	4.9	5.5				
	5.3	2.5	6.3	4.4	8.0	11.0	9.5	12.7				
Mean	5.6	2.75	6.55	5.0	9.55	8.85	7.2	9.1				
BP14	3.6	9.0	7.4	7.2	6.3	7.7	8.2	-	6.8	11.6	9.2	8.2
	-	7.6	8.7	8.1	-	12.8	9.8	-	10.0	13.1	-	9.5
Mean	3.6	8.3	8.05	7.65	6.3	10.25	9.0	-	8.4	12.35	9.2	8.85
BS14	10.8	6.8	16.0	1.8	-	7.1	8.4	3.4	5.1	11.8	8.3	4.2
	7.4	8.7	-	-	-	13.0	9.4	3.6	7.7	14.9	9.7	10.2
Mean	9.1	7.75	16.0	1.8	-	10.05	8.9	3.5	6.4	13.35	9.0	7.2

Table 10.13 Mean peak forces applied to the skin wounds in the four treatment groups (N/cm).

Group	Day-6	n	Day-14	n
CP	2.4±0.2	8	7.4±0.8	8
CS	2.5±0.2	8	6.8±0.8	8
BP	1.8±0.2	12	8.4±0.7	11
BS	1.9±0.2	12	8.5±1.2	11

Comparison	t	U	p
CP vs CS, Day-6	0.39	30.5	ns
CP vs CS, Day-14	0.54	26	ns
BP vs BS, Day-6	0.11	69	ns
BP vs BS, Day-14	0.07	60.5	ns
CP vs BP, Day-6	2.47	17	<0.05
CP vs BP, Day-14	0.92	31	ns
CS vs BS, Day-6	2.38	21	<0.05
CS vs BS, Day-14	1.03	32	ns
CP, Day-6 vs Day-14	6.54	0	<0.001
CS, Day-6 vs Day-14	5.05	3	<0.01
BP, Day-6 vs Day-14	10.0	0	<0.001
BS, Day-6 vs Day-14	5.67	6	<0.001