METABOLIC STUDIES IN PATIENTS UNDERGOING THORACIC SURGERY

Being a thesis presented for the Award of the

Degree of Doctor of Philosophy

in the University of Surrey

by

A. Boroumand-Naini, B.Sc., M.Sc. (London).

September, 1978

Department of Biochemistry,
University of Surrey,
Guildford, Surrey

ABSTRACT

1. The metabolic changes following thoracic surgery in three groups of patients, (oesophageal cancer, lung cancer and hiatus hernia) have been studied.

2. Fasting levels of plasma glucose in patients with tumours of the lung or oesophagus were within the normal range and were no different from those found in patients with hiatus hernia, before operation.

3. Hyperglycaemia occurred after oesophagectomy, oesophagogastrectomy and herniorrhapy. Operations of the lung, such as pneumonectomy or lobectomy did not lead to an immediate rise in blood sugar level after surgery.

4. Post-operative hyperglycaemia was accompanied by the fall in the levels of plasma glucogenic amino acids. Evidence is presented in support of the idea that post-operative hyperglycaemia is the result of increased glucose production rather than the decrease in glucose utilization.

5. In contrast to the plasma insulin concentrations which remained unchanged immediately after surgery, the levels of plasma 11-hydroxy-corticosteroids rose immediately after operation and that was accompanied by the same rise in the levels of plasma FFA.

6. Plasma insulin concentration rose significantly and the rise was not proportional to the level of blood glucose on the second post-operative day. Since the urinary excretion of ketone bodies was also high on the same day, there was evidence of post-operative insulin resistance.

7. Elevated plasma levels of glucagon coincided with hyperglycaemia in oesophageal cancer patients but did not occur in lung cancer patients in whom there was no hyperglycaemia.

8. The plasma free tryptophan level in patients with oesophageal or lung cancer tended to be lower than in patients with hiatus hernia. Furthermore, the concentration of plasma free tryptophan rose after surgery and this rise was associated with a fall in the level of plasma total tryptophan.

9. There was no significant correlation between the level of plasma tryptophan and the rate of urinary excretion of N'-methylnicotinamide (NMN) in patients with oesophageal cancer. The significance of these findings has been discussed in relation to the metabolism of tryptophan.

10. The concentration of copper in the plasma was found to be elevated in patients with oesophageal cancer. Thoracic surgery was not associated with a consistent change in the level of plasma copper.

11. There was a transient fall in the level of plasma zinc after operation and this was associated with a similar fall in urinary excretion in hiatus hernia and oesophageal cancer.

12. Urinary levels of cyclic AMP or GMP in patients with tumours of the lung or oesophagus were no different from those found in patients with hiatus hernia. Cyclic GMP increased after surgery, and was higher in patients with malignancy than in patients with hiatus hernia.

13. Post-operative parenteral nutrition prevented the fall of plasma amino acids and led to a rise of plasma albumin. It also diminished the urinary losses of nitrogen on the second post-operative day.

In loving memory of my father, Hossain

and to my mother, Trab.

ACKNOWLEDGEMENTS

I would like to express my deep gratitude to Professor J.W.T. Dickerson for his guidance, constant encouragement and constructive criticism. I would also like to thank Mr. M. Brown and Mr. R. Rowlandson (Milford Chest Hospital) for allowing me to study patients under their care, Sister R. Watson and other nursing staff of F Ward, Milford Chest Hospital for their help in collecting blood and urine specimens. I am thankful to Miss E.A. Drury for the measurement of blood thiamin, Dr. P. Wood for determination of urinary cyclic nucleotides and Mr. D. Tsiolakis for plasma glucagon estimations.

I would like to express my sincere appreciation to my wife, Saidah, for her help, encouragement and patience throughout the period of my training.

Finally, my thanks to Mrs. M. Lewis and Mrs. J. Agombar for typing and improving the presentation of my thesis.

INDEX

CHAPTER ONE

INTRODUCTION

1. BIOCHEMISTRY OF INJURY

1.1 Historical aspects

As Johnston (1964) has pointed out surgical operation or other form of physical injury in a previously healthy person initiates a series of metabolic and endocrine processes which are associated with recovery and normal convalescence.

As long ago as 1794 John Hunter wrote with a rare perception: 'there is a circumstance attending accidental injury which does not belong to disease, namely, that the injury alone, has, in all cases a tendency to produce both the disposition and means of cure'.

In 1904 Hawk and Gies confirmed the observation of Bauer (1872) and Jürgensen (1885) that haemorrhage caused an increased elimination of nitrogen, and demonstrated that even the operation of venesection without 'blood letting' is sufficient to cause an appreciable though slight increase in the output of nitrogen and sulphur in the dog.

Cannon (1929) was the first to recognise the importance of the endocrine system in the response to injury. He introduced the concept of a neuroendocrine response to stress and described an increase in the activity of the sympathetic nervous system and in the output of adrenalin-like substances. Cuthbertson (1932) reported that a rise in the excretion of nitrogen in the urine occurred in patients with

fractures and described the so-called 'catabolic response' to trauma. The amount of nitrogen excreted and the duration of the negative nitrogen balance was found to depend on the severity of the injury, and was greater after severe injuries such as fractures of long bones than after minor trauma.

In a further paper in 1942 Cuthbertson separated the metabolic response to injury into an early shock or 'ebb' phase and a subsequent catabolic or 'flow' phase. The 'ebb' phase is associated with a decrease in oxygen consumption and body temperature which are both consequences of the reduction in cellular metabolism. The 'flow' phase is associated with increased nitrogen loss, increased resting energy expenditure and an increased glucose turnover. The magnitude of these changes is roughly correlated with the severity of injury. The negative nitrogen balance reaches a maximum three to eight days after injury, and muscle is thought to be the source of this nitrogen loss.

In order to give a comprehensive picture of the metabolic changes following injury it is necessary to discuss its effects on energy, fat, carbohydrate, protein, water and electrolyte and hormonal balance.

1.2 Effect of injury on energy metabolism

Changes in energy expenditure resulting from trauma, including the minimal trauma of elective surgery, are now well known. It is however, difficult to measure basal metabolic rate (BMR) in patients after injury or operation. In most studies, therefore, the resting

metabolic expenditure (RME) was measured in order to obtain simply the energy output at rest rather than energy output under a standard condition (BMR) which required at least 12 hours fasting. Uncomplicated abdominal surgery does not produce any change in RME (Tilston, 1974). Multiple fractures however, increase the RME by 10 to 30%, and burns by up to 100% or even more. In burns patients water loss is a major factor contributing to the increase (Tilston, 1974). Total energy expenditure does not however, follow the same pattern. Thus Kinney, Duke, Long and Gump (1970) found that total energy expenditure was 120% of RME on the day before elective surgery, 105% of RME on the first day after the operation and gradually rose to 120% of RME by 12 to 14 days.

Controversy still exists as to the exact nature and cause of the change in energy expenditure in injury and this largely centres around the extent to which it can be accounted for by increased protein catabolism.

In the pioneer studies of Cuthbertson (1932) the nitrogen loss following human injury was considered to be an indication that the body was breaking down protein for use as fuel to meet its increased energy needs. The suggestion that protein was the major source of the increased RME (Cuthbertson and Tilston, 1969) was based on the fact that in both the early human studies on fracture patients (Cuthbertson, 1932) and later studies on rats with experimental fracture of the long bones (Cairnie, Campbell, Pullar and Cuthbertson, 1957) there was a parallel rise and fall of nitrogen loss and energy metabolism. However,

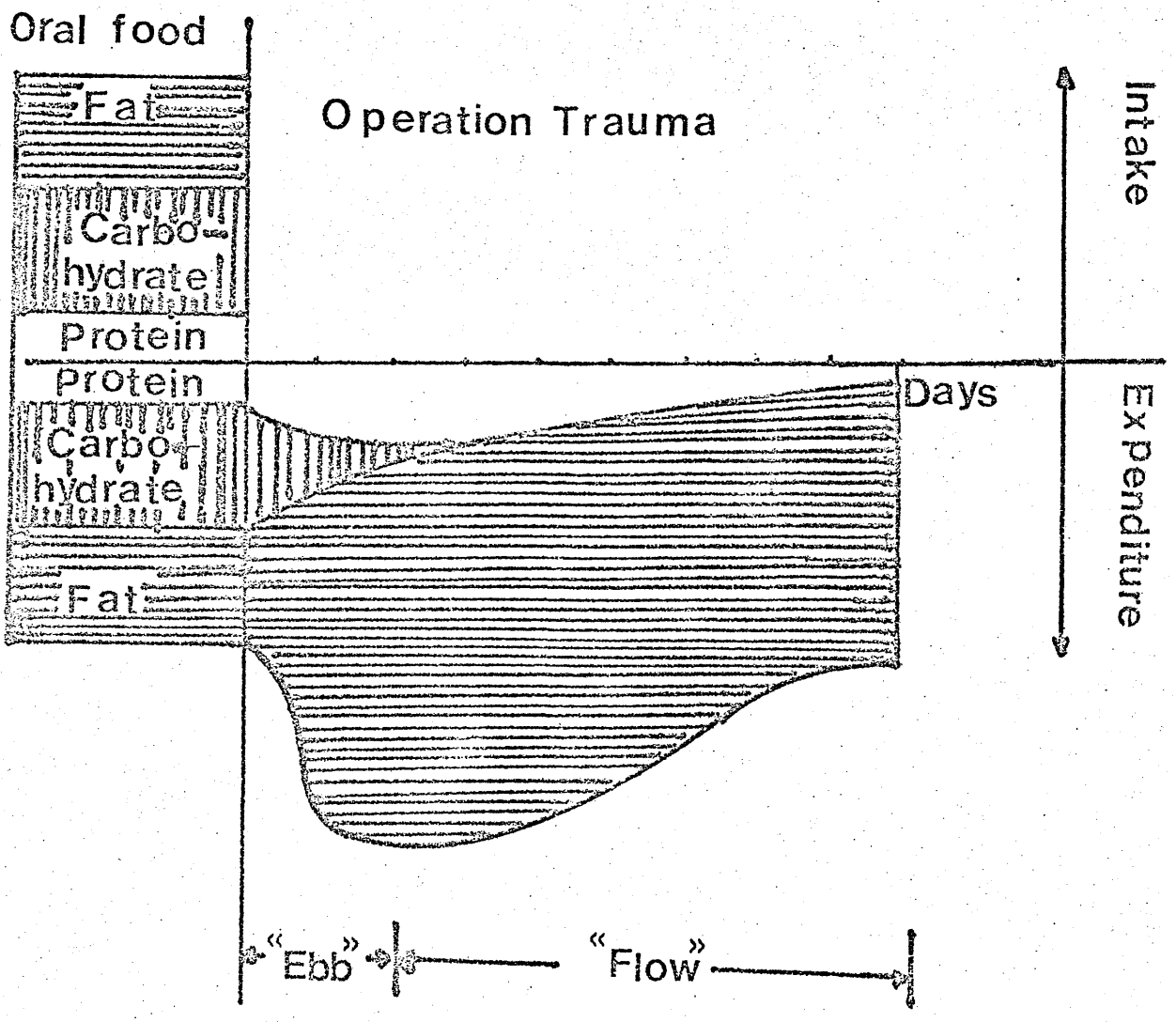
the later work of Kinney, Long and Duke (1970) using indirect calorimetry in humans, and that of Caldwell (1970), using direct calorimetry in rats, seems to be against this view. The contribution of protein to the energy expenditure following most surgical conditions is only about 15% (Duke, Jorgensen, Long and Kinney, 1970) and this is not significantly higher than that found under conditions of starvation (12-15%). On this evidence, Kinney et al. (1970) have concluded that the clinical impression that the weight loss seen after surgery is a result of the body using protein as means for providing fuel to meet increased resting energy demand, seems unlikely. These investigators suggest that the increased nitrogen excretions following injury results from the increased gluconeogenesis necessary to provide carbohydrate intermediates for synthesis purposes, including the synthesis of glucose for subsequent oxidation by tissues such as the brain which cannot utilize fat, though it can utilize ketone bodies.

1.3 Effect of injury on fat metabolism

A substantial part of the body stores of energy is present as triglyceride (fat) in adipose tissue. This store may well amount to some 8 to 15 Kg in a healthy man and from 10 to 20 Kg in a healthy woman. In a very emaciated patient the store is reduced to about 1 Kg. This body fat is the main source of energy after operation (Fig. 1.1). The small contribution of the body's carbohydrate reserve provides sufficient fuel in an injured patient for less than 24 hr without gluconeogenesis from protein. Moore (1959) has described the large loss of adipose tissue which can occur following injury. It has also

Fig.1.1. Energy balances after operation and trauma

(Wretlind, 1976)



been reported that after uncomplicated surgery fat produced 75-90% of the energy while protein accounted for the remainder (Duke et al., 1970).

Triglycerides are composed of one molecule of glycerol bound by ester linkages to three molecules of fatty acid. Most triglyceride is derived from glucose and under the influence of adrenalin glucagon or growth hormone breakdown of triglyceride into glycerol and free fatty acids occurs (FFA), and these are released into the blood stream and are available as tissue fuel. Lipolysis is increased during fasting but very greatly enhanced following injury. Allison, Tomlin and Chamberlain (1969) reported that pre-operative infusion of glucose lowered the plasma level of FFA. During operation, however, the basal level of FFA was elevated and the stimulus of operation caused a continuing rise in spite of glucose infusion. Increased lipolysis has been reported in patients with fracture of the leg or pelvis (Le Pisto, 1976, reported by Kinney, 1977). These workers studied the blood lipid changes in 43 patients and found that the serum triglyceride concentration rose over 4 days, while a small fall in the concentration of cholesterol and phospholipid was noted in 8 of the patients who were diagnosed as having fat 'embolism syndrome', an uncommon disease associated with obstruction of the microcirculation by fat globules. High plasma levels of FFA resulting from increased lipolysis have also been observed in patients with major burns (Carlson, 1970). It was also noted that in burned patients the rise in the plasma FFA appeared to be proportional to the area of the burn, while at the same time the plasma triglyceride level was relatively normal.

It is not clear if the elevation of the plasma FFA level after trauma is useful or harmful to the patients. Studies in dogs with high plasma FFA levels induced by injury have shown that a raised level of plasma FFA was associated with an increase in the triglyceride content of the liver. Moreover, this increase in liver triglyceride was proportional to the rise in plasma FFA level (Carlson, 1970). From his results Carlson concluded that the increased mobilization of FFA seen in response to trauma was in fact an 'excessive' mobilization of FFA, as the production was greater than that required to meet the energy demands. These findings, coupled with the observation that patients who die after severe trauma very often have extremely fatty livers (Carlson, 1970), has made it desirable to prevent the elevation of plasma FFA level which follows injury.

Studies carried out in patients with burns have shown, that both the administration of fat, and treatment in a warm dry environment, reduces plasma FFA level (Davies and Liljedahl, 1976). However, the mechanism by which the increased energy input reduces the level has not yet been fully elucidated. It has been suggested (Davies and Liljedahl, 1976) that in burns patients treated in a warm environment it is simply due to a reduced rate of energy expenditure, as shown by a reduction in oxygen consumption.

Extensive investigations have been carried out in recent years to study the fate and rate of metabolism of fat emulsion administered to the patients with burns. Many of these studies involve the intravenous fat tolerance test. In this test 10% Intralipid is given intravenously

in volumes of 1 ml per Kg body weight and the rate of disappearance of the emulsion from the bloodstream is determined. Carlson and Hallberg (1963) found that the rate of disappearance of fat emulsion from the blood is characterised as having one linear phase and one logarithmic phase, and it is possible to determine the two rate constants of these phases. Carlson (1970), believes that the rate constant K_1, of the linear phase reflects the total available enzyme activities which are responsible for removing the fat emulsion from the bloodstream while the constant K_2 of the logarithmic phase, reflects a number of factors, such as the distribution and flow of substrate passing the enzyme sites. These rate constants alter in response to trauma and are considerably increased in patients after abdominal surgery (Hallberg, 1965) and in patients with extensive burns (Wilmore et al., 1973).

These findings have led Carlson (1970) to suggest that it would be safe to administer the intravenous fat emulsion to patients after trauma, because apparently there is an increased capacity to remove exogenous lipids from the bloodstream. Nevertheless, from the studies conducted to compare the effects of intravenous infusion of fat and carbohydrate on nitrogen balance, it appears that in the early stage of post-operative period carbohydrate spares more nitrogen than fat. Jeejeebhoy, Anderson, et al. (1976) have reported that in patients undergoing abdominal surgery infusion of glucose led to a better nitrogen balance during the first days after operation, but thereafter glucose and fat were equivalent in their protein sparing effects.

1.4 Effect of injury on carbohydrate metabolism

It has long been known that injury and operation induce a diabetic type of state, associated with glucose intolerance (Thomsen, 1938; Hayes and Brandt, 1952; Drucker, Miller et al., 1953). Evans and Butterfield (1951) described the 'pseudo diabetes' of burns, in which non-ketotic diabetic coma may occur in previously non-diabetic patients.

Studies of glucose tolerance after injury or abdominal surgery have shown that glucose tolerance is impaired and this has generally been interpreted as an indication that the entry of glucose into cells is reduced (Allison, Prowse and Chamberlain, 1967; Allison, Hinton and Chamberlain, 1968; Allison, Tomlin and Chamberlain, 1969). Studies of the insulin response to glucose infusion during, and after, upper abdominal operation (Giddings, 1974), however, produced evidence in support of the conclusion previously presented by Hayes and Brandt (1952), that the change in post-operative glucose dynamics is due to increased gluconeogenesis rather than to decreased peripheral glucose utilisation. Using ^{14}C labelled glucose, Kinney et al. (1970) have shown that in patients undergoing abdominal surgery glucose oxidation is essentially unchanged in minor degrees of trauma. These observations were in agreement with those of Long, Kinney and Geiger (1971) who demonstrated that patients with burns or multiple skeletal injuries oxidised glucose at normal or increased rates in the presence of a stable circulation. These workers have also noted that glucose turnover was increased above normal along with the associated hyper-

metabolism. Intravenous glucose tolerance tests carried out in burns patients (Wilmore, Mason, and Pruitt, 1976) have shown that although the fasting blood glucose concentration in these patients was elevated, the rate constant for glucose disappearance was similar to that obtained in normal individuals. Furthermore, in these patients, hypermetabolism and negative nitrogen balance occurred in association with the normal insulin response to glucose. Wilmore et al. (1976) have suggested that the elevated blood glucose observed in thermally injured patients was a consequence of increased hepatic production, and not of altered peripheral glucose disappearance. This suggestion has received further support from the studies of Aoki, Brennam, Muller and Cahil (1974) who reported that plasma amino acid concentrations in severely injured patients were depressed, and the half-life of labelled alanine, a measure of hepatic gluconeogenesis, was reduced by 50%. However, the mechanism by which injury enhances the rate of gluconeogenesis in the liver has not yet been fully elucidated. In the opinion of Wilmor et al. (1976) the increased hepatic gluconeogenesis observed in injured patients is caused by the increased levels of glucagon and cathecolamines, not by a decrease in fasting insulin or by a dampened insulin response.

1.5 Effect of injury on protein metabolism

1.5.1 General picture

The early observations that total urinary nitrogen increases considerably following trauma (Cutherbertson, 1930) have been amply confirmed by subsequent workers (Howard, Parson et al., 1944; Peters,

1948; Flear and Clarke,1955; Kinney, 1959; Moore, 1959) and much experimentation has been undertaken to clarify the causes of the urinary losses.

Partition of the urinary nitrogen showed that the increase was mainly due to an increase in the amount of urea excreted, whilst partition of the sulphur-containing compounds showed that the increase was due to a similar proportional increment in the excretion of inorganic sulphate (Cuthbertson, 1932). These findings suggested that skeletal muscle was the chief source of the extra nitrogen, and this view was supported by the concomitant rise which occurs in the excretion of magnesium (Walker, Morgan and McCowan, 1964) and zinc (Fell and Canning, 1971). In the same way that the fall in nitrogen excretion in starvation reaches a minimal plateau at 5 to 6 days (Martin and Rabinson, 1922), so after trauma of moderate severity nitrogen losses reach a maximal plateau over a similar period (Howard, Wintermitz et al., 1944). The level of nitrogen excretion is dependent upon a number of factors including age, sex, and the degree to which energy deprivation contributes to the continuing losses. The catabolic process is less marked in the elderly and in females (Peaston, 1974) and may be largely suppressed if the prior nutritional status verges on starvation (Munro and Cuthbertson,1943; Chalmers and Munro, 1945; Calloway, Grossman, Bowman and Calhoum, 1955; Browne, 1944).

1.5.2 The nature of the disturbance in protein metabolism

(i) Whole body protein turnover.

Nitrogen balance techniques give information about net exchanges

of nitrogen with the environment, but isotopic tracers are needed to examine the dynamics of nitrogen within the body. Sprinson and Rittenberg (1949) used 15N-glycine for this purpose and calculated that the rate of protein synthesis in normal adult man is approximately 300 mg/day. Waterlow and his colleagues (Waterlow, 1967) have developed a different approach using a continuous infusion of labelled amino acid. O'Keefe, Sender and James (1974) reported measurements made on five patients in which ^{14}C-leucine had been given as a continuous infusion before and after abdominal surgery. They found that following operation there was a decrease in protein synthesis with no increase in protein breakdown. However in Kinney's view (Kinney, 1977) this study is difficult to interpret since the patients received a normal diet pre-operatively and were given only water and electrolytes in the post-operative period. It is therefore possible that the negative nitrogen balance after operation, and the decrease in protein synthesis, were a result not of injury, but of the withdrawal of dietary protein. In further work from the same laboratory (Crane, Pirou, Smith and Waterlow, 1977) labelled glycine was given orally every four hours for 32 hours to eleven patients before and after elective orthopaedic surgery. In this study a constant protein intake was maintained throughout the investigation. The results were essentially the same as those of O'Keefe et al. (1974), for the rate of protein synthesis fell after operation, with no change in the calculated rate of protein breakdown. These workers postulate a block in muscle protein synthesis as being responsible for the catabolic loss of nitrogen after injury. However, Waterlow and Sender (1976), believed that the main criticism

of both the studies cited above is that the trauma was not very severe, so that the negative nitrogen balance was small. In fact, studies by Long, Jeevanandam and Kinney (1977) suggested that in acutely ill surgical patients with sepsis a negative nitrogen balance could be the result of an increased rate of protein synthesis with an even greater increase in protein breakdown.

(ii) Plasma proteins

Cuthbertson and Tompsett (1935) reported that injury was followed by a fall in the concentration of albumin and an increase in that of certain globulins. The changes in the electrophoretic pattern of serum proteins from injured patients have been discussed by Owen (1967); Werner and Cohnen (1969); Fleck (1976) and Davies (1976). All investigators agree that after trauma the albumin concentration falls to a minimum at around the third to the sixth day and that it gradually returns towards a normal concentration over days or weeks after injury, depending upon the severity of the injury. However studies by Ballantyne and Fleck (1973a) have shown that the fall in the albumin concentration observed after injury could be minimised if the patients were maintained at high environmental temperature (e.g. at 30°). They reported that the fall in serum albumin in patients with long bone fracture was much less at 30° than at 20°. The mechanism by which treatment in a warm environment prevents the fall in plasma albumin, like that which prevents a rise in plasma FFA after injury, has not yet been decribed. It has been shown that exposure to warm, dry air will minimise the net catabolism of protein in the rat after burn injury (Caldwell, 1962) or

fracture of the femur (Campbell and Cuthbertson, 1967) and in man after long bone injury (Cuthbertson, Smith and Tilstone, 1968). In these studies, however, protein metabolism was assessed chemically mainly by measurements of the excretion of metabolites in urine.

Ballantyne and Fleck (1973b) have studied albumin turnover using ^{125}I-labelled albumin in eleven patients with fractures who had been treated at an environmental temperature of 20^{o} and in nine patients treated at 30^{o}. They found that the plasma albumin concentration fell to a minimum on day 5 after injury at 20^{o}, but that there was no significant alteration in those patients treated at 30^{o}. However, the rate of albumin catabolism was the same at either environmental temperature. These workers concluded that the fall in plasma albumin in fracture patients treated at 20^{o} could not be ascribed to increased catabolism of albumin.

The concentration of fibrinogen rises occasionally very considerably, after injury (Cuthbertson and Tompsett, 1935; Crockson, Payne, Ratcliff and Soothill, 1966; Davies, Liljedahl and Reizenstein, 1970; Koj, 1970). Increases of up to twice the upper limit of the normal range and maintained for nine days have been reported after operation on the femur in elderly patients (Davies et al., 1970).

Alpha 1 antitrypsin and alpha 1 acid glycoprotein both increase fairly rapidly up to 50% (Ballantyne and Fleck, 1973a; Crockson et al., 1966) and it is the rise in these proteins in spite of a decrease of about 50% in the alpha 1 lipoprotein (Werner and Cohnen, 1969) which probably accounts for the early increase in total alpha 1 globulins.

Alpha 2 globulins, including ceruloplasmin and heptoglobulin, are increased by 50-100% (Crockson et al., 1966; Werner and Cohnen, 1966) after injury. The beta-globulins such as transferin and beta-lipoprotein consistently show a decrease of 25 to 50% (Werner and Cohnen, 1966). The immunoglobulins IgG, IgA, and LgM are seen not to change significantly (Ballantyne and Fleck, 1973a) unless there is a source of infection, as in burns, where the increase may be considerable (Prendergast, Feninchel and Daly, 1952).

(iii) Plasma amino acids

Changes in blood amino acid concentrations have been described shortly after injury. Engel, Winton and Long (1943) reported that haemorrhagic shock in the rat was characterized by a raised blood concentration of amino nitrogen and considered that this was due partly to an increased breakdown of protein in the peripheral tissues and partly to a decreased ability of the liver to dispose of amino acids due to the accompanying anoxia and decreased metabolic activity of this tissue. Studies by Van Slyke, Philips et al. (1951) in dogs showed that haemorrhagic shock caused decreased urea formation, whilst Burt (1954) reported that solutions of amino acids infused into injured dogs were poorly tolerated. Both these studies provided further evidence in support of the conclusion presented by Engel et al. (1943).

Several studies on the metabolic response of man to injury present different aspects. In an examination of 177 battle casualties, Green, Stoner, Whiteley and Eglin (1949) reported that the concentrations of plasma amino nitrogen was not raised, and indeed tended to bear an inverse relationship to the elevated blood sugar concentrations.

Similarly, Schreier and Karch (1954) (quoted by Cuthbertson, 1964) observed in man that the serum amino acids were usually lower 3 to 4 hours after operation than before, although in 4 out of 5 patients the isoleucine and leucine levels rose. In this connection, Schonheyder, Bone, and Skjoldborg (1974), using ion exchange chromatography, reported a study of plasma essential and non-essential amino acids after abdominal surgery. The concentrations of plasma amino acids fell, and while the concentrations of branched chain amino acids as well as phenylalanine rose above basal levels by two days after operation, the levels of other amino acids remained low until the seventh day after operation. These observations were more or less in agreement with the more recent work of Dale, Young et al. (1977) who found that immediately after an abdominal operation of moderate or extensive nature, the concentrations of most plasma amino acids fell promptly. The concentrations of non-essential amino acids continued to fall for the first two days or more while those of the essential amino acids returned to a higher concentration than immediately after operation. Furthermore, there appeared to be no correlation between these changes and the length of anaesthesia or the severity of the operation.

In an attempt to find an explanation for the postoperative changes in plasma branched chain amino acids, Wedge, Campos et al. (1976) have studied the relationship between the branched chain amino acids of plasma, urinary excretion of nitrogen and the concentration of circulating ketone bodies in a group of 16 male patients following skeletal and soft tissue injury, and compared them with similar measurements on four adults undergoing elective skin grafts. They

found that the concentrations of the branched chain amino acids rose shortly after operation and reached twice the normal value by 4 days. In contrast, the concentrations of plasma alanine, glycine and glutamic acid were depressed from the 4th to 7th days. Moreover, after operation, increased concentrations of branched chain amino acids were accompanied by hyperketonaemia and an elevated excretion of urinary nitrogen. Wedge et al. (1976) have suggested that the changes in the concentrations of branched-chain amino acids after injury indicate a decreased uptake of amino acids by muscle, or an excessive release from muscle due to an imbalance between protein synthesis and metabolism. Post-operative malnutrition has also been suggested (Shonheyderet al., 1974) as a cause of the changes found in the concentrations of plasma amino acids after surgery. This suggestion is not, however, supported by the fact that malnutrition and protein deprivation result in a rise in the concentrations of non-essential amino acids and a fall in those of essential amino acids.

(iv) Muscle free amino acids

Animal experiments have shown that the concentrations of several free amino acids are considerably higher in the cells than in the extra cellular fluid (Herbert, Coulson and Hernandez, 1966; Munro, 1970; Adibi, 1971). Higher free amino acid concentrations in muscle tissue than in plasma have also been reported in man (Zachman, Cleveland, Sundberg and Nyhan, 1966; Bergstrom, Furst, Nore and Vinnars, 1974). These workers have shown nearly 80% of the total free amino acid pool in the total body are found in skeletal muscle. The development of a rapid needle biopsy technique by Bergstrom (1962) has made it possible

to investigate the pattern of free amino acids in muscle tissue under different pathological conditions. Using this technique, Vinnars, Bergstrom and Furst (1965) have studied changes in the free amino acids of muscle tissue in five patients following uncomplicated abdominal operation. They found that after operation the concentrations of essential amino acids in the muscle remained unchanged, while those of non-essential amino acids fell at 3 days after operation. However, the magnitude of postoperative alterations in muscle amino acid pattern appears to be related to the severity of injury and trauma. Thus studying muscle amino acid changes in seven patients who had severe complications such as spesis or peritonitis, Vinnars et al. (1976) found that the size of both the essential and non-essential amino acid pool decreased, though the concentrations of phenylalanine was increased. Moreover, the phenylalanine/tyrosine ratio in muscle was markedly increased. Increased phenylalanine concentration in the muscle observed after injury is in contrast with that seen in starvation which has been reported to be decreased (Vinnars et al., 1976). These workers have suggested that changes in amino acids following moderate and severe injury are distinctly different from those of starvation.

1.6 Effect of injury on water and electrolyte balance

Surgical operation or physical injury affects the normal balance of water and electrolyte in the body. According to Zimmerman (1972), there is, in fact, no distortion of fluid and electrolyte metabolism to which surgical patients are not subject. However, the main types of water and electrolyte disorders that are particularly common in injured patients

can be grouped as follows:-

1.6.1 Isotonic changes in fluid volume

(i) Extracellular deficit.

Major losses of extracellular fluid (E.C.F.) occur when there is a diminished intake or an increased loss of water. It is customary for the consumption of food and water by most surgical patients to be restricted for 12 hours or more before, and for up to several days after surgery. The need for restriction, however, varies with the site and nature of the operation and in Wilkinson's view (Wilkinson, 1969) is often more lengthy and severe than is probably necessary. The significance of water depletion is often underemphasized as the fall in the water content of the body is not associated with a fall in electrolytes. Nadal, Pederson and Maddock (1941) studied the effects of water deprivation in normal subjects, and found that when the intake of water was stopped for three days the body weight fell steadily and the daily volume fell to about 600 ml., but it's specific gravity rose to about 1036. There was no change in the packed cell volume or in the concentration of sodium in the plasma, but that of non-protein nitrogen rose. When water was given, the body weight rose, there was a diuresis and the specific gravity of the urine fell as also did the concentration of non-protein nitrogen in the plasma. Experimental studies of the effects of water depletion in man have also been reported by Black, McCance and Young (1944) and by Winkler, Danowski, Elkinton and Peters (1944). These studies, however, were concerned with the pure syndrome induced by a reduction in the intake of water while loss of water continued through the skin, lungs and kidneys. Under these conditions, loss of water is quickly reduced by a fall in urine output. The volume of urine which

is formed during water depletion depends on the amount of osmotically active solutes derived from the diet. Protein foods are degraded to urea which requires excretion by the kidney. Whereas fat and carbohydrate yield mainly water and carbon dioxide. On a normal diet, water deprivation quickly induces a situation in which the water available for urine formation is inadequate to excrete the normal solute load, even at maximal urine concentration. In injured patients the situation is complicated by the fact that due to the stress following injury, the body metabolism is altered so that the kinds of solutes which are derived from tissue catabolism may differ considerably from those induced by water deprivation in normal subjects. However, when the water loss is in excess of 4 litres, the blood volume diminishes, the pulse speeds up and the blood pressure falls. Initially, the withdrawal of water from the tissues to maintain the blood volume results in loss of tissue turgor, loss of elasticity of the skin, and fall in intra-occular pressure. The diminished volume of body fluid stimulates the secretion of aldosterone (Bartter, 1958) and Na is thus conserved, so that the loss of fluid falls predominantly on the intracellular fluid (I.C.F.) rather than the E.C.F. (Walker and Johnston, 1971). Water depletion is indicated by a urine volume below 700 ml/day and thirst in the conscious patient. It can be corrected by oral or intravenous administration of 5% Dextrose.

(ii) Extracellular fluid excess.

E.C.F. excess in association with surgery was frequently seen in earlier days when "physiological saline" was used routinely as a hydrating solution. After the recognition by Coller, Campbell et al. (1944) of the tendency for patients to retain sodium in this period, excessive use of sodium chloride was discontinued, and, as a result,

postoperative oedema became far less common (Zimmerman, 1972) and is most frequently seen in combination with protein depletion. In this situation, as with dehydration the serum levels of ions, do not give any indication of the overall excess of extracellular electrolytes that may be present. Serial determinations of body weight are of importance and will demonstrate incipient fluid retention long before clinical oedema is apparent (Zimmerman, 1972).

1.6.2 Disturbances of extracellular tonicity

(i) Water intoxication and the low sodium syndrome

This is the direct opposite of water depletion and in this condition the body water is increased without an increase in the electrolyte content. The recognition of the sodium retention which normally occurs during the early postoperative period led to a tendency to administer isotonic glucose solutions instead of saline at this time. However, during the first week after operation, the excretion of water is slower than normal and therefore, if an excessive amount of intravenous 5% glucose is administered, a large proportion of the injected water may be retained which will produce hypotonicity of E.C.F. followed by a movement of water into the cells thus causing them to swell. This particularly affects the cerebral cells and may cause serious mental changes (Walker and Johnston, 1971). Water intoxication has been described after rectal administration of water (de Takats, 1931) and after excessive drinking of water in the immediate postoperative period (Wilkinson, 1969). It may also arise in conditions in which fluid and electrolyte loss is replaced by water only. A particular example is when gastric suction is used with the replacement of the electrolyte-rich

fluid by intravenous 5% dextrose (Walker and Johnston, 1971). Water intoxication is associated with low serum sodium levels and neurological changes are frequently seen at serum sodium levels below 125 mmol/litre and almost always at 115 mmol/litre (Zimmerman, 1972). Since hyponatraemia by itself impairs the kidney's ability to respond to a water load (McCance, 1936), it has been suggested that this syndrome represents a self-perpetuating cycle which can be interrupted only by introduction of a sodium load for excretion (Zimmerman, 1972). These observations emphasise the important role of the sodium ion in the maintenance of normal water distribution.

(ii) Chronic hypernatraemia

In contrast to acute hyponatraemia associated with water intoxication, which is followed by impaired function of the central nervous system, chronic hyponatraemia develops when the sodium depletion occurs over a long period of time and normally is not associated with disorders of the central nervous system (Black, 1967; Zimmerman, 1972). Chronic hyponatraemia is frequently seen in malnourished patients and also is very common after operations (Moore, 1954; Zimmerman, Casey and Bloch, 1956). In this condition a low plasma sodium concentration is accompanied by a low plasma protein level. Recognition of a reduced plasma protein level is very important since plasma sodium does not respond to hypertonic saline, and indeed, administration of large volumes of sodium-containing solutions results in oedema which would lead to further surgical complications (Black, 1967; Zimmerman, 1972). It has been suggested (Black, 1967) that under these circumstances saline should be given only when a low plasma sodium is accompanied by evidence of low plasma or E.C.F. volume. Administration of plasma,

or salt-free albumin, has also been reported to be of beneficial value and leads to improvement and normal electrolyte concentrations in hyponatraemia (Zimmerman, 1972).

(iii) Hypernatraemia

The retention of sodium after injury is one of the most constant features of the metabolic response to trauma and is mediated through the release of aldosterone. It begins immediately after injury, reaches its maximum by the second day, and lasts for 4-6 days or even longer, depending on the age of the patient and on the severity of trauma (Walker and Johnston, 1971). Retention of sodium is accompanied by retention of water. This prevents a rise in plasma sodium occurring during a normal response to surgery. There are, however, circumstances under which the plasma sodium concentration does rise. For example, hypernatraemia has been found in infants whose feeds were made with insufficient water (Simpson and O'Duffy, 1967). It has also been reported in patients to whom sodium sulphate had been administered for the treatment of hypercalcaemia (Heckman and Walsh, 1967).

Since hypernatraemia fundamentally expresses hyperosmolality, it can be induced by a high intake of other solutes as well as sodium salts (Black, 1967). In this connection Zimmerman (1972) has reported that hypernatraemia observed in surgical patients is most commonly the result of high osmotic loads of non-electrolyte materials. A particular example is when gastric and jejunal tube-feeds containing high concentrations of carbohydrate and protein or amino acids were used without sufficient available water for the excretion of the solutes. These observations have led to the suggestion (Zimmerman, 1972) that it is mandatory that such feeds when they are used, are accompanied

by intravenous infusions of sodium-free 5% glucose solution.

1.6.3 Disturbances of acid-base equilibrium

Metabolic distortions of acid-base balance which may occur in the practice of surgery can be considered under the following headings:-

(i) Metabolic alkalosis

In metabolic alkalosis the reduction in hydrogen ion concentration may be due either to an absolute increase in the quantity and concentration of extracellular bicarbonate or to the loss of hydrogen ion from the body. Under normal conditions of health the body has a great capacity to tolerate large doses of bicarbonate. Thus Van Goidsenhoven, Gray, Price and Sanderson (1954) found that patients without renal and metabolic disease could tolerate doses of sodium carbonate of up to 140 g/day for 3 weeks. The main body defence mechanism against metabolic alkalosis is the renal excretion of HCO_3^-. Pitt (1963) found that the kidneys normally reabsorb 24-28 mmol HCO_3^-/litre of glomerular filtrate thus stabilising the plasma HCO_3^- at 24-28 mmol/litre. Any excess HCO_3^- is excreted in the urine. In surgical patients metabolic alkalosis is commonest when there is pyloric obstruction and the loss of acid by the repeated vomiting of gastric secretions is accentuated by the excessive consumption of sodium bicarbonate (Wilkinson, 1969). When vomiting and suction are repeated or prolonged, the situation is complicated by the loss, in addition to hydrogen and chloride, of significant amounts of sodium and potassium (Davies, Jepson and Black, 1956). Renal function is impaired, and simple replacement of chloride is inadequate to correct the alkalosis (Black, 1967). In these circumstances it has been

suggested (Roberts, Randall, Philbin and Lipton, 1954), that it is necessary to correct sodium and potassium before treatment of the alkalosis.

(ii) Potassium deficiency.

Control of body potassium differs from that of sodium in that the kidneys do not deal with potassium in the same way as sodium. Excretion of potassium in the urine continues even when the intake of food is restricted or ceases, and this continuing loss leads to slow depletion of the body potassium. A few hours after operation, or injury, there is usually an increase in the urinary excretion of potassium, as part of the normal metabolic response to injury. The urinary potassium excretion is maximal in the first 24 hours and lasts for 2 to 3 days (Johnston, 1964). As there is no intake during that period this loss reflects a negative potassium balance. Moreover, in so far as intravenous administration of saline or glucose solutions increase the urinary potassium loss (Wilkinson, 1969), the administration of such solutions to surgical patients may greatly contribute to potassium depletion. However, the amount of potassium lost after injury, or operation, is related to the general state of nutrition and to the size of the muscle mass, which contains over 75% of the total potassium in the body. In Wilkinson's view (Wilkinson, 1969) the loss of 200 mmol in 2 or 3 days is well tolerated by most patients, and is a common event after major operations such as partial gastrectomy. Negative potassium balance is quickly corrected when a normal dietary intake is resumed (Walker and Johnston, 1971). However, if normal dietary intake is not resumed for a few days after operation and potassium loss remains high, a potassium salt should be administered preferably by mouth, to prevent potassium depletion.

(iii) Metabolic acidosis

The metabolic production of actual and potential hydrogen ion is a normal phenomenon. It is accentuated by a diet rich in sulphur and phosphorus (i.e. in protein); by a diet which allows the incomplete oxidation of fat (a ketogenic diet); and by forced exercise, which leads to incomplete combustion of carbohydrate to lactic acid rather than to carbon dioxide and water. This normal production of hydrogen is dealt with by continuous or increased activity of the lungs and kidneys. Reaction of the acid with HCO_3^- liberates CO_2 which is excreted by the lungs. However much of the acid load is excreted in the urine along with the cations Na^+, K^+, Mg^{++} and Ca^{++}, and in a severe prolonged metabolic acidosis the body content of these cations may be diminished.

Under pathological conditions, the rate of acid production may rise to a level beyond the body's homeostatic mechanism to control. For example, the excessive protein breakdown in fever, trauma, starvation, or dehydration can lead to excessive hydrogen production and to acidosis (Black, 1967).

In surgical patients metabolic acidosis may arise when there is the loss from the body of secretions possessing a high sodium concentration (Zimmerman, 1972). For example, in patients with pancreatic fistulae a large amount of pancreatic juice may be lost. Since pancreatic juice has a sodium concentration comparable to that of plasma, excessive loss would lead to metabolic acidosis. Under these circumstances, especially when there is increased protein breakdown due to trauma and/or starvation, administration of sodium bicarbonate might become necessary to avoid a metabolic acidosis.

(iv) Magnesium deficiency

Magnesium is the cation present in second largest amount in the intracellular fluid and has an important role to play in relation to enzyme activity. All reactions involving phosphate transfer, including all stages of oxidative phosphorylation, are magnesium dependent (Fell and Burns, 1976). The adult human weighing 70 Kg contains approximately 833 to 1200 mmole of magnesium (Widdowson and Dickerson, 1964; Duckworth and Warnock, 1942). About 55% is present in bone and about 27% in muscle. The plasma concentration normally ranges between 0.75 to 1.05 mM (Wacker and Parisi, 1968).

The question of replacement of the magnesium ion and the rare occurrence of magnesium deficiency syndromes are matters of considerable interest. Although the intake of magnesium in the average diet is said to be about 7.5 to 20 mmole/day (Seelig, 1964), the normal person appears able to conserve this ion effectively in the presence of a deficient intake. Studies of Jones, Manalo and Flink (1967) suggest that as little as 0.15 mmole/day would maintain a normal adult in magnesium balance, that a magnesium-deficient diet can be tolerated for as long as 38 days, and that there is no significant obligatory loss by normal kidneys and gastrointestinal tract over that period of time. Nevertheless, magnesium deficiency has been reported in a number of pathological conditions, such as malabsorption (Gerlach, Morowitz and Kirsner, 1970), chronic alcoholism with malnutrition (Vallee, Wacker and Ulmer, 1960), prolonged magnesium-free parenteral feeding in association with prolonged losses of gastrointestinal secretions (Gerst, Porter and Fishman, 1964; Baron, 1961; Broughton, Anderson and Bowden,

1968). It has also been suggested that even in the absence of measurable losses, blood levels fall in association with surgical operations (Sawyer, Drew et al., 1970). However, in Zimmerman's view (Zimmerman, 1972) major operations, particularly gastrointestinal operations, present a threat to effective magnesium levels. This occurs most frequently in patients who have diarrhoea, gastrointestinal drainage or loss of function of large portions of the intestinal tract for long periods, and also with parenteral maintenance without inclusion of magnesium ions. Zimmerman suggests that parenteral administration of magnesium is indicated when the blood level falls below the normal range of 0.75 to 1.25 mM or when the urinary excretion of magnesium is less than 1.5 mmole/day.

1.7 The endocrine response to injury

Injury or surgical operation initiates a series of metabolic or endocrine changes which are associated with normal recovery, repair, and convalescence. However, according to Johnston (1974) the mechanism by which the endocrine and metabolic response is activated after injury has not been defined clearly and the mode of action and physiological role of the various hormones in maintaining homeostasis after injury is not fully understood.

Hinton, Allison, Littlejohn and Lloyd (1971) have suggested that some of the metabolic changes which follow injury could be mediated by a change in endocrine pattern in which a diminished secretion or an effectiveness of the anabolic hormone, insulin is accomapnied by secretion of the catabolic hormones, cortisol, catecholamines, and

glucagon. This hypothesis is diagrammatically shown in figure 1.2. In order to give a comprehensive picture of the hormonal changes following injury it is necessary to discuss its effect on the secretory activity of the endocrine glands.

1.7.1 Adrenal cortex secretions

The most striking aspect of the endocrine response involves the adrenal cortex. Three types of hormones are produced, namely, glucocorticosteroids, mineralocorticoids, and adrenal metabolites of the sex hormones. The glucocorticoids have important metabolic functions, and cortisol is the main member of this group production of which is increased after injury. The mineralocorticoids are secreted in relatively small amounts and aldosterone is the principal hormone in this group; its production is elevated after operation. The adrenal metabolites of the sex hormones have very little metabolic function (Johnston, 1964) and their output barely alters in response to trauma.

(i) Cortisol

Physical injury or surgical trauma stimulates the secretion of ACTH (Cooper and Nelson, 1962) which in turn produces an increased output of adrenocortical hormones. The plasma cortisol level rises rapidly during a surgical operation and a peak is reached at between 4 and 6 hours, and thereafter the level returns slowly towards the resting value (Sandberg, Eik-Nes, Samuel and Tyler, 1954). The height of the response depends not only on the rate of production of cortisol, but also on the rate of conjugation, metabolism and excretion of the steroid by the body (Johnston, 1967).

The excretion of cortisol and its metabolites in the urine after

RESPONSE TO INJURY

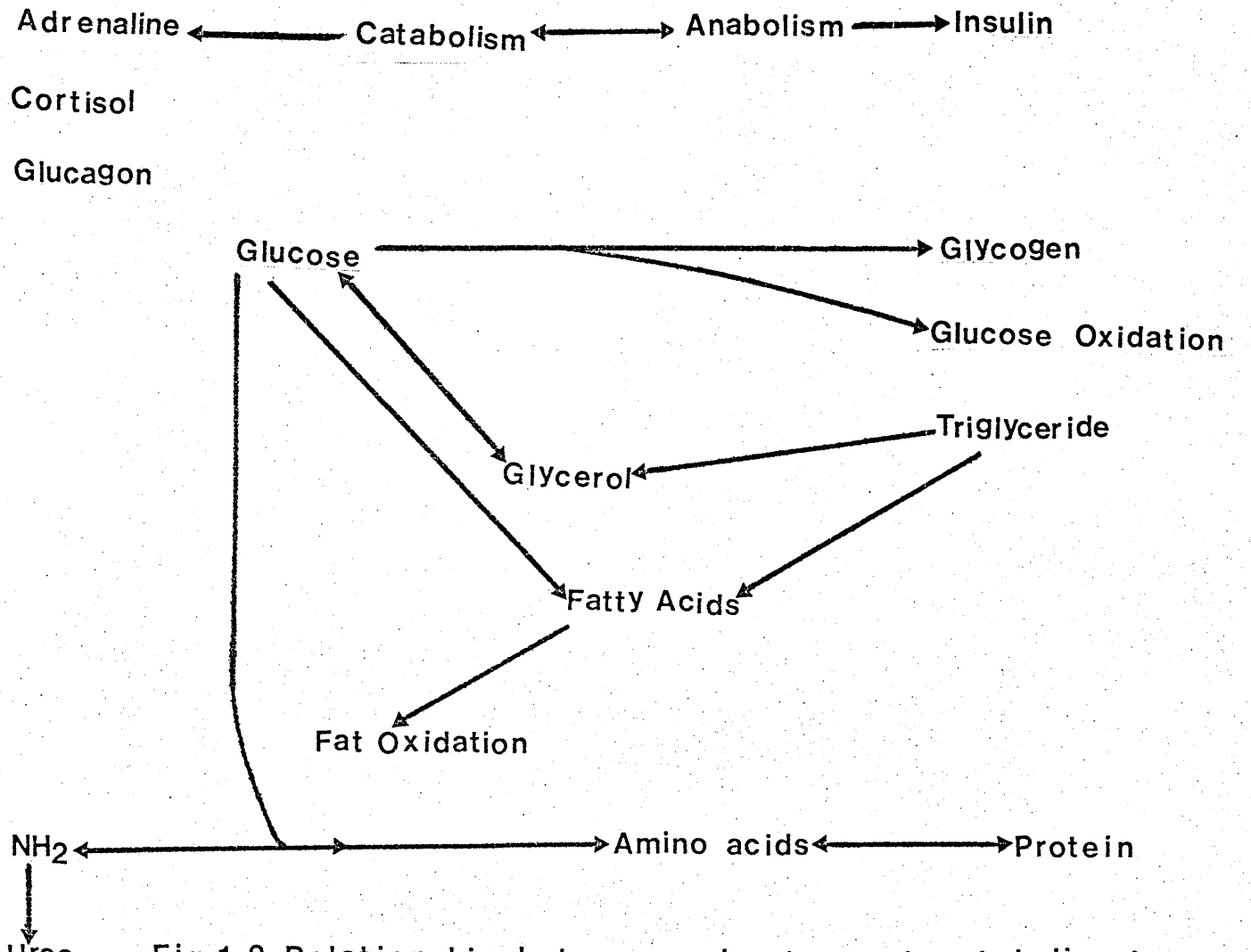


Fig.1.2. Relationship between endocrine and metabolic changes after injury (Woolfson, 1977)

trauma is increased for a much longer period than that during which the plasma cortisol is raised and provides another measurement of the total adrenal response (Moore et al., 1955). Cortisol in the plasma is either bound to protein or is free. The free component is filtered in the urine and its measurement is believed to provide a more accurate picture of changes in adrenocortical activity (Espiner, 1966). When the free cortisol in the urine was measured before and after various kinds of surgical operation, a wide range of response was found (Espiner, 1966). A thirtyfold increase in cortisol excretions was found after a major operation such as abdominoperineal resection of the rectum. Bilateral stripping of varicose veins produced a similar extensive response, while hernia operations and other minor procedures produced only a small increase. These observations led to the suggestion that the extent of the adrenocortical response may depend upon the amount of tissue damaged at the time of the injury (Espiner, 1966).

(ii) Aldosterone

Raised aldosterone levels were first detected in surgical patients by means of bioassay techniques. The urine of postoperative patients was found to have sodium-retaining properties when injected into adrenalectomized rats (Llaurado, 1955). Further indirect evidence of the role of aldosterone in postoperative electrolyte changes came from studies using the aldosterone blocking agent spironolactone. Thus, Johnston (1964) measured the daily balance of sodium in six patients after abdominal operation. Three were given spironolactone (400 mg/day by intramuscular injection) and the remaining three acted as controls. The patients given spironolactone all excreted large amounts of sodium after operation compared to the controls. Johnston (1964) concluded

that a sodium-retaining hormone was active after abdominal operation and that this activity could be abolished by an aldosterone blocking agent.

Studies using direct estimation of aldosterone in the urine after operation have confirmed the results of indirect techniques and an increased output has been reported after surgery (Hume et al, 1962). However, aldosterone secretion is controlled among other things by acute changes in blood-volume. Walker (1965) has shown that intensive monitoring of blood volume during and after open heart surgery reduces the secretion of aldosterone when compared with operations of comparable severity during which no monitoring takes place. High blood levels of potassium have been found also to stimulate aldosterone release directly (Moran, Rosenberg, and Zimmerman, 1959).

(iii) The permissive role of the adrenal cortex.

Surgical trauma is followed by changes in the metabolism of protein, carbohydrate and electrolytes which are similar to those found in Cushing's syndrome or after large doses of cortisone (Johnston, 1967). A correlation was also found between the extent of the postoperative negative balance of nitrogen and the amounts of cortisol and its metabolites found in the urine (Moore et al., 1955). These observations suggested that in the postoperative patients the adrenocortical response was a direct cause of the changes in metabolism. Further studies, however, produced evidence to show that all the postoperative metabolic events could not be explained in terms of increased adrenocortical activity. In this connection, Johnston (1964) has found that the metabolic response to major surgery in patients who had already had their adrenals or pituitary

removed earlier, follows the normal course when constant maintenance doses of cortisol were given throughout the period of stress. In the same study nitrogen excretion and adrenal functions were also compared in undernourished and well nourished subjects after surgery. It was found that adrenal responses were similar in extent while the nitrogen loss in the poorly nourished patients was greatly reduced. Munro (1966) investigated the mechanism of these differences in an animal study, and found that the administration of steroids to protein-depleted animals produced an increased nitrogen loss, whereas severe injury in the presence of protein depletion had no effect on nitrogen excretion.

Evidence such as this led Ingel (1952) to develop the concept of the permissive rather than causative role of the adrenal cortex in the metabolic response to injury. In other words, adrenocorticoids were necessary for normal metabolic changes to occur, but they do not cause the changes, and both responses can vary independently of each other. However, Alberti, Batstone and Johnston (1977) have recently put this view in question. These workers reviewed the original experiments which relegated cortisol to this passive role, and concluded that an important criticism of these studies, apart from their failure to use the physiological glucocorticoids of the rat, corticosterones in physiological dosage, was the narrow definition of the term catabolism and the long-term nature of the studies. In contrast to previous workers who have equated catabolism with tissue on total body protein loss, these workers defined catabolism as including glycogen breakdown in the liver and other tissues; gluconeogenesis; mobilisation of gluconeogenic precursors from extrahepatic tissues; proteolysis in extrahepatic tissues; and

lipolysis with the mobilisation of glycerol, NEFA and hence ketone bodies.

In order to examine the possible active role of cortisol after injury, Alberti et al. (1977) induced an increase in plasma cortisol in normal subjects by injecting ACTH in the form of Synacthen-depot (1 mg/day intramuscular) for three days. They found that the plasma cortisol concentrations achieved were similar to those found in their severely burned patients. Furthermore, the fasting concentrations of several hormones and metabolites were altered. Thus blood glucose concentration was increased as was serum insulin, but glucose tolerance was impaired despite an exagerated insulin response. Concentrations of the gluconeogenic precursors, lactate, alanine and pyruvate were also grossly elevated.

These findings in normal subjects supported their hypothesis that the rise in cortisol found after trauma contributes to the increase in the circulating concentrations of gluconeogenic precursors. On this evidence they suggested that the metabolic response to injury could be explained on the basis of an early phase dominated first by cortisol and catecholamines, with glucagon over-riding these effects in the second phase. Moreover, each of these hormones plays an individual and active part in the response to trauma.

1.7.2 Adrenal medulla

Two catecholamines, namely, adrenaline and noradrenaline are secreted by the adrenal medulla. Catecholamines are closely associated with carbohydrate metabolism. They cause the mobilisation of glucose from glycogen (Sutherland and Robison, 1966; Krebs, Delange, Kemp

and Riley, 1966) and free fatty acids from adipose tissue (Steinberg, 1966; Fredholm, 1970). The stimulation of both glycogenolysis and lipolysis by catecholamines appears to be mediated by activation of adenyl cyclase with a resulting increase in intracellular cyclic AMP (Exton, Lewis, Robison and Park, 1971; Burns, Langley and Robison, 1971). It has also been shown that catecholamines inhibit the release of insulin in response to a glucose load (Porter, 1969; Mayhew, Wright and Ashmore, 1969). More recently Christensen, Alberti and Brandsborg (1975) have reported a 3 to 4-fold increase in blood lactate concentration following an infusion of adrenaline.

Alterations in the secretion of catecholamine following injury have been studied. Thus, Pekkarinen (1960) reported that the urinary excretion of catecholamines, measured chemically, was increased for many days after severe injury. Other workers using bio-assay methods have found a similar, but less prolonged, rise after uncomplicated major abdominal operations (Halme, Pekkarinen and Turnon, 1957). Increased secretion of catecholamines have also been reported in patients with burns (Wilmore et al, 1974a). However, Alberti et al. (1977) believe that further measurements are required to pinpoint the timing of the changes in catecholamine excretion following injury since they are critical to the interpretation of the changes in fuel homeostasis. These workers have also postulated that the immediate phase of the metabolic response to injury is dominated by catecholamines.

1.7.3 Thyroid

Postoperative changes in thyroid function have been the subject of considerable investigation. Some aspects of the metabolic response

to injury have suggested the participation of thyroxine (Goldenberg, Lutwak, Rosenbaum and Huys, 1956). The resting metabolic expenditure after operation of moderate severity is increased by 10%, but total energy expenditure is unaltered due to a reduction in activity (Tweedle and Johnston, 1971). After severe injury, metabolic expenditure and oxygen consumption is also increased (Kinney et al., 1970). Furthermore, hyperglycaemia is found after major trauma and the rate of glucose utilisation may change after operation (Johnston, 1964).

It has been suggested that the changes observed after injury or operation are similar to those due to excessive circulating thyroid hormone (Johnston and Bell, 1965). Direct evidence of alteration in thyroid function following operation has come from human experimental studies (Johnston and Bell, 1965). These workers first reported that after an abdominal operation the plasma concentrations of protein-bound iodine rose within six hours, and the high levels were maintained for three days after surgery. The capacity of the thyroid to take up iodine after abdominal surgery was also investigated by these workers, and a marked reduction was observed following an oral dose, though it tended to return to normal levels within the next two days. In the same study Johnston and Bell, investigated the effects of intravenous infusions of hormones known to be increased after injury, on the rate of thyroid uptake of iodine. Their results showed that ACTH, and epinepherine had no effect in this respect and therefore they concluded that the postoperative reduction in iodine uptake by the thyroid could not be attributed to the increased circulating levels of either of these catabolic hormones. Moreover, the fall rather than the rise in iodine uptake by the thyroid suggested that TSH activity was not increased in

the postoperative period. This was later confirmed by direct radio-immunoassay estimation of TSH after operation (Johnston, 1972).

The rate of production and utilisation of thyroid hormones appears to be enhanced after operation. Thus, Blomstedt (1965) has found that the rate at which the tissues use free thyroxine and excrete it in the urine is increased after abdominal surgery. The plasma concentration of free thyroxine is believed to be the most accurate assessment of thyroid activity as this is the fraction which can penetrate cell membranes and exert a metabolic effect (Johnston, 1972). Free thyroxine exists in equilibrium with thyroxine bound to globulin, albumin and prealbumin. Therefore, any changes in the concentration of thyroid-binding proteins would lead to an increase in free hormone. In this connection, Kirby and Johnston (1971) reported that thyroid-binding prealbumin (TBPA) levels fell after abdominal surgery and were found low when there was an increase in free thyroxine concentration. The fall in the plasma concentration of TBPA has been shown to be due to an acute reduction of synthesis of the protein (Schwartz and Roberts, 1957; Blomstadt, 1965). Based on this evidence, Johnston (1972), proposed that the postoperative increase in triiodothyronine is related to the fall in TBPA releasing free thyroxine which is preferentially bound to thyroid-binding globulin (TBG) thus releasing triiodothyronine. According to this hypothesis, TBPA holds and controls the release of free T4 for metabolic purposes in the tissues. However in Johnston's view this hypothesis suffers objection from the fact that only about 15% of the endogenous T4 is bound by TBPA and its control over the release of free T4 seems to be far less important than TBG and therefore, the changes in the binding capacity of TBPA

cannot account for changes in the concentrations of free T4 found in ill patients. Moreover, while no change in TBG binding capacity has been observed after elective abdominal surgery (Kirby and Johnston, 1971), others (Harvey, 1971) have found depressed TBG values in seriously ill patients and have demonstrated an inverse relationship between TBG capacity and the free thyroxine fraction in plasma.

Thyroxine secretion from the thyroid, and its clearance from the plasma is increased after injury. Thus Harland, Orr and Richards (1972) reported that after abdominal operation thyroxine release was increased by 25%. In this connection Woeber and Harrison (1971) have shown that the clearance of exogenously labelled T3 and T4 from plasma was accelerated during stress and acute infection in monkeys. The increase occurred within 8 hours and it was concluded that this was not related to any changes in binding sites. Nevertheless, in spite of accelerated clearance of exogenous hormone, endogenous labelled T4 remained unchanged in the serum. These findings have led to the suggestion that the transport of the exogenous unbound hormone into the cell is accelerated in stress (Woeber and Harrison, 1971).

1.7.4. Pancreas

(i) Insulin secretion

Abnormalities in carbohydrate metabolism following surgical operation were first investigated by Johnston (1964). He found a marked reduction in the rate of glucose utilisation immediately after abdominal operation as indicated by a fall in the rate of glucose disappearance from the blood following an intravenous injection of glucose. Further studies of the elevation of this reduced glucose utilisation showed that

the maximum fall occurred on the first postoperative day, with normal values being almost reached by the third day (Johnston, 1964). From his observations, Johnston postulated that the mechanism of these changes was endocrine in origin. Evidence in support of this view came three years later when Allison, Prows and Chamberlin (1967) described a failure of insulin secretion in the acute phase of injury. These workers extended their observation to patients with burns (Allison et al., 1968) in whom a standard 25 g i.v. glucose tolerance test (Samols and Marks, 1965) was performed during the shock phase of injury and repeated one to two weeks later. It was found that in the acute phase of injury there was a failure of insulin response to a glucose load, associated with marked glucose intolerance, and that, this response was proportional to the severity of the injury which could last up to 72 h. Subsequently there was persistent glucose intolerance associated with abnormally high insulin levels, suggesting resistance to the action of endogenous insulin (Allison, 1974). These abnormalities observed in patients with burn injury were confirmed by others (Alberti et al., 1977). Similar results have been obtained in studies of patients undergoing abdominal surgery (Allison et al., 1969).

The suppression of insulin release which follows injury has been suggested to be mediated by catecholamines. Coore and Randle (1964) found that adrenaline prevented the release of insulin from rabbit pancreas in vitro. Similar observations have also been made in man (Porte, Grober, Kuzuya and Williams, 1966). Furthermore, using α and β adrenergic blockers, Porte et al. (1966) were able to show that this phenomenon was due to catecholamine inhibition of cell secretion.

Nevertheless, the cause of the insulin resistance which follows injury has not been described. In this connection, Ross, Johnston, Welborn and Wright (1966) studied this phenomenon in patients undergoing abdominal operation and were unable to correlate it with the increased secretion of hormone antagonists such as cortisol and growth hormone. However, inactivation in the plasma or resistance at the cell membrane has been suggested as causing insulin antagonism in injury (Allison, 1974).

(ii) Glucagon secretion

It is now well accepted that glucagon has a hyperglycaemic action resulting from stimulation of hepatic glycogenolysis. In addition studies with perfused liver have demonstrated that glucagon also enhances gluconeogenesis as indicated by increased incorporation of amino acids (Garcia, Williamson and Cahill, 1966) and lactate (Exton and Park, 1968) into glucose, and augmented hepatic urea formation (Garcia et al., 1966). Observations in intact man and animals also support a gluconeogenic effect as evidenced by augmented hepatic uptake of amino acids (Shoemaker and Van Itallie, 1960; Kibler, Tylor and Myers, 1964) and increased urinary excretion of nitrogen (Salter, Ezrin, Laidlaw and Gornall, 1960; Marliss, Aoki et al., 1970). Glucagon has also been implicated in the regulation of lipid metabolism. Thus, administration of small doses of the hormone to anaesthetised dogs resulted in an elevation of plasma free fatty acids (Lefebvre, 1966). In contrast, Lefebvre (1965) observed that administration of large doses of glucagon to normal man resulted in an initial fall in plasma FFA followed by a secondary rise. It has been suggested that the early fall in FFA in probably not a direct effect of glucagon, but a consequence of glucagon-stimulated insulin

secretion demonstrable when glucagon is administered in a pharmacological but not in a physiological dose (Bondy and Felig, 1974).

The development of radioimmunoassay techniques in recent years has made it possible to study glucagon response to stress of different pathological origin. It has been suggested (Lindsey, Santeusanio et al., 1975) that plasma glucagon levels rise whenever need for endogenous fuel production is increased, as in starvation (Aguilar-Parada and Eisentraaut, 1969) or after major trauma such as fracture (Meguid, Brennan et al., 1973) or burns (Wilmore et al., 1974b). More recently, Russell, Walker and Bloom (1975) studied alterations in glucagon concentration in patients undergoing abdominal operation. These workers reported a significant rise in plasma glucagon during abdominal surgery. In contrast, Giddings, O'Connor et al. (1975) studying a heterogenous group of patients undergoing different kind of operation observed no change in glucagon concentrations during operation. They did, however, observe a significant rise on the second postoperative day. The discrepancy between the findings in these two studies could be attributed to the type of surgical procedure involved. In this connection, Bloom et al.(1974) have shown that vagal stimulation resulted in an increased glucagon production. On this evidence, therefore, it could be postulated that the elevated plasma glucagon concentration observed during abdominal surgery (Russell et al., 1975) was a result of vagal stimulation during the course of the operation.

2. NUTRITIONAL PROBLEMS OF SURGICAL PATIENTS

In the preceding paragraphs the alterations in energy, fat, carbohydrate and protein metabolism following injury or surgical operation were discussed. In this section an attempt is made to discuss the impact of these changes on the overall nutritional status of the injured patients.

The original observations of Sir Cuthbertson in 1930, that negative nitrogen balance is developed after injury, prompted many investigators to study the nature of this nitrogen loss and its possible prevention. Howard and his associates (Howard, Parson et al., 1944; Howard, Bigham et al., 1944; 1946) compared the nitrogen balance of patients in convalescence following fractures of the long bones to that of patients undergoing elective operations on the skeleton. They found that the magnitude of the negative nitrogen balance in fracture patients was much greater than that of patients undergoing surgery on the skeleton. Furthermore, on the basis of their results, it was concluded that a high protein-high-calorie diet did not exert any appreciable sparing effect at the peak of negative nitrogen balance following trauma in healthy and previously active patients. They did, however, observe that severely emaciated patients who showed no significant change in nitrogen metabolism following multiple fractures were kept in nitrogen equilibrium one week after injury with a daily intake of 6 g nitrogen and 900 calories per 1.73 M^2.

Inasmuch as the increase in protein catabolism which follows trauma occurs at a time when the energy intake is at its lowest, interest has been focussed on the contribution of starvation to the metabolic response.

Werner, Habif, Randall and Lockwood (1951, cited by Randall, 1976) studied this phenomenon and showed that when patients undergoing either ventral or inguinal herniorrhaphy or elective cholecystectomy were given pre-operative parenteral nutrition (consisting of 18 to 24 calories/Kg/ day of 10% glucose with 12.5 to 14.4 g of nitrogen as amino acid), their slight negative nitrogen balance was increased by less than 1 g/ day following operation. When patients subjected to partial gastrectomy for duodenal ulcer were given a similar pre-operative intravenous nutritional regimen, nitrogen losses during the immediate postoperative period were larger than following herniorrhaphy or cholecystectomy. However, the losses were considerably less than those noted in another group of patients maintained postoperatively on routine parenteral fluid consisting of 5% dextrose with sodium and potassium chloride. These results led Werner et al. (1951) to conclude that starvation played the major role in the postoperative nitrogen loss under the condition of their studies. Further support for this conclusion came from the investigation of Abbott and Albertson (1963), who studied a series of volunteers and patients undergoing operations ranging in severity up to partial gastrectomy and with or without complications. They found little difference in nitrogen losses between the two groups which had similar intakes (50 calories and 0.2 g nitrogen as amino acids per Kg. body weight). However, when a complication such as infection was present, thereby adding greatly to the stimulus for catabolism, it was no longer possible to obtain equilibrium at this level of intake and as a result a significant negative nitrogen balance developed. They also were able to show that an adequate intake of protein and calories given intravenously could abolish or greatly minimise the loss of

nitrogen. Similar findings were reported by Wadstrom and Wiklund (1964), who gave a daily intake of 0.1 g.N/Kg and 35 calories/g immediately after operation and reduced the negative nitrogen balance to 1.0 g nitrogen/day. These observations were essentially similar to those of Johnston, Marino and Stevens (1966) showing reductions in the negative nitrogen balance by using a daily intravenous intake of 0.18 g.N/Kg and 47 calories/Kg. In Clark's view (Clark, 1967) these levels of intake were very near the normal and indicated that relatively little catabolic drive was present.

As the severity of the trauma increases, the stimulus to protein catabolism becomes greater. This has been clearly shown by Sorroff, Pearson and Artz (1961), who reported that in patients with burns 3-4 times the normal amount of protein were required to restore the nitrogen balance to normal. From their findings they suggested that in burn injuries, the body's ability to utilise the administered protein was not decreased. Clark (1967) recalculated the results of Sorroff et al. (1961) and showed that the requirement to restore nitrogen equilibrium during the early phase of burn injury was 0.5 g/Kg body weight, while the corresponding value at the late phase of catabolism was only 0.16 g. Thus indicating the relationship between the severity of trauma and protein requirement. These observations led Clark (1967) to suggest that almost all negative nitrogen balance which follows operation or injury is due to a low calorie intake and can be reversed by adequate nutrition of normal composition. This view is now shared by the others (Lee, 1974). However, despite these observations and the fact that over the last fifteen years great advances have been made in introducing improved intravenous nutrient solutions and minimising their

toxicity, a large number of surgical patients are suffering from malnutrition of varying degree. On the basis of both anthropometric measurements and serum albumin, Bistrian, Blackburn et al. (1974) observed a striking prevalence of protein-energy malnutrition (PEM) amongst the patients on the surgical wards of a large city hospital in the United States. They reported that hypoalbuminaemia was present in 30 of 56 patients, and in 15, (27%) of these the serum albumin level was in the severely depleted range (less than 2.8 g/100 ml). They also noted that serum albumin levels became subnormal in at least 3 of 10 subjects after admission to the hospital. Moreover, a close correlation was observed between serum albumin and mid-arm muscle circumference. Similarly, Hill, Blackett et al. (1977) studied 105 surgical patients in a teaching hospital in Great Britain and reported a prevalence of PEM (55%) as judged by arm muscle circumference and plasma albumin, amongst patients who had undergone major operations and had been staying in the hospital for more than a week.

A partial explanation for the general failure to recognise the extent of these problems has been suggested (Bistrian and Blackburn et al. , 1975) to be the widespread reliance on weight for height as the routine measure of nutritional status, while the more sensitive anthropometric measures like arm muscle circumference are seldom used. Additionally, in these worker's view, the significance of low serum albumin levels as a marker of significant protein deficit has not generally been appreciated.

However, these findings point to the fact that the nutritional support of hospitalised patients, as Butterworth has put it, is far from ideal (Butterworth, 1974). He like others (Bistrian et al. 1974;

Randall, 1975; Hill et al., 1977) believes that little attention is being given to the nutritional status of ill patients and calls for the greater emphasis on nutrition in medical curricula.

3. CONCLUSIONS AND PLAN OF PRESENT STUDIES

The review of the literature showed that although the metabolic disturbances following bone fractures and burns have been extensively studied, those that follow elective surgery have been studied either in patients subjected to heterogenous groups of operations or those undergoing abdominal surgery. No attempt appears to have been made to elucidate the changes which follow particular operations other than those on the abdomen. Furthermore, in contrast to the considerable amount of information which is available on changes in plasma protein concentrations and urinary nitrogen excretion after injury, very little is known about the postoperative changes in the pattern of plasma amino acids.

Whilst all amino acids are involved in protein synthesis, some also participate in other reactions, and play other roles, which are important in the normal functioning of particular systems. One such amino acid, tryptophan, seemed to be of particular interest because of its involvement in the synthesis of serotonin (5-hydroxytryptamine). In recent years it has been found that the concentration of free tryptophan in the plasma exerts some control on the entry of this amino acid into the brain and hence upon the synthesis of serotonin (Fernstrom and Wurtman, 1971; Dickerson and Pao, 1975). It has also been found that the concentration of tryptophan in the plasma falls in malnourished

animals (Dickerson and Pao, 1975). No previous work was found in which this matter had been specifically investigated in postoperative patients.

In the light of these conclusions, it was decided to study some aspects of protein, energy and hormonal metabolism in patients undergoing different types of operation. Patients undergoing thoracic surgery were chosen for a number of reasons. Patients with oesophageal disease seemed to be particularly worthy of study. Those in which the oesophagus is resected for cancer often have a history of weight loss prior to seeking advice, and thus often come to surgery in a relatively malnourished condition. This is not the case, however, in patients with a hiatus hernia for they tend to be overweight. Operations for both these conditions involve the alimentary tract. Surgery of the lung also involves a thoracotomy, and thus patients having lung resections for cancer were also investigated.

Aspects of these three conditions and their treatment relevant to these studies will now be described.

3.1 Epidemiology, pathology, surgical treatment and prognosis of oesophageal cancer, lung cancer and hiatus hernia

3.1.1. Cancer of the oesophagus

(i) Epidemiology

The incidence of carcinoma of the oesophagus shows wide variations from one country to another. The 1947 survey in the United States revealed a rate of 8.3 and 1.9 per 100,000 population for male and female whites and 9.7 and 2.2 for male and female non-whites

(Ackerman and del Regato., 1970). In contrast, Marcial, Tomo et al. (1966) have reported a remarkably high incidence of oesophagus carcinoma in Puerto Rico (13.7 for males and 5.6 for females per 100,000). Over 90% of all cases were among patients over 50 years of age, with a peak incidence between 60 and 69 years.

It has long been recognised that carcinoma of the oesophagus is prevalent amongst Chinese and this has been shown to be true for Chinese residents in Java (Kouwenaar, 1950). In South Africa, Higginson and Oettle (1960) and Burrell (1962) have reported a high incidence among the Bantus of the Transkei region. In England, Scotland and Scandinavia, the occurrence of this form of cancer among underpriveleged women has been connected with the occurrence of the Plummer-Vinson syndrome (Ahlbom, 1936), a nutritional deficiency now being corrected by the addition of iron and vitamins to the baking flour (Ackerman and del Regato, 1970). Burrell et al. (1966) have attempted to explain the recent rise in the incidence of oesophageal cancer among the Bantus by the presence of a carcinogenic substance in their food. Carcinoma of oesophagus has also been reported in association with malabsorption. Thus Wright and Richardson (1967) reported four cases of thoracic oesophageal cancer in patients with a malabsorption syndrome. From these observations, Ackerman anddel Regato (1970) have suggested that the differences in sex ratios and racial incidence could possibly be explained by nutritional variations as influenced by other factors such as alcoholism.

(ii) Pathology

Ochsner and De Bakey (1941) collected 8572 cases of oesophageal cancer from the medical literature and found that 20% developed in the upper, 37% in the middle and 43% in the lower third of the oesophagus.

Some carcinomas develop in a bulky form that rapidly closes the lumen of the oesophagus. Others are superficially ulcerated and spread in the wall of the oesophagus without much obstruction (Ackerman and del Regato, 1970). In this connection, Mathews and Schnabel (1935) in a review of 237 autopsies on patients with cancer of the oesophagus, found twenty-two with no obstruction.

(iii) Complications

The most common symptom associated with oesophageal neoplasms is dysphagia. This may be accompanied by a sensation of substermal fullness with or without pain. The dysphagia may first be associated with solid food and later with soft foods and then even with liquids. In addition to swallowing difficulty, weight loss almost invariably occurs in the later stages of the disease and may go on to severe emaciation (Ellis, 1972). Vomiting and regurgitation often take place soon after eating. Iron deficiency anaemia is common and is sometimes severe.

(iv) Treatment

Surgical treatment: The first successful resection of a carcinoma of the oesophagus was performed by Torek over 60 years ago (Torek, 1913). Since then a number of refinements and modifications have been made in the techniques of oesophagectomy. However, the basic principle of the operative procedure is excision of the neoplasm including wide resection of adjacent areas of the oesophagus and the regional lymph nodes (Adkins, 1972). One of the limitations of this procedure is the proximity to the tumour mass of vital structure such as the aorta, heart and trachea. Consequently, there are limitations in the extent of the resection (Adkins, 1972). However, despite this limitation some surgeons advocate

total oesophagectomy regardless of the level of the tumour in the thorax (Parker, 1967 cited by Adkins, 1972).

Palliative procedures: When there are indications of metastasis or advanced cardiovascular disease, radical surgery is not attempted. Under these circumstances radiation therapy is preferred. Another approach to the palliation of an inoperable carcinoma of the oesophagus is the use of a rigid tube that is pushed through the obstructing neoplasm, thus allowing the patient to swallow. However, it has been reported that such tubes encourage necrosis and bleeding and might cause ulceration (Ackerman and del Regato, 1970).

(v) Prognosis

The results of treatment of malignant tumours of the oesophagus have been generally unsatisfactory, and the average life expectancy of these patients used to be very short, with about 25% dying within six months and 75% within one year (Greenwood, 1926). In recent years, a greater proportion of patients have received treatment, but according to Ackerman and del Regato (1970), the overall survival of these patients remains dismally low. For example, only 1.4% of Nakayama's 2382 patients (Nakayama, 1964) survived five years and only 1.7% of 1109 patients registered from 1950 to 1958 at the Puerto Rico Cancer Registry survived five years (Martinez, 1964). From 1951 to 1960, Franklin, Burn and Lynch (1964) studied 129 patients with carcinoma of the oesophagus at the Hammersmith Hospital in London. Ninety-one of these patients were explored, but only fifty-eight had a resection, with thirty-six of them dying within one month and the remaining twenty-two living over one year. In another series of 208 patients seen by Sturdy (1965) at the Royal Gwent Hospital in Newport,

Wales, seventy-one were submitted to radical surgery, with nine surviving two years and five surviving five years.

3.1.2 Lung cancer

(i) Epidemiology

Carcinoma of the bronchus has become the most frequent form of cancer in men in the United States and many other countries (Denoix and Gelle, 1955). In 1968 approximately 60,000 Americans developed cancer of the lung. The incidence in males in Denmark doubled between 1943-1947 and 1953-1957, whereas the incidence in females increased by only two-thirds (Ackerman and del Regato, 1970).

The increase in cancer of the lung has led to the extensive clinical, epidemiologic and laboratory investigations. The aetiology of lung cancer has not yet been defined but it has been suggested that it is an environmental product of modern civilisation (Ackerman and del Regato, 1970). Ashley (1967) found that individuals working in coal and textile industries had an increased incidence of bronchitis and a decreased incidence of lung cancer. On this evidence he suggested that chronic lung disease associated with the inhalation of dust could protect the lung against carcinogenic substances. Furthermore, there is adequate experimental and epidemiologic evidence to incriminate various organic and inorganic industry-related chemicals as causes of cancer of the lung (Hueper, 1959) with definite risk to specific worker groups. This is supported by experimental investigations in which cancer of the lung has been produced in animals exposed to radioactive metals, nickel, chromium, and arsenic (Ackerman and del Regato, 1970). Moreover, the evidence collected supports an important relationship between the inhaling of tobacco smoke and cancer of the bronchus (Oettle, 1963;

Bocker, 1963). The carcinogenicity of tobacco smoke condensates on the skin of animals has also been shown (Van Duren, 1958). Air pollution has also been suggested to have an important role to play in relation to lung carcinoma (Stocks, 1966).

(ii) Pathology

Bronchogenic carcinoma arises most often in and about the hilus of the lungs. About three quarters of the lesions take origin from the lower trachea and first, second, and third order bronchi. Histologically, bronchogenic carcinomas are divided into three types in the following approximate distribution (Robins, 1974).

1. Squamous cell carcinoma, 70 per cent.
2. Adenocarcinoma, 10 per cent.
3. Undifferentiated carcinoma, 20 per cent.

Squamous cell carcinoma is found exclusively in men while adenocarcinoma occurs about equally frequently in men and women. Moreover, there is no clear correlation between the smoking history and the occurrence of adenocarcinoma of the lung, and as Robins (1974) has pointed out, in contrast to squamous cell carcinoma, there has been no significant increase in the frequency of this pattern over the recent past. One of the interesting clinical aspects of bronchogenic carcinoma is the occasional undifferentiated tumour which produces hormones such as ACTH, ADH, gonadotrophins, and parathormone. Sufficient ACTH, for example, may be elaborated by these tumours to induce Cushing's syndrome (Lipseet, 1965; Azzopaadi and Williams, 1968). It has been speculated that in such neoplasms, the genetic code for ACTH synthesis and the other hormones becomes depressed in the course of the carcinomatous transformation (Robins, 1974).

(iii) Treatment

Surgery: In 1933, Dr. Evarts A. Graham performed the first successful pneumonectomy for carcinoma of the lung. His patient, a physician, lived twenty-nine years without evidence of cancer and outlived Dr. Graham who died of carcinoma of bronchus (Reported by Ackerman and del Regato, 1970). Over the last forty years, pneumonectomies have been performed on an increasing number of patients. The usually elderly patients who survive a pneumonectomy face a diminishing pulmonary reserve (Adams, Parkins et al., 1957). Lobectomies have also been performed in the treatment of the peripheral carcinoma of the lung where they have proved to be successful (Churchill, Sweet and Wilkins, 1958). Moreover, lobectomies are believed to have less operative mortality, and for this reason there has been an increasing trend toward utilisation of this procedure (Ackerman and del Regato, 1970).

Chemotherapy: Carcinoma of the lung has been the subject of numerous trials of chemotherapeutic agents as adjuvant to surgery (Curreri, Ansfield, 1958; Curreri, 1962). Nitrogen mustard, a powerful alkylating agent, has been used extensively (Ackerman and del Regato, 1970). However, in Slack's view (Slack, 1970) administration of nitrogen mustard may relieve superior vena cava obstruction but this is invariably of short-term benefit. Neither has it proved advantageous to add a chemotherapeutic agent at the end of an operative procedure.

(iv) Prognosis

According to Robins (1974), lung cancer is one of the most insidious and aggressive neoplasms in the whole realm of oncology. In the usual case, it is discovered in the sixth decade of life. Despite all efforts at early diagnosis by frequent radiological examination of the chest,

the five-year survival rate is of the order of 7 percent (James, 1966).

In general, the adenocarcinoma and squamous cell patterns tend to remain localised longer and have slightly better prognosis than undifferentiated tumours which usually are bulky, invasive lesions by the time they are discovered (Robins, 1974). In a recent analysis of 4000 cases, Bignall and Martin (1972) reported that the five-year survival of males was approximately 10 percent with squamous cell carcinoma and adenocarcinoma, but only 3 per cent with undifferentiated lesions.

3.1.3 Hiatus hernia

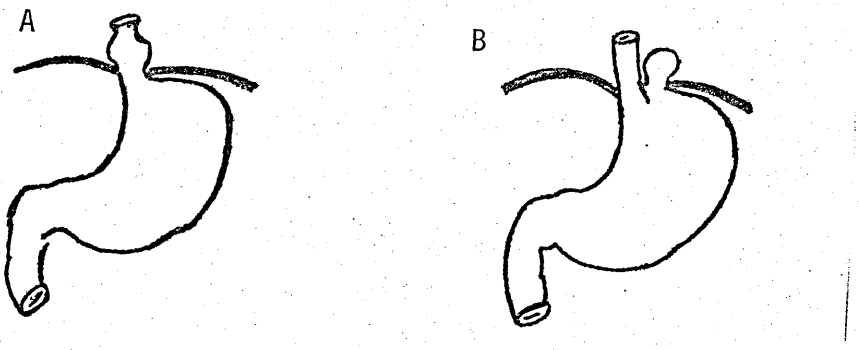
(i) Aetiology

In recent years oesophageal hiatus hernia and its sequel, reflux oesophagitis, have been increasingly recognised as common causes of dyspepsia and dysphagia (Davidson, Passmore et al., 1975). The diaphragm has several openings through which herniation of abdominal viscera into the thorax can occur. Of these openings the most important is the oesophageal hiatus through which the oesophagus passes and to which it is loosely attached. In middle-aged and elderly people particularly, this attachment weakens so that herniation of the oesophagus and stomach results. It is most common in middle aged obese females and the gross incidence in females is three times that in males (Naish and Read, 1974).

(ii) Pathology

There are two types of hernia, sliding and rolling (Fig.1.3)

Figure 1.3 Hiatus hernia



A. Sliding type. A small sac is seen above the diaphragm

B. Rolling type. Most of the fundus of stomach lies above the diaphragm and displaces the lower oesophagus.

A. Sliding hernia. The gastrooesophageal junction protrudes upwards through the hiatus and a bag of stomach comes to lie within the chest. This is the most common form, and 80 to 90 per cent of all hiatal hernia are of this type (Hagarty, 1960).

B. Rolling hernia. In this form part of the cardia of the stomach prolapses through the hiatus alongside the oesophagus. This type is less common, and about 10 per cent of hiatal hernias are of this type.

(iii) Complications

Acid and pepsin pass through the incompetent oesophagogastric sphincter to produce reflux oesophagitis. Loss of blood in the vomit may lead to anaemia and ulceration and fibrosis would lead to stricture of the lower oesophagus. Heartburn may occur after meals but is also, and typically, associated with a change of posture, such as bending down at housework, or lifting or straining, which produces a rise of intra-abdominal pressure. It may occur on lying down at night and may waken the patient, who obtains relief by sitting up. The patient may also complain of more severe pain or the sensation of food sticking which may amount to dysphagia.

(iv) Treatment

Symptomless hernias require no active treatment, but the patient should be encouraged to keep slim, to abstain from fatty foods which stimulate gastric secretion, to avoid straining, and raising intra-abdominal pressure, and to sleep on a firm mattress with the head of the bed higher than the foot (Naish and Read, 1974). However, if the patient does not respond to medical measures and continues to suffer from reflux and pain, or have oesophagitis of more than slight degree surgical treatment is employed. The principle of surgical repair is to ensure a length of at least 4 cm. of intra-abdominal oesophagus and to prevent this segment from returning to the chest.

CHAPTER TWO

PATIENTS AND METHODS

1. PATIENTS

The patients used for the studies in this thesis were all admitted to Milford Chest Hospital and were under the care of two Consultant Thoracic Surgeons. The ward into which they were admitted has an attached intensive care unit to which the patient is taken following operation and in which he or she remains for 2 to 4 days or longer, depending on the nature of the operation and upon clinical progress. Brief clinical details of the patients included in the study, and who gave their signed permission to participate in the research, are given in Table 2.1. Hiatus hernia is rather more common in women than in men and of the nine patients studied 7 were women. The average age of these patients was 61.6 years with a range of 49 to 71 years. Eleven patients with oesophageal cancer were available for study. These consisted of 7 men and 4 women with an average age of 65.7 years and a range of 54 to 76 years. The eight patients studied with lung cancer were all men and their average age was 60.2 years with a range of 53 to 67 years. The differences between the average ages of the groups were not significant. All the patients were studied for 14 days.

Fasting blood samples and 24 hour urine collections were obtained before operation and at 2, 7 and 14 days after operation. A blood sample was also obtained immediately after the operation. Blood samples were heparinized and the plasma separated within 45 minutes of the blood being drawn. The plasma was stored at -35° until analyzed. Urine samples were collected in bottles containing 25 ml of glacial acetic acid. After mixing and measuring the volume, aliquotes were stored at -15° C.

for hiatus hernia, oesophageal cancer and lung cancer

Patient	Sex	Body weight (Kg)	Age (years)	Diagnosis	Surgical procedure
			Hiatus hernia		
F.E.	Female	54	62	hiatus hernia	hiatus hernia repaired by left thoracotomy
G.L.	Female	72	64	"	"
F.B.	Male	76	56	"	"
R.L.	Male	77	50	"	hiatus hernia repaired by fundoplication
F.V.	Female	48	67	"	hiatus hernia repaired by left thoracotomy
D.S.	Female	79	65	"	"
P.C.	Female	77	71	"	"
E.S.	Female	58	70	"	hiatus hernia repaired by pascical reinforcement
L.H.	Female	64	49	"	hiatus hernia repaired by left thoracotomy
			Oesophageal cancer		
R.D.	Male	69	64	Carcinoma of oesophagus	resection by right thoracotomy
N.H.	Female	64	67	"	"
L.J.	Male	68	64	"	"
M.E.	Male	63	55	"	oesophago-gastrectomy
G.B.	Male	59	66	"	"
J.P.	Male	81	76	"	"
E.G.	Female	46	76	Sarcoma of the proximal stomach	left thoracotomy and oesophago-gastrectomy
P.T.	Male	64	54	Carcinoma of oesophagus	oesophago-gastrectomy
E.H.	Female	46	68	Carcinoma of cardia	"
L.T.	Female	46	66	Carcinoma of oesophagus	"
G.W.	Male	59	67	"	"
			Lung cancer		
R.W.	Male	78	63	Bronchial carcinoma	right pneumonectomy
T.F.	Male	83	67	Carcinoma of right lung	upper lobectomy
L.B.	Male	77	66	"	lower and middle lobectomy
G.S.	Male	83	54	"	lower lobectomy
J.S.	Male	90	53	carcinoma of left lung	left thoracotomy and pneumonectomy
P.M.	Male	73	58	"	upper lobectomy
P.V.	Male	65	61	Carcinoma of right lung	"
R.S.	Male	75	60	"	"

2. ANALYTICAL METHODS

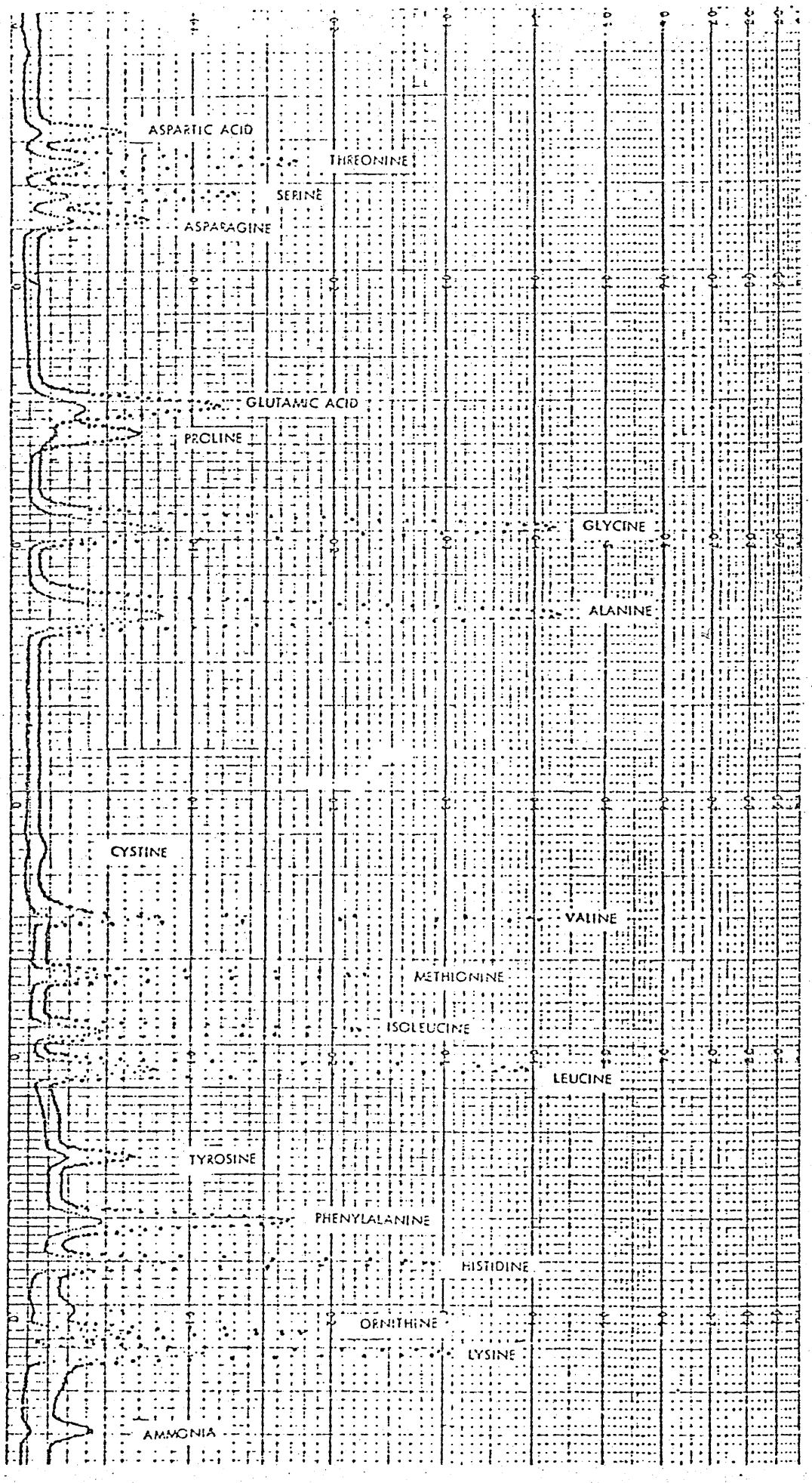
2.1 Determination of plasma free amino acid by amino acid analyzer

Plasma was deproteinised according to the method described by Dickinson et al. (1965). Plasma (0.5 ml) was mixed in a centrifuge tube with 2 ml of sulphosalicylic acid (3%), and the resulting protein precipitate was removed by centrifugation at 2,000 x G. The supernatant (1 ml) was used without further treatment for the quantitative determination of individual amino acid on an LKB 410 Amino Acid Analyzer (LKB, Biochrom Ltd., England). Acidic, neutral, and basic amino acids were separated on a single column (725 x 9 mm) packed with a cation exchanger (Aminex^{R}, BioRad Laboratories, California). Sodium citrate buffers with different ionic strength and pH were used as eluents, and the flow rate was maintained at 60 ml per hour throughout the analysis.

For quantitative evaluation of each amino acid, the curve with the highest absorption value was used. For most amino acids this is the 570 nm curve. For proline and hydroxyproline, however, the highest absorption is to be found at 440 nm.

A standard solution containing 2.5 µ moles of each of 18 amino acids was obtained from LKB (LKB Biochrom, England), and was further diluted 1:25 prior to the analysis (final concentration, 100 nmoles/ml of each of the 18 amino acids). The accuracy of this technique was evaluated and found to be within 3%. A typical amino acid chromatogram is shown in Figure 2.1.

Figure 2.1 Standard Amino Acid Chromatogram



2.2 Determination of plasma total tryptophan (Denkla and Dewey, 1967)

Reagents: Reagents were TCA/$FeCl_3$: 6×10^{-5} M $FeCl_3$ in 10% TCA; 10% TCA (w/v); and formaldehyde : 1.8% (w/v) aqueous formaldehyde; tryptophan stock solution : 250 nmoles per ml of 0.1 N NH_4OH.

Procedure: Heparinised plasma (0.05 ml) was pipetted into a polyethylene centrifuge tube using an Oxford Pipette (Oxford Laboratories, California) and 1.8 ml TCA/$FeCl_3$ was added, mixed, and the mixture centrifuged at 20,000 r.p.m. for 10 minutes. The supernatant was decanted completely into a graduated glass-stoppered tube and 0.2 ml of formaldehyde was added. The stoppered tubes were placed in a boiling water bath for 1 hour. The tubes were cooled to room temperature and 10% TCA was used to replenish to the 2 ml mark the fluid lost during incubation. The fluorescence of the product was read at excitation and emission wavelengths of 373 and 452 nm respectively, in a Perkin Elmer spectrofluorimeter. The standard curve for the determination of tryptophan is shown in Figure 2.2.

2.3 Determination of plasma free tryptophan

The method used for the estimation of plasma free tryptophan was essentially that described by Baumann, Duruz and Heimann (1974). Fresh plasma samples (0.5 ml) were first ultrafiltered by centrifugation for half an hour at 1000 G and room temperature in CF-50 Amicon^R membrane cones. Duplicate 0.1 ml aliquots of the ultrafiltrate were assayed for tryptophan as described above. The precision of the method was evaluated and it was found that 90% $\pm$ 3 (mean $\pm$ SD) of the added

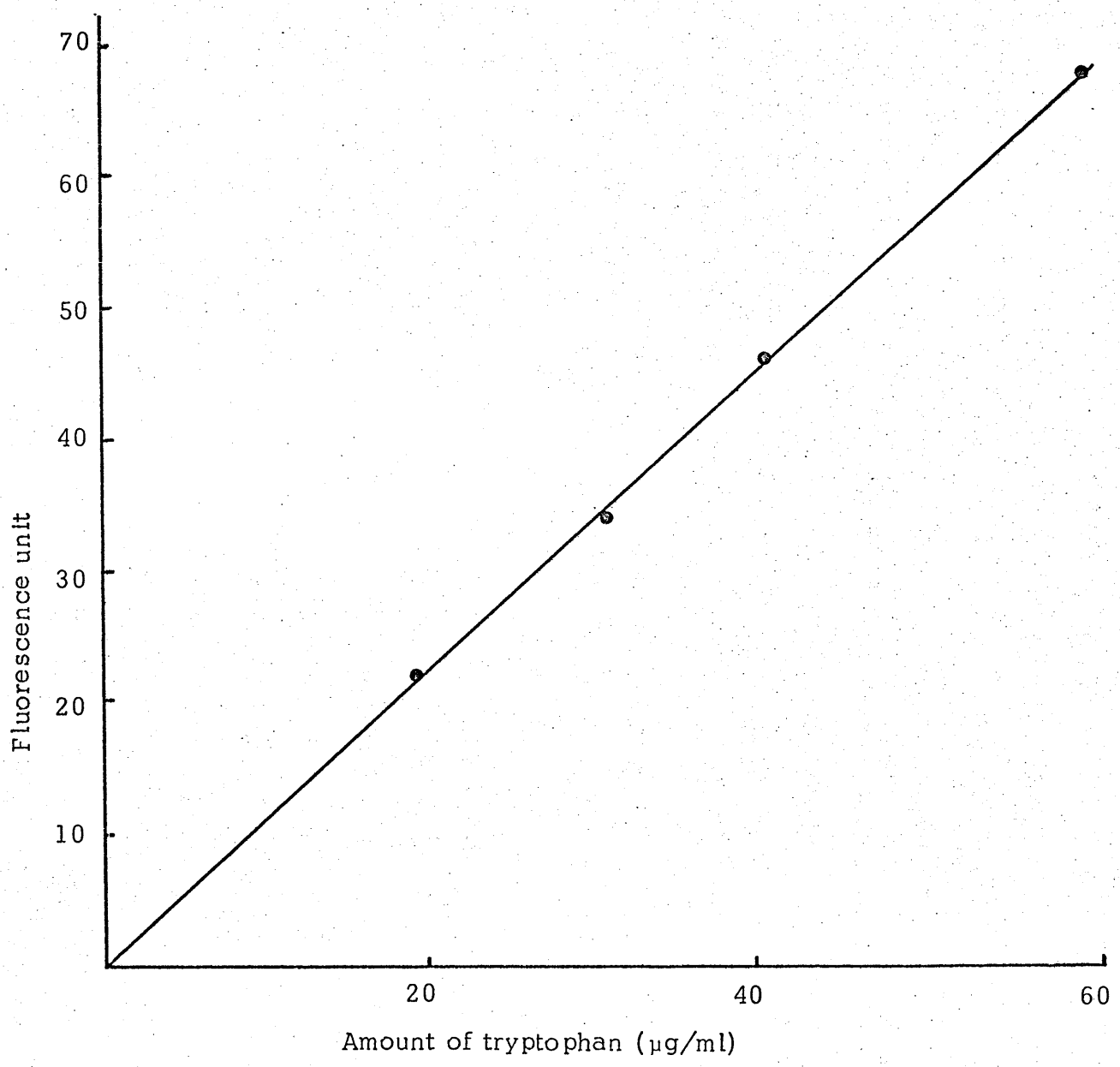


Figure 2.2. Standard curve for determination of tryptophan

tryptophan could be recovered after filtration.

2.4 Determination of plasma total protein (Gornal, Bardawill and David, 1949)

Biuret reagent: This solution was prepared by dissolving 1.5 g of copper sulphate ($CuSO_4$, $5H_2O$) and 6.0 g of sodium potassium tartrate ($NaKC_4H_4O6,4H_2O$) in 500 ml of water and adding 300 ml of 2.5 N sodium hydroxide. The reagent keeps well when stored in a polyethylene bottle and room temperature.

Procedure: Into the test tubes containing 5 ml of biuret reagent plasma samples (0.05 ml) were added, mixed and incubated for 30 minutes at room temperature. The developed colour was then measured at 540 nm in an SP500 spectrophotometer. A standard curve (Figure 2.3) was prepared using human plasma protein solution supplied by Sigma Company.

2.5 Determination of plasma total globulins (Neely, Pollack, and Cupas, 1975)

Globulin reagent: This reagent was prepared by dissolving 15 g copper sulphate in 15 ml water and then transferring it to 800 ml of 11.8 M (85% w/v) lactic acid. After mixing, 110 ml of concentrated sulphuric acid was carefully added with stirring. After cooling to room temperature 1.1 g of glyoxylic acid was added. When the glyoxylic acid had completely dissolved, the solution was diluted to 1000 ml with 11.8 M lactic acid and mixed thoroughly. This solution was stable for several months at room temperature.

Procedure: Into the test tubes containing 5 ml of globulin reagent were added 0.05 ml aliquots of plasma with an Oxford pipette (Oxford

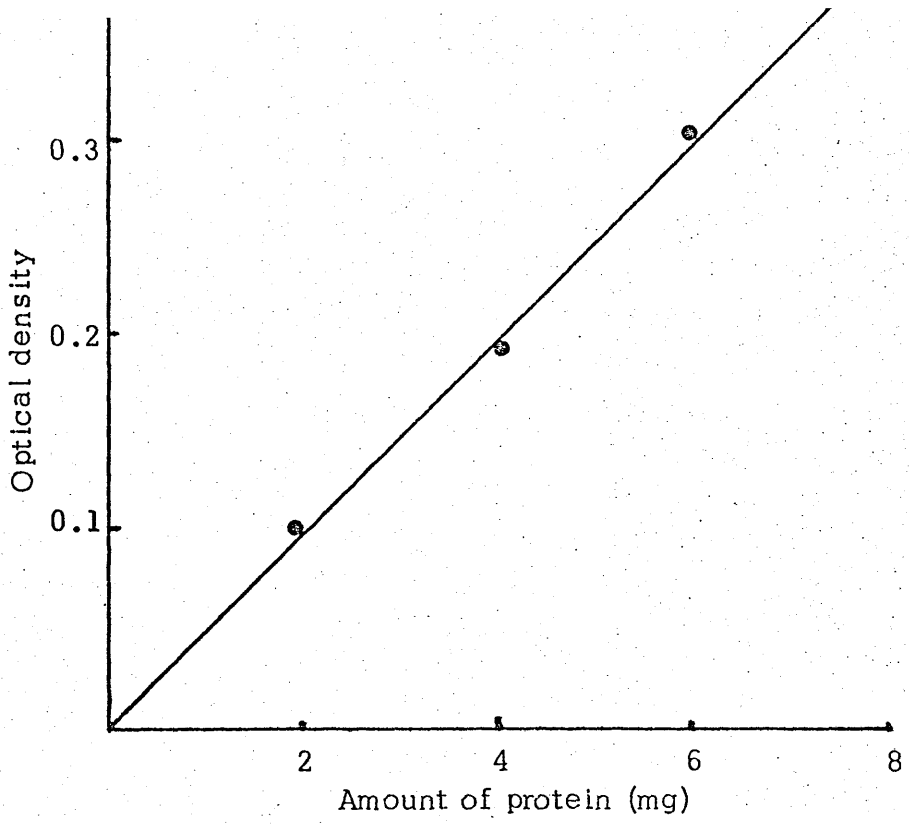


Figure 2.3 Standard curve for determination of plasma total protein

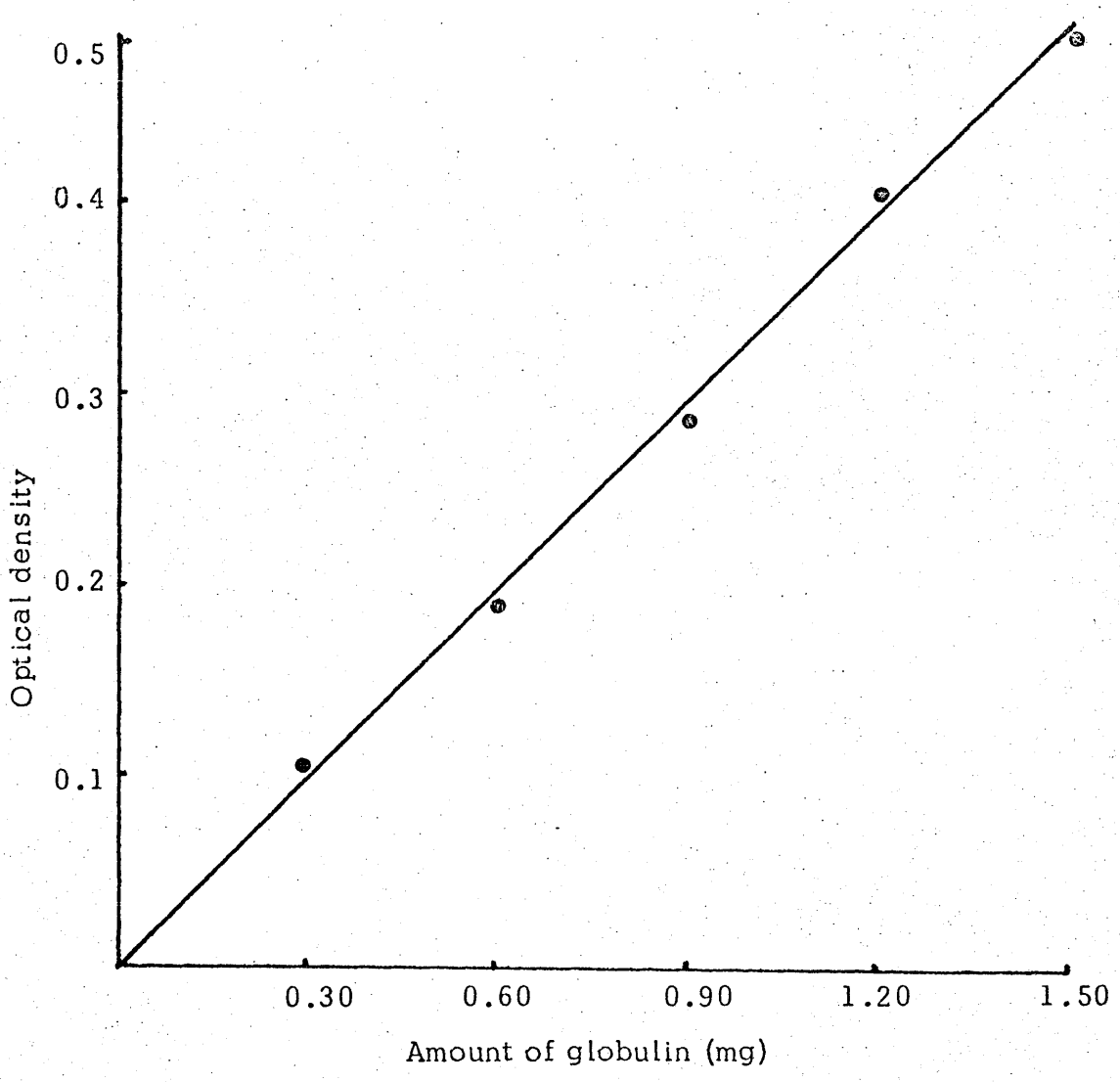


Figure 2.4 Standard curve for determination of plasma globulin

Laboratories, California). After mixing thoroughly on a vortex mixer, the tubes were capped with marbles and placed in a boiling water bath for 10 minutes. They were then cooled to room temperature and the colour read at 565 nm on a Unicam 500 spectrophotometer. A standard curve (Figure 2.4) was prepared using a globulin standard solution obtained from Sigma.

2.6 Determination of total plasma 11-Hydroxycorticosteroid

The 11-Hydroxycorticosteroids in plasma were measured by the modified method described by Mattingly (1962). Cortisol was extracted from plasma by shaking with methylene chloride. The extract was mixed with a sulphuric acid-ethanol mixture (7:3, v/v; fluorescence reagent) to develop the fluorescence. The fluorescent product is unstable and therefore the timing of the fluorescence reading was important.

The assay procedure for 11-hydroxycorticosteroids was as follows: 0.5 ml of heparinised plasma was pipetted into a glass-stoppered tube. 0.5 ml of distilled water and 4.0 ml of purified methylene chloride were added. The corticosteroids were extracted into the methylene chloride by mixing on a rotary shaker for 10 minutes. 3.0 ml of methylene chloride extract was carefully transferred to a 5 ml glass stoppered tube, and 1.5 ml of fluorescence reagent added noting the time of addition, and mixed by vigorous shaking for 30 seconds. The acid phase (lower) was transferred to a fluorimeter cell and the fluorescence was measured at 475 nm and 515 nm activation and emission wavelengths respectively at exactly 25 minutes after the fluorescence reagent was added. A blank using 0.5 ml of distilled water and standards (cortisol in ethylalcohol) were prepared

and carried through the same procedure. A standard curve for the determination of 11-hydroxycorticosteroids is shown in Figure 2.5.

2.7 Determination of plasma insulin

Plasma insulin concentrations were measured by a single antibody-radioimmunoassay procedure, developed by Amersham, Radiochemical Centre.

Human insulin standards 0-160 units per ml in phosphate buffer, pH 8.6 were included and run in parallel to the tests for each determination. A standard curve for determination of plasma insulin is shown in Figure 2.6.

2.8 Determination of plasma glucose

The method used was a modification of that described by Werner and Wirlinger (1970). Plasma (0.1 ml) was deproteinised by the addition of 1 ml perchloric acid (0.33 N) The precipitated protein was removed by centrifugation at 2000 x G for 10 minutes. Supernatant (0.1 ml) was transferred to a test tube to which 5 ml of enzyme solution (glucose oxidase) 10 U/ml; peroxidase 0.8 U/ml, in phosphate buffer, 100 mM, pH 7.0, obtained from Boehringer) was added and mixed. After incubation at room temperature for 20 minutes, the developed colour was measured at 620 nm using a Unicam SP500 spectrophotometer. A blank (0.1 ml water) and standards (0.1 ml of solutions containing 20-200 mg/100 ml glucose) were carried through the same procedure. A standard curve for the determination of glucose is shown in Figure 2.7.

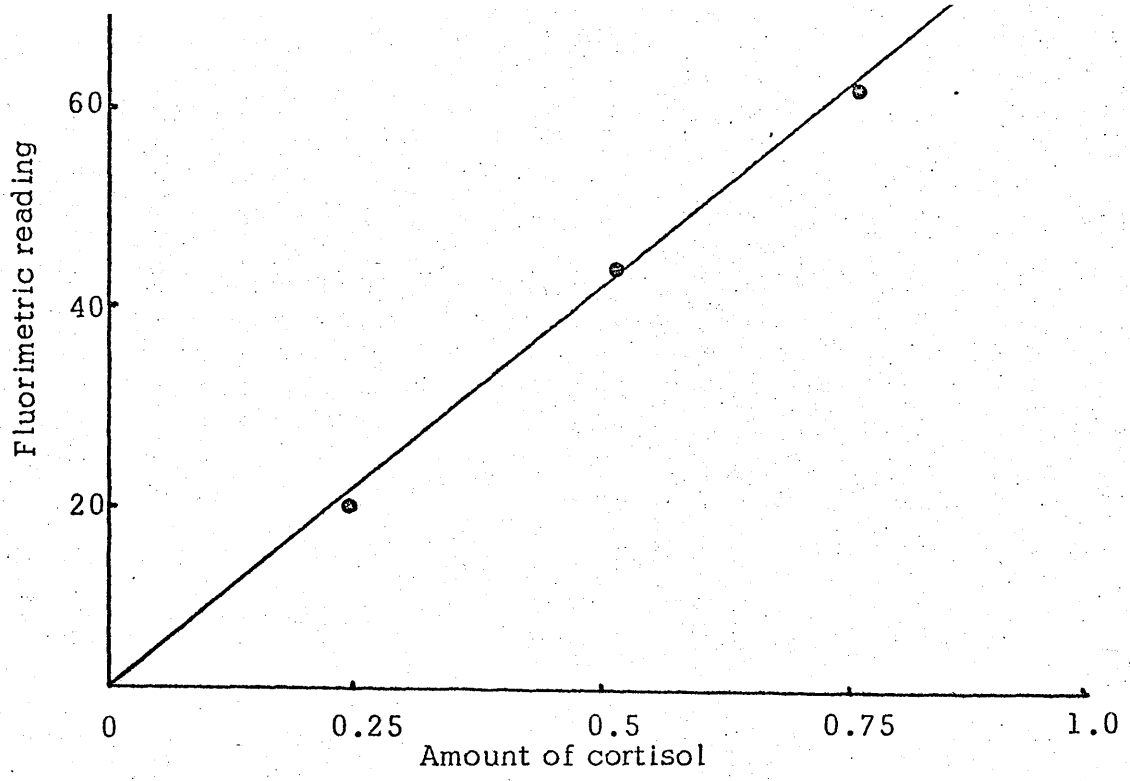


Figure 2.5 Standard curve for determination of 11-hydroxycorticosteroid

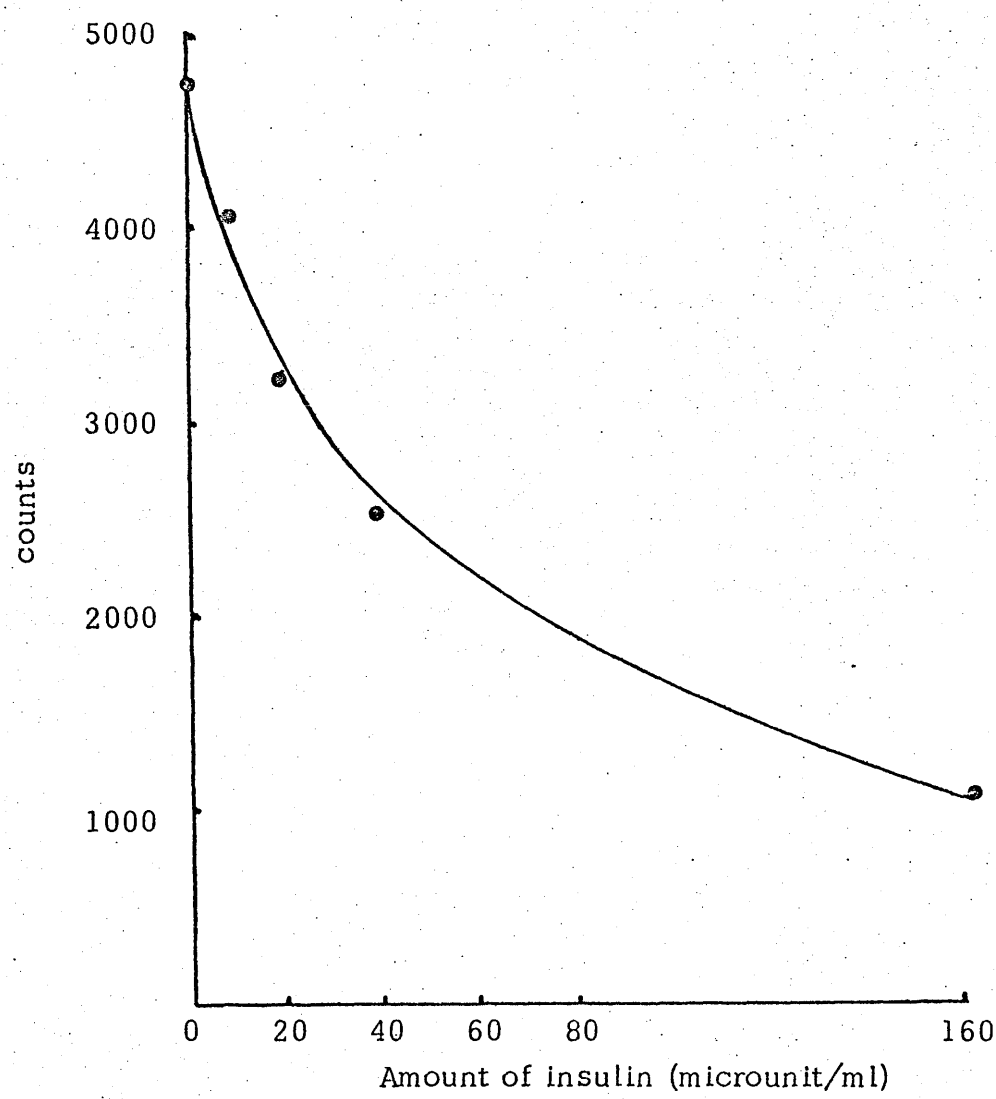


Figure 2.6 Standard curve for determination of plasma insulin

2.9 Determination of plasma free fatty acids (Duncombe, 1964)

Reagents: The reagents were copper reagent:9 vol. of aqueous 1 M triethanolamin, 1 vol 1 N acetic acid and 10 vol 6.45% (w/v) Cu $(NO3)_2$; and Diethyldithiocarbamate reagent: 0.1% (w/v) sodiumdiethyl-dithiocarbamate (Analar) in redistilled butanol. Both the above reagents were stored in the refrigerator and used within a week.

Procedure: Chloroform (5 ml) were pipetted into a 10 ml stoppered centrifuge tube, together with 0.2 ml of plasma and 1 ml of the copper reagent. After stoppering, the tube was shaken on a rotary shaker for 10 minutes and then centrifuged for five minutes. The aqueous phase was separated by a water aspirator, leaving the chloroform phase free of particles. Chloroform phase (2 ml) was pipetted into a clean dry tube followed by the addition of 0.2 ml of diethyldithiocarbamate reagent and after mixing, the absorbance was read at 440 nm on an SP500 spectro-photometer. A reagent blank with water and standards of palmitic acid in chloroform were also run through the same procedure. A standard curve for determination of plasma free fatty acid is shown in Figure 2.8.

2.10 Determination of copper and zinc in plasma and urine

Plasma zinc can be measured by atomic absorption spectrophotometry following dilution of the serum with water. Using this method, plasma was diluted 1 to 5 with water and at this dilution interference of the atomic absorption signal by the serum matrix was removed. Zinc and copper concentrations were therefore measured directly.

The measurement of metals in urine is complicated by the interference

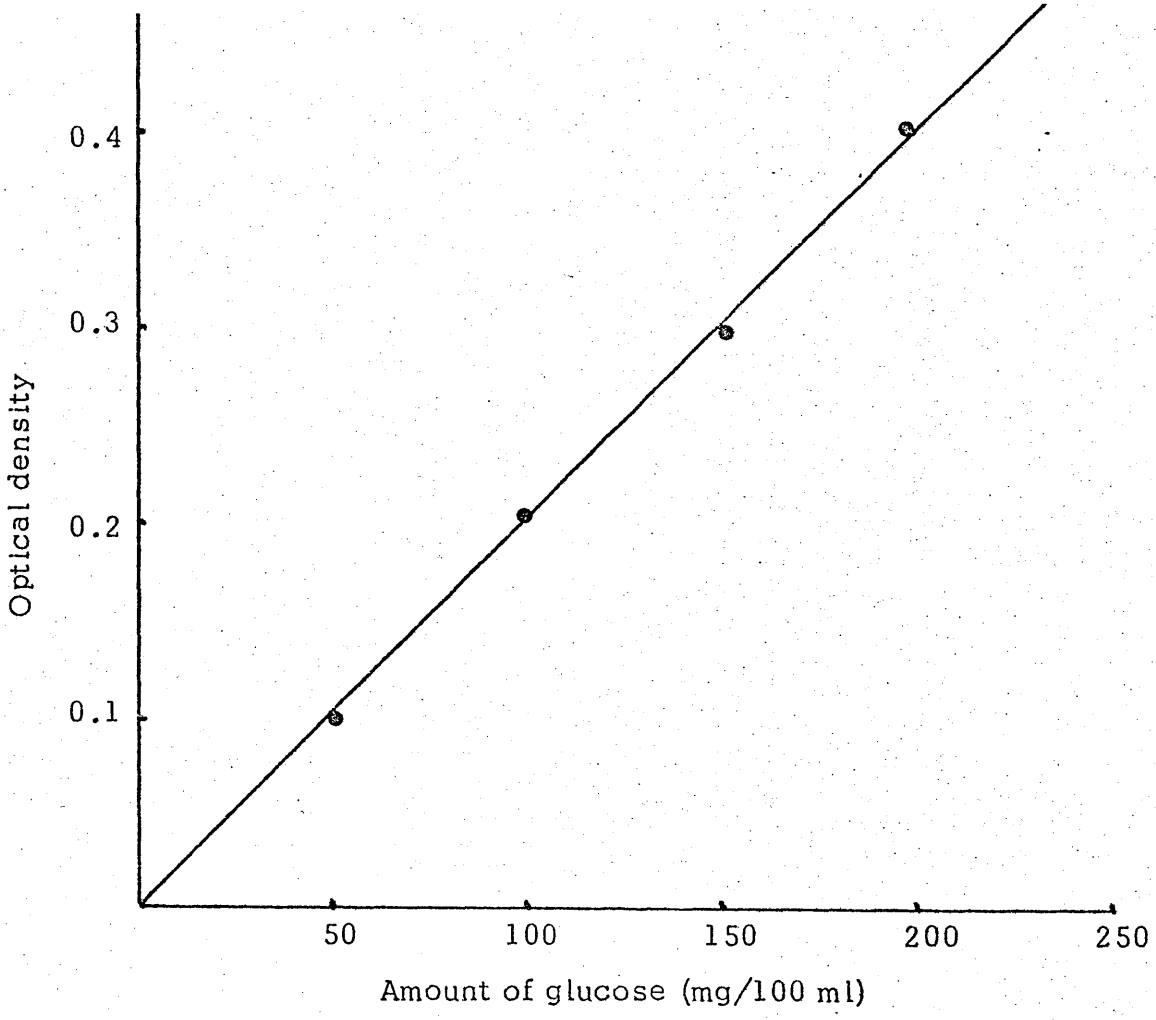


Figure 2.7 Standard curve for determination of plasma glucose

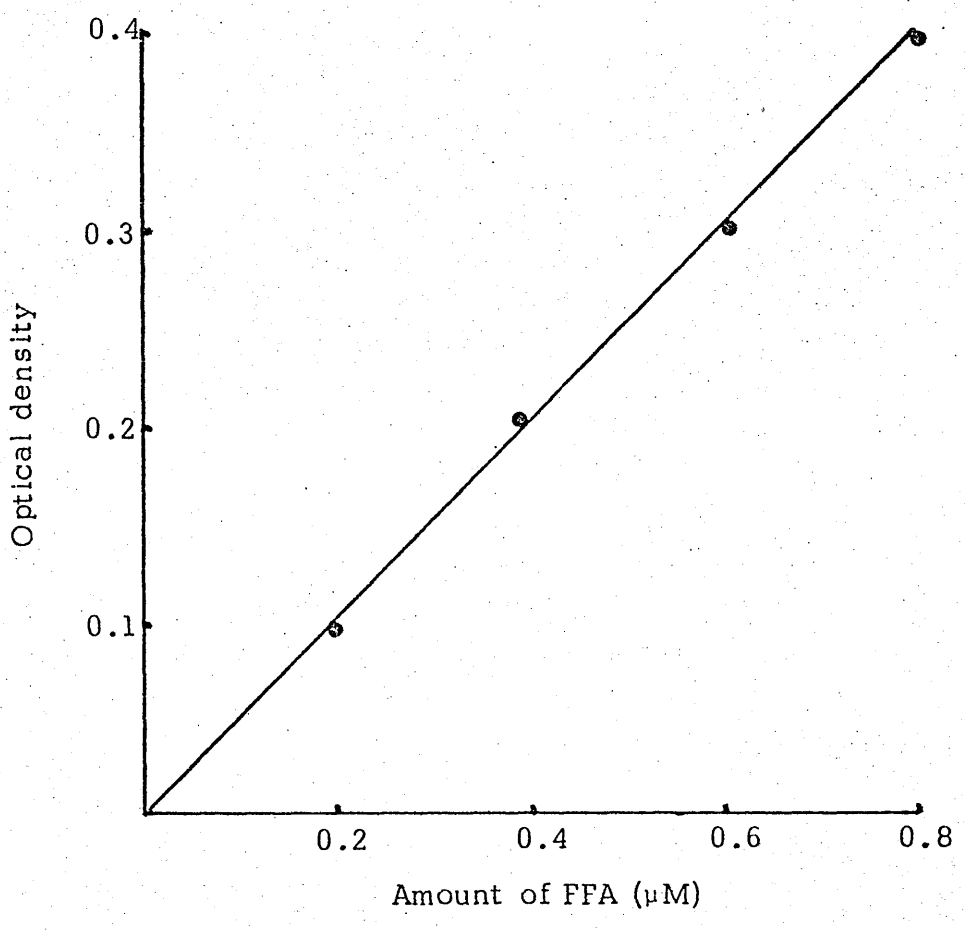


Figure 2.8 Standard curve for determination of plasma FFA

of the urine matrix with the atomic absorption signal. To eliminate this effect, standard solutions of copper and zinc were prepared in a normal urine for the concentration of the standard curve. Thus any matrix effect is identical for standards and tests. The instrument used was an IL353 Atomic Absorption Spectrophotometer. The instruments' setting was as follows

Setting	Copper	Zinc
Wavelength	324.7 nm	213.9 nm
Slit width	320 nm	320 nm
Lamp current	5 mA	5 mA
PM voltage	530 V	530 V
Chart speed	20 mm/min	20 mm/min
Chart range	20 mv	20 mv

Typical standard curves for the determination of copper and zinc are shown in Figures 2.9 and 2.10.

2.11 Determination of urine total nitrogen concentration

Total nitrogen in the urine was measured by a modification of the micro Kjeldahl method as described by Wootton (1964).

Reagents: The reagents were sulphuric acid (conc.); sulphuric acid (N/100); sodium hydroxide (40% w/v); ammonium sulphate solution (1 mg N/ml in water); and boric acid-indicator (prepared by mixing 1 ml of a solution containing 0.8 mg methyl red and 0.2 mg methylene blue per 100 ml ethanol, with 100 ml of 1% boric acid).

Procedure: Duplicate aliquots of 0.2 ml urine were pipetted into

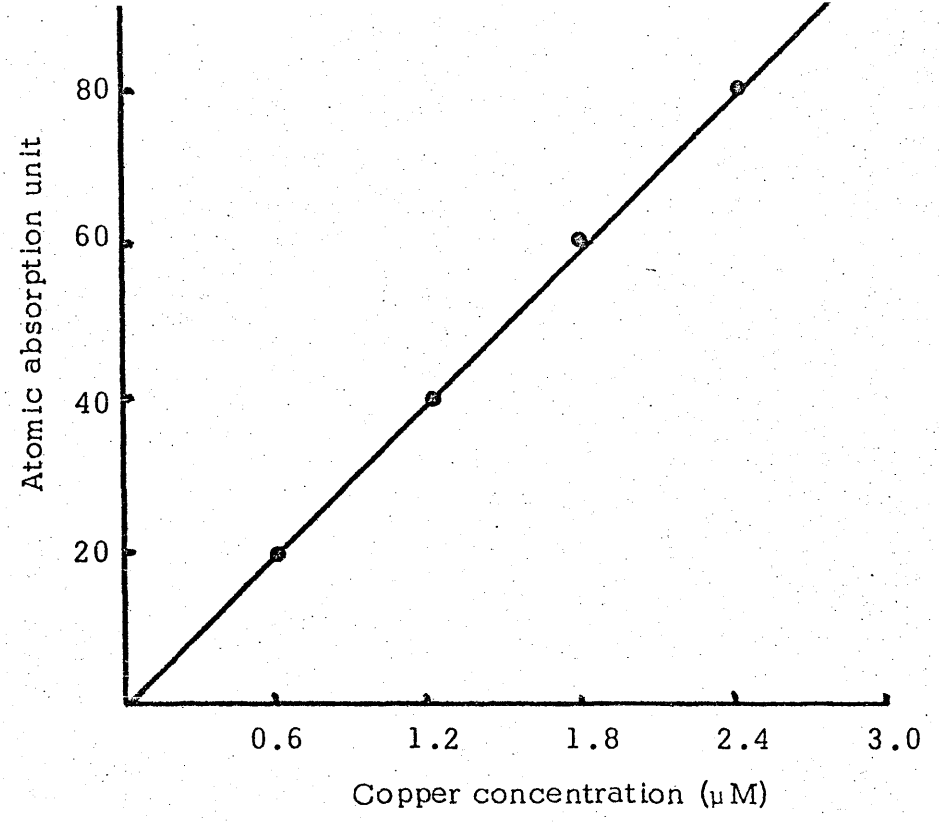


Figure 2.9 Standard curve for the determination of copper

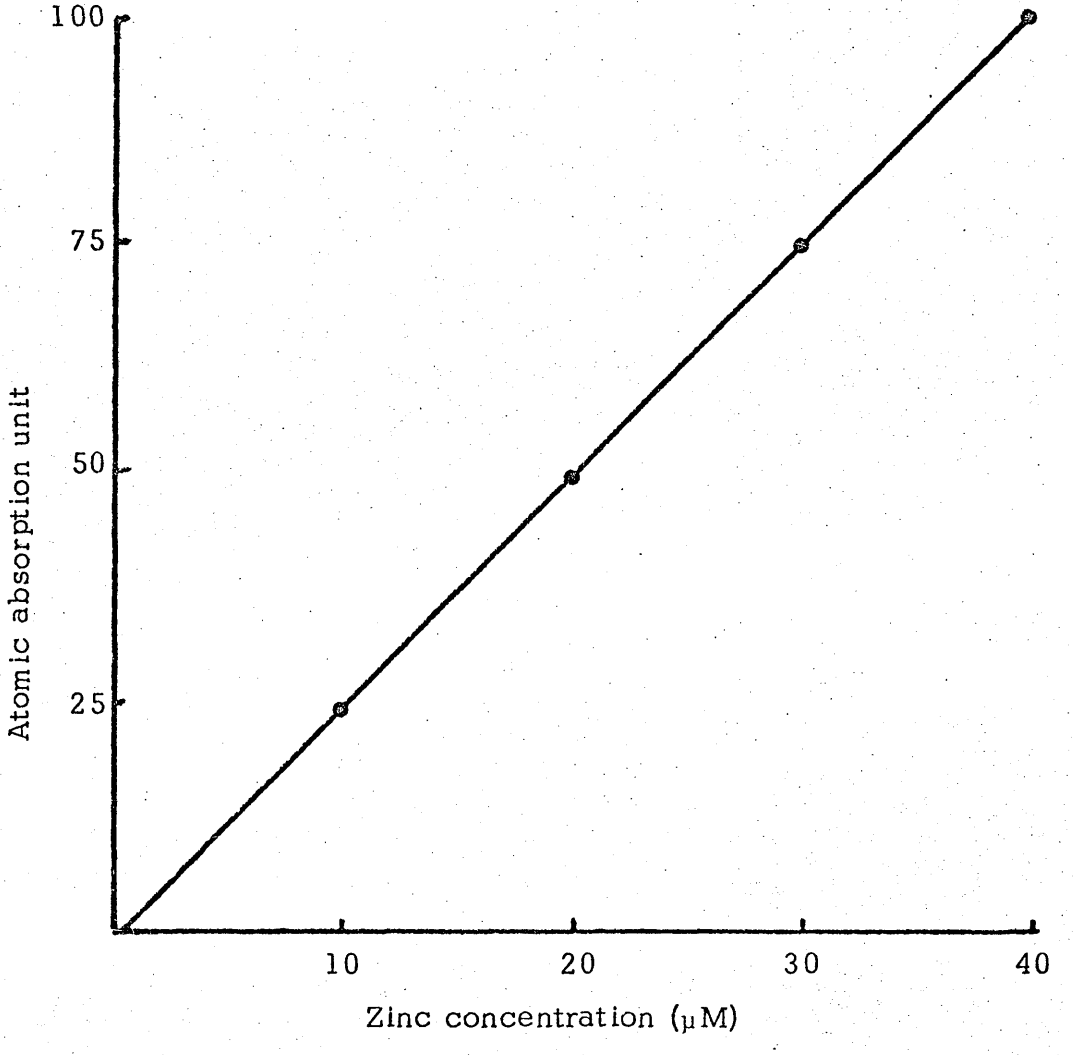


Figure 2.10 Standard curve for the determination of zinc

micro-Kjeldahl flasks (Gallenkamp, England), followed by 3 ml concentrated sulphuric acid and one tablet of Kjeldahl catalyst (British Drug Houses, England). The contents of the flasks were heated on an electric coil heating rack for about 2 hours until the liquid was colourless. The digested solutions were allowed to cool to room temperature, and then diluted to 25 ml with distilled water. Portions (5 ml) of this solution were transferred to a Markham Still (Gallencamp, England). The delivery tube of the apparatus was arranged below the surface of 5 ml of boric acid-indicator solution in a 100 ml conical flask. Sodium hydroxide solution (10 ml) was added and steam distilled for 5 minutes. The contents of the receiver were titrated back to the original grey colour with N/100 sulphuric acid, using a 10 ml burett. The nitrogen concentration in the urine was then calculated as follows:

$$A = B \times 3.5$$

where, A is the nitrogen concentration (g/L), and B is the millilitre N/100 sulphuric acid used for titration.

2.12 Determination of urine urea-nitrogen concentration

The concentration of urea in the urine was measured using an automated diacetyl technique on a Technicon Autoanalyzer, as described by Wootton (1964).

2.13 Determination of urine creatinine concentration

The concentration of creatinine in the urine was measured, using an automated alkaline picrate method as described by Wootton (1964).

2.14 Determination of urinary amino acid nitrogen concentration

The method used for the estimation of urine amino acid nitrogen concentration was essentially that described by Khachadurian, Knox and Cullen (1960).

Reagents: The reagents were sodium carbonate buffer (0.2 M, pH 10.5); EDTA (0.24% w/v, in sodium acetate buffer, 0.15 M pH 3.7); ninhydrin reagent (Prepared by mixing following reagents: 0.5 ml of 1% ninhydrin in 0.5 M acetate buffer, pH 5.5; 1.20 ml of glycerol; and 0.2 ml of 0.5 M acetate buffer, pH 5.5); and standard solutions of glycine containing 5 to 25 mg N per ml.

Procedure: To 0.2 ml of urine 0.2 ml of carbonate buffer was added and evaporated in a dessicator over concentrated sulphuric acid. After leaving overnight the dry residue was dissolved in 2 ml EDTA. Duplicate 0.2 ml aliquots were mixed with 5 ml ninhydrin reagent, mixed and heated in a boiling water bath. After 15 minutes the absorbance of the developed colour was measured at 570 nm on a SP500 spectrophotometer. A reagent blank with water and standards of glycine solutions were also run through the same procedure. A standard curve for amino acid nitrogen determination is shown in Figure 2.11.

2.15 Determination of ketone bodies in urine

The method used for the determination of ketone bodies in the urine was that described by Natelson (1963).

Reagents: The reagents were barium hydroxide (0.3 N); zinc sulphate (5%); potassium dichromate (5%); salicylic aldehyde (20%,

w/v in ethanol); and sulphuric acid (2 N).

Procedure: Urine (1 ml) was pipetted into each of two centrifuge tubes, followed by 5 ml water and 2 ml zinc sulphate solution. The tubes were mixed and centrifuged at 2,000 x G for 2 minutes. Supernatant (7 ml) were transferred to a Markham micro-kjeldahl distilling flask, followed by 2 ml of potassium dichromate and 3 ml of sulphuric solution, and steam distilled for 10 minutes. To the 5 ml of distillate 4 ml barium hydroxide and 1 ml of salicylic aldehyde was added, mixed and incubated at 37°C for 1 hour. The absorbance of the developed colour was measured at 465 nm in a SP500 spectrophotometer. A reagent blank with water and standards of acetone solutions were also run through the same procedure. The standard curve for the determination of the ketone bodies is shown in Figure 2.12.

2.16 Determination of sodium and potassium in urine

The concentrations of sodium and potassium in the urine were determined by the flame photometric technique, following the procedure described by Wootton (1964).

2.17 Determination of alkaline-ribonuclease activity in the urine

Free alkaline (pH 7.8) ribonuclease activity in the urine was determined by a modification of the method described by Roth (1968). The assay mixture contained 0.3 ml freshly prepared of 0.07 M veronal acetate buffer (pH 7.8); 0.2 ml RNA substrate (1% solution of grade A yeast RNA, obtained from Boehringer); and 0.2 ml of urine which had been diluted to 25% of its original concentration with the veronal acetate

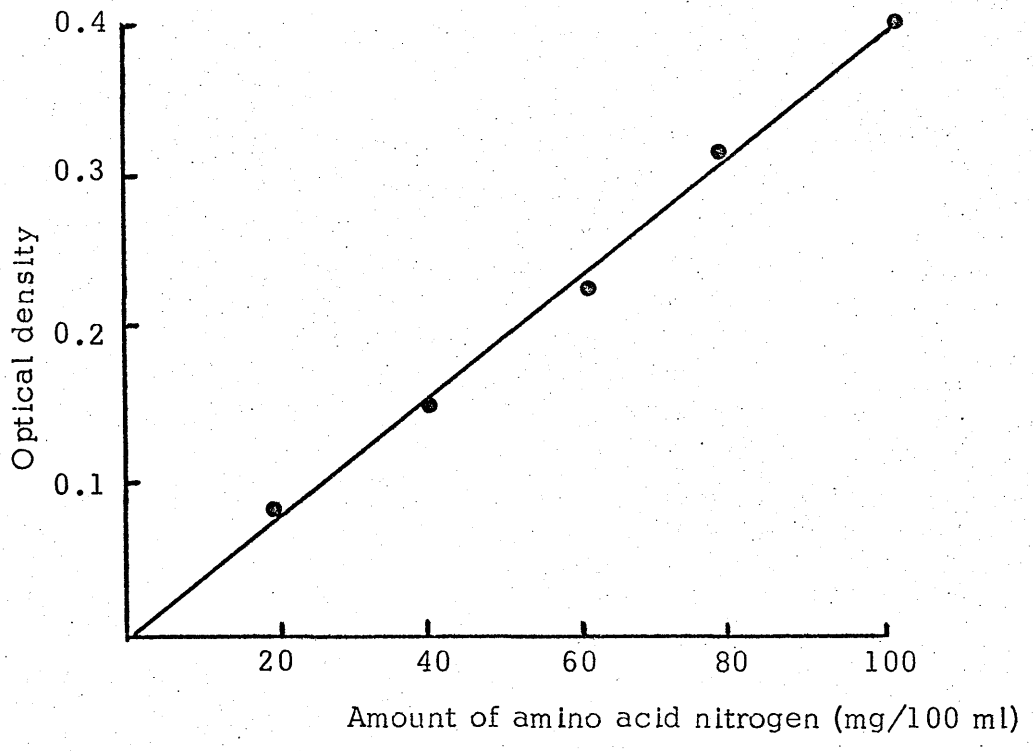


Figure 2.11 Standard curve for determination of urinary amino acid nitrogen

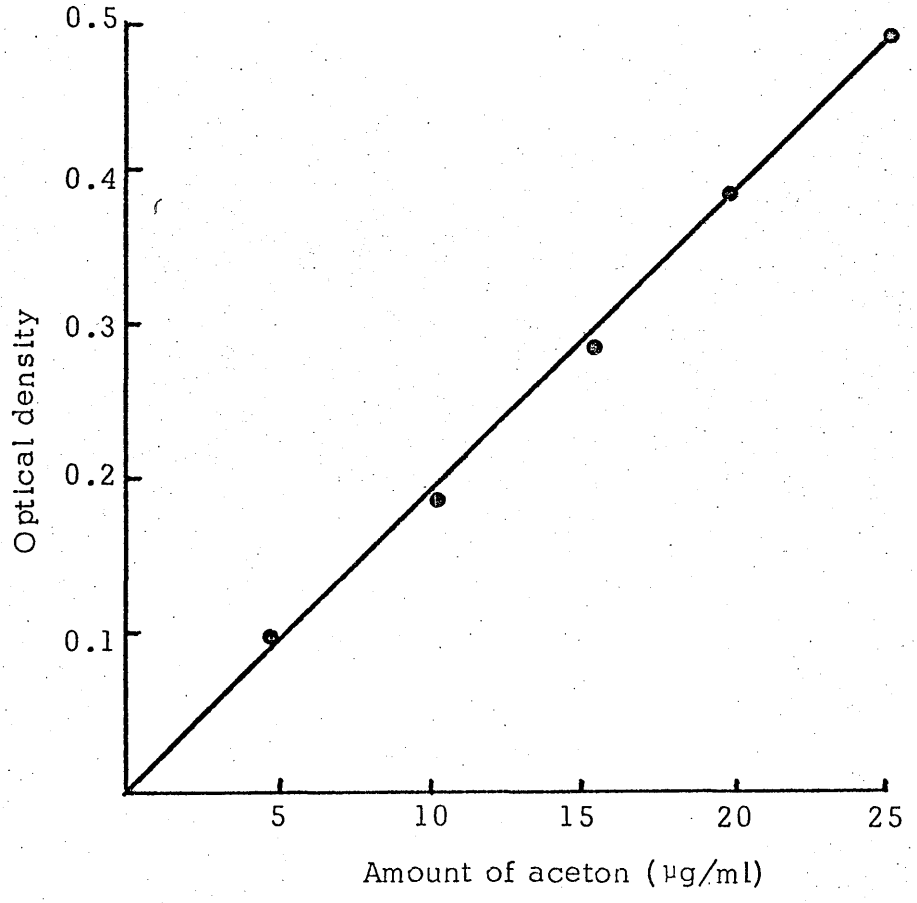


Figure 2.12 Standard curve for determination of urinary ketone bodies

buffer before assay. The incubation mixture was incubated for 30 minutes at 37° C and the reaction stopped by the addition of 0.6 ml of cold acid-alcohol-lanthanum chloride reagent prepared according to Roth (1968). Following 20 minutes centrifugation at 1,000 x G in the cold, the supernatant from samples and their zero time controls were decanted, diluted 1:40 with distilled water, and read against distilled water at 260 nm on an SP500 spectrophotometer. Units of ribonuclease activity were arbitrarily defined as the Δ O.D. of one extinction per thirty minutes incubation measurement. The data were ultimately expressed in units per mg creatinine.

2.18 Determination of 5-hydroxyindolacetic acid (5HIAA) in urine

Since the spectrophotometric technique for the measurement of 5HIAA was found unsatisfactory, a sensitive spectrofluorimetric method was employed. The method used was essentially that described by Contractor (1966).

Preparation of column: G-10 Sephadex (1 g) was slurried in 0.1 N HCl and poured into a glass column (internal diameter 1 cm, height 35 cm). The Sephadex was allowed to settle by gravity on a base formed by a pledget of cotton wool.

Procedure: Urine (1 ml) mixed with 10 ml of 0.1 N HCl was allowed to drain through the Sephadex column which was then washed with 10 ml of 0.1 N HCl, followed by 5 ml of distilled water. The absorbed 5HIAA was eluted with 5 ml of 0.02 N NH_4OH, and an aliquot (2 ml) of this eluate, was mixed with 2 ml of 6 N HCl. The fluorescence intensity

of this solution was determined in a Perkin Elmer spectrophotofluorimeter set for activation at 295 nm and fluorescence at 535 nm. Since the fluorescence intensity of the solution was found to decrease with time after the addition of 6 N HCl, the estimation was carried out within 15 minutes of the addition of the acid. One drop of a saturated solution of potassium persulphate in water was next added to the solution and mixed thoroughly. After 3 minutes, the fluorescence of the mixture was again determined, and this value, which represented the urine 'blank', was subtracted from the original fluorescence to give the corrected fluorescence of 5HIAA. Recovery experiments carried out by this method showed, that 97% $\pm$ 3 (mean $\pm$ SD) of the added 5HIAA to the urine could be recovered. A standard curve for determination of 5HIAA ~~is shown~~ is shown in Figure 2.13.

2.19 Determination of N'-methylnicotinamide (NMN) in urine

The method used for the determination of NMN was a modification of the method of Carpenter and Kodicek (1950).

Reagents: The reagents were methyethylketone-$MNCl_2$: prepared by the addition of 1 ml aqueous M/10 $MNCl_2$ to 500 ml of the purified ketone; sodium hydroxide 6 N; hydrochloric acid 6 N; and potassium dehydrogen phosphate 20% (w/v).

Procedure: Urine (0.2 ml) was pipetted into the matched calibrated test tubes followed by 0.8 ml of water and 0.5 ml of ketone solution and mixed. After 5 minutes 0.3 ml of HCl was added, mixed, and heated for 5 minutes in a boiling water bath, and then cooled in cold water. Potassium hydrogen phosphate solution (1 ml) was next added and the

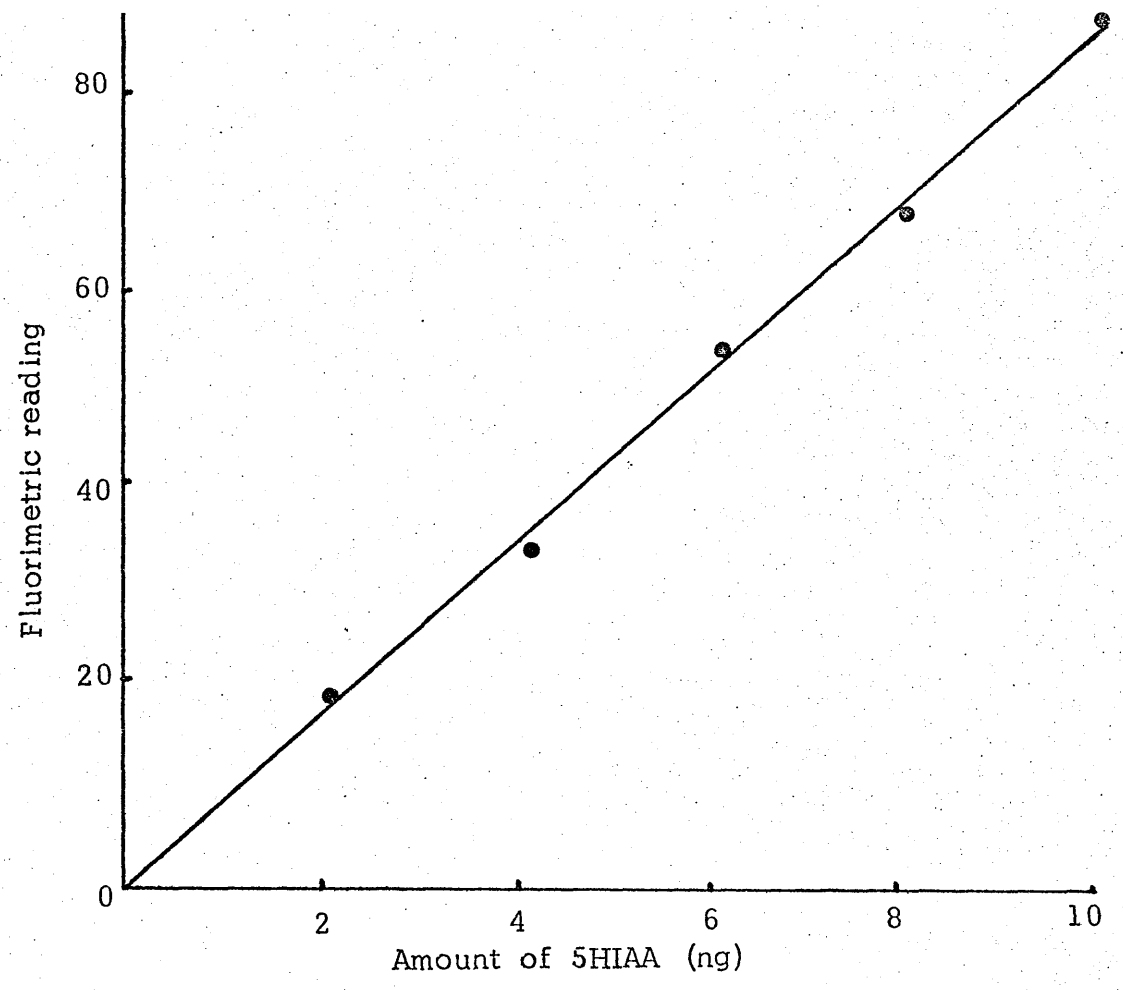


Figure 2.13 Standard curve for determination of urinary 5HIAA

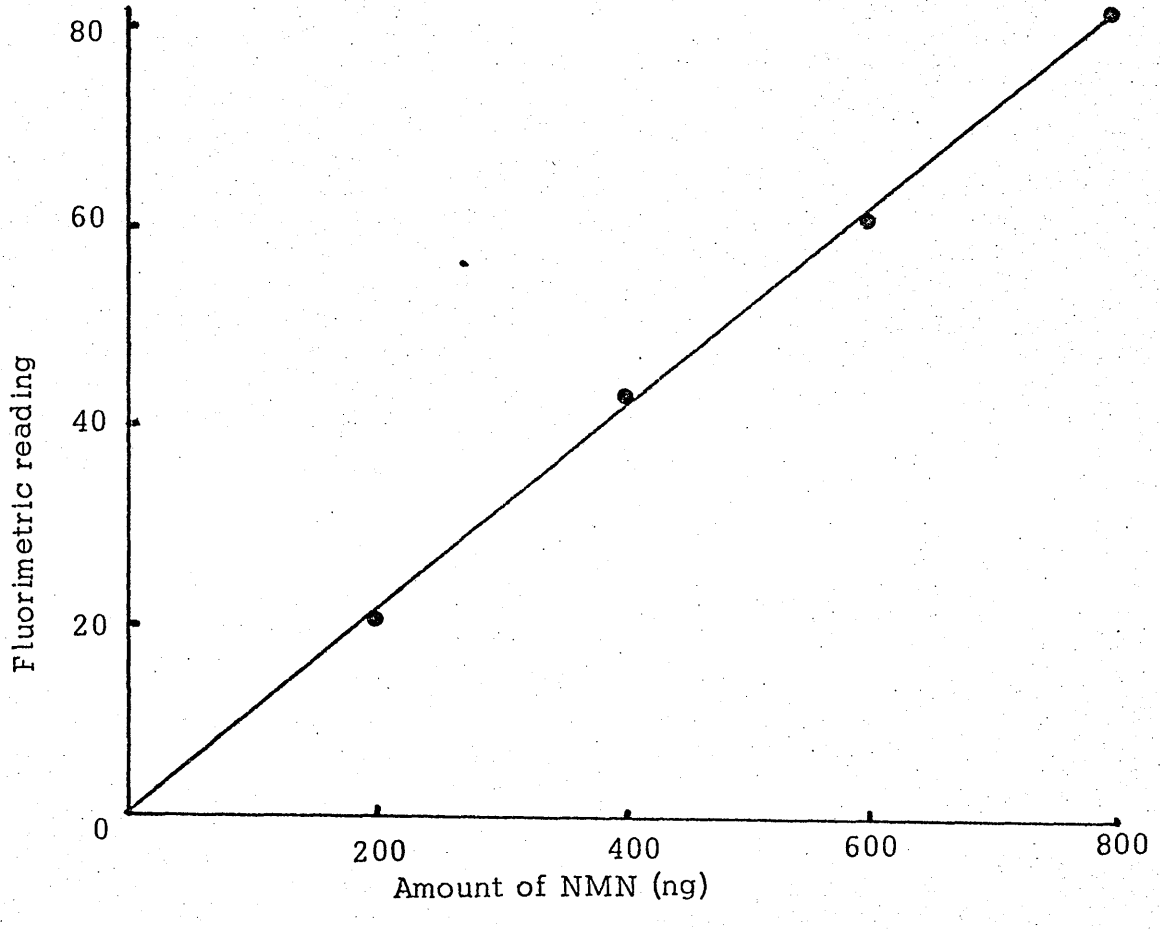


Figure 2.14 Standard curve for determination of urinary NMN

volume made up to the 10 ml with distilled water. The fluroescence was measured in a Perkin Elmer spectrophotofluorimeter set for activation at 440 nm and fluorescence at 575 nm. A reagent blank with water and standard with NMN (Sigma) were also run through the same procedure. A standard curve for NMN determination is shown in Figure 2.14.

Statistics

All results are expressed as average $\pm$ SEM. Student paired 't' test was used for the comparison between values of before and after operation and student 't' test was used when the values of cancer patients were being compared with those of patients with hiatus hernia.

CHAPTER THREE

AMINO ACID AND PROTEIN METABOLISM IN PATIENTS UNDERGOING SURGERY BECAUSE OF HIATUS HERNIA, OESOPHAGEAL CANCER AND LUNG CANCER

1. INTRODUCTION

The mobilization of tissue protein in the tumour-bearing host to meet the requirement of the neoplasm has been shown to result in alterations in the nitrogenous constituents of blood. Thus Babson (1954) observed a progressive rise in the level of nonprotein nitrogen in the blood of tumour-bearing rats throughout the period ot tumour growth. Similarly Wu and Bauer (1960) reported that in rats large increases in blood amino acid concentrations accompanied tumour growth.

Hypoalbuminaemia is a common observation in patients with malignant tumours (Mider, Alling and Morton, 1950; Steinfeld, 1960; Mariani, Strober et al., 1976). Initially, decreased albumin synthesis was thought to be the sole cause for the hypoalbuminaemia (Steinfeld, 1960; Waldmann, Trier and Fallon, 1963). Recently, new metabolic turnover techniques have been used to demonstrate that excessive loss of serum protein into the gastrointestinal tract may also contribute to the observed hypoalbuminaemia in some patients (Waldmann, Broder and Strober, 1977).

The primary interest of this study was the investigation of protein and amino acid metabolism in patients with carcinoma of oesophagus before and after surgical treatment, since these patients are frequently malnourished as a result of reduced food intake and quite often have a protracted period of recovery after surgery, which involves an extensive operative procedure such as oesophagectomy or oesophagogastrectomy.

After oesophagectomy fat absorption has been reported to be impaired (Shils and Gilat, 1966; Shils, 1971) and this is thought to be due to the sacrifice of the vagus nerves at operation. On this evidence it seemed important to study changes in protein and amino acid metabolism in patients undergoing surgery for oesophageal cancer. Similar studies have been done in patients with a non-malignant disease of the oesophagus, hiatus hernia, since surgery for this disease also requires thoracotomy and the handling of a part of the alimentary tract. These patients then served as controls for the oesophageal cancer patients. Surgical treatment of lung cancer, whilst requiring thoracotomy, does not involve handling the gut. This chapter describes the patterns of plasma proteins, amino acids, urea nitrogen, zinc, copper and urinary excretion of nitrogen-containing compounds, zinc, copper, potassium and urinary activity of ribonuclease in these three groups of patients.

2. RESULTS

Before operation, patients with oesophageal cancer, but not those with lung cancer, had significantly lower plasma protein concentrations than patients with hiatus hernia (Table 3.1). After operation the concentrations of total protein fell in both oesophageal cancer and hiatus hernia patients whereas they did not change significantly in the lung cancer patients. In fact, the concentration of total protein was never significantly below the pre-operative level in the lung cancer patients up to 14 days after the operation. Whereas in the other two groups the concentrations remained below the pre-operative value up to the 7th post-operative day. Up to this time, the values in the oesophageal cancer patients were lower than those in the hiatus hernia patients.

The findings for plasma albumin (Table 3.2) were similar to those for total protein except that the concentrations remained lower than the pre-operative values up to the 14th day after operation. The post-operative concentrations of globulin (Table 3.3) were significantly lower than the pre-operative values only in the oesophageal cancer patients, and in these patients they remained low until the 7th day.

The pre-operative concentrations of each of the essential amino acids (Table 3.4) were similar in both groups of cancer patients and in those with hiatus hernia. No significant changes were found in the concentrations of isoleucine in any of the groups following surgery. The concentrations of leucine, valine and lysine were significantly higher at 14 days post-operatively in the two groups of cancer patients

than in the hiatus hernia patients. In the latter patients, however, the concentration of valine was significantly lower at 14 days after operation than before operation. In the case of threonine the concentration was lower after operation at each stage examined. In the oesophageal cancer patients the concentration of this amino acid fell markedly following surgery and gradually rose up to 14 days by which time the value was not significantly different from the pre-operative value. In each group, the concentration of phenylalanine rose to a value that was significantly higher than the pre-operative value by the second post-operative day. The concentration of methionine fell immediately after operation in the hiatus hernia and oesophageal cancer patients but did not change significantly in the lung cancer patients.

Of the non-essential amino acids (Table 3.5), the pre-operative plasma concentrations of alanine and arginine were significantly lower in patients with lung cancer than in those of the other two groups. After operation the concentrations of glutamine fell in all patients and remained low throughout the post-operative period. The concentrations of glutamic acid fell immediately after surgery in patients with hiatus hernia and oesophageal cancer whereas the level rose in lung cancer patients. The levels, however, returned to pre-operative value in all patients by 14 days after operation.

The concentration of glycine fell immediately after surgery in patients with hiatus hernia and oesophageal cancer but not in those with lung cancer. In the latter group the level fell on the second post-operative day and in both groups of cancer patients the levels remained low until the seventh post-operative day. By the 14th day after surgery,

the glycine concentrations in the plasma of all patients were comparable to those of basal pre-operative values.

In patients with hiatus hernia and oesophageal cancer, but not in those with lung cancer the concentrations of alanine, arginine and histidine fell immediately after surgery and remained low throughout the post-operative period. The post-operative changes in the concentrations of arginine and histidine followed the same pattern as that of alanine.

In all patients the concentrations of serine fell immediately after operation and remained low until the second day. By the 14th day after surgery the concentrations had risen to values which were not significantly different from those before operation.

In general, the post-operative patterns of change were such that plasma amino acids could be grouped into three profiles:-

Profile 1. Contained those amino acids whose concentrations fell markedly after surgery. These included glutamine, alanine, glycine, arginine and theronine. In one of the hiatus hernia patients (Mrs. G.L.) however, the concentrations of alanine fell immediately after operation, but rose again and was found to be surprisingly high on the second and seventh post-operative day (1.6 and 1.7 mmole/l respectively. Maximum normal limit 0.6 mmole/l).

Profile 2. Contained those amino acids whose concentrations were moderately affected by surgery. These included histidine, serine, and glutamic acid. The concentration of glutamic acid fell in hiatus hernia and oesophageal cancer patients, but rose in patients with lung

cancer immediately after operation.

Profile 3. Was exhibited by those amino acids whose concentrations changed only slightly or not at all after surgery. These included phenylalanine, lysine, valine, isoleucine and leucine.

Pre-operative concentrations of plasma urea-nitrogen were found to be similar in both cancer patients and patients with hiatus hernia (Table 3.6; Figure 3.1). After operation, however the plasma urea-nitrogen in lung cancer patients, but not in the other two groups, rose and was significantly higher than the pre-operative level on the 2nd, 7th and 14th post-operative day. On the 14th day the levels in patients with hiatus hernia were significantly lower than before operation. The plasma urea nitrogen concentrations in oesophageal cancer patients was not affected by surgery. Pre-operative concentrations of plasma zinc were similar in both groups of cancer patients and in patients with hiatus hernia (Table 3.7 and Figure 3.2a). Moreover, the levels did not change significantly immediately after surgery but did fall significantly in all patients by 2 days. In patients with hiatus hernia and oesophageal cancer, but not in those with lung cancer, the post-operative fall of plasma zinc was coincident with a low level of excretion of zinc in the urine (Table 3.8 & Fig3.2b)Plasma copper concentrations, however, presented a different pattern of change. Before operation the concentrations in oesophageal cancer patients were higher than those in the other two groups (Table 3.9). By 2 days after surgery, the levels had fallen significantly in the oesophageal cancer group and remained low until the seventh day. In contrast, in hiatus hernia patients the levels were elevated on the 7th and 14th post-operative days. No significant changes were found in the patients with lung cancer. The pre-operative excretion

of copper in the urine (Table 3.10) was similar in all the patients and remainded so during the course of the study. Before operation, there was no difference between any of the groups in the excretion of potassium (Table 3.11). By 2 days after surgery the levels in the patients with hiatus hernia and oesophageal cancer, but not in those with lung cancer, had risen significantly, but they returned to the pre-operative level by 14 days. Conversely, the excretion of sodium had fallen significantly in all patients by the second post-operative day (Table 3.12). The levels however, recovered and reached the value which was not significantly different from that before operation by 14 days after surgery. The urinary excretions of nitrogenous compounds before and after surgery are shown in Tables 3.13, 3.14 and 3.15 and Figures 3.3 and 3.4. Before operation the concentrations of total-nitrogen and urea-nitrogen were similar in cancer patients to those in patients with hiatus hernia (Figure 3.3), but the levels of amino-nitrogen in oesophageal cancer group, and creatinine-nitrogen in both groups of cancer patients were higher than those in patients with hiatus hernia (Figure 3.4). Furthermore, the levels of total-nitrogen and urea-nitrogen in hiatus hernia and lung cancer patients showed a tendency to increase in the early post-operative period but by 14 days after surgery their levels were found to be below the pre-operative levels. In oesophageal cancer patients, but not in the other two groups, there was a progressive decrease in the levels of total-nitrogen and urea-nitrogen following surgical operation. In lung cancer patients, but not in the other two groups, the concentrations of amino-nitrogen were found to be elevated on the second and seventh post-operative days (Figure 3.4).

Before operation, the activities of urinary alkaline ribonuclease were similar in cancer patients to those in patients with hiatus hernia (Table 3.16). By two days after operation, the activities rose to levels which were significantly higher than pre-operative values, and the biggest rise occurred in patients with hiatus hernia. The levels, however, fell to the pre-operative value by 14 days after operation.

Table 3.1 Plasma total protein concentrations (g/100 ml) in patients undergoing surgery for hiatus hernia, oesophageal cancer and lung cancer.

Values are means ± S.E.M. for the number of patients shown in parentheses

		Before operation	Immediately after operation	Time after operation (days) 2	7	14
Hiatus hernia	(10)	6.5 ± 0.3	5.6 ± 0.3*	5.3 ± 0.2***	5.6 ± 0.2***	5.7 ± 0.3
Oesophageal cancer	(11)	5.7 ± 0.2	4.9 ± 0.2**	4.6 ± 0.2 †***	4.7 ± 0.2*††	5.4 ± 0.2
Lung cancer	(8)	6.08 ± 0.3	5.6 ± 0.2	5.7 ± 0.2	5.8 ± 0.2	6.2 ± 0.2

Statistical significance of differences between values of before and after operation shown * where $P < 0.05$, ** where $P < 0.02$ and *** where $P < 0.01$.

Statistical significance of differences between values for cancer patients and that of patients with hiatus hernia shown † where $P < 0.05$ and †† where $P < 0.01$.

Table 3.2 Plasma albumin concentration (g/100 ml) in patients undergoing surgery for hiatus hernia, oesophageal cancer and lung cancer.

Values are means ± S.E.M. for the number of patients shown in parentheses

		Before operation	Immediately after operation	Time after operation (days) 2	7	14
Hiatus hernia	(10)	4.07 ± 0.2	3.3 ± 0.3*	3.2 ± 0.2*	3.1 ± 0.3*	3.0 ± 0.4*
Oesophageal cancer	(11)	3.2 ± 0.2 †	2.7 ± 0.2*†	2.6 ± 0.3*	2.4 ± 0.2***	2.7 ± 0.2*
Lung cancer	(8)	3.5 ± 0.2	3.2 ± 0.2	3.2 ± 0.2	2.9 ± 0.2**	3.1 ± 0.3

Statistical significance of differences between values of before and after operation shown * where $P < 0.05$, ** where $P < 0.02$ and *** where $P < 0.01$.

Statistical significance of differences between values for cancer patients and that of patients with hiatus hernia shown † where $P < 0.05$.

Table 3.3 Plasma globulin concentration (g/100 ml) in patients undergoing surgery for hiatus hernia, oesophageal cancer and lung cancer.

Values are means ± S.E.M. for the number of patients shown in parentheses

		Before operation	Immediately after operation	Time after operation (days) 2	7	14
Hiatus hernia	(10)	2.4 ± 0.1	2.4 ± 0.1	2.1 ± 0.1	2.6 ± 0.1	2.7 ± 0.1
Oesophageal cancer	(11)	2.5 ± 0.1	2.1 ± 0.2**	2.0 ± 0.1**	2.2 ± 0.2*	2.7 ± 0.1
Lung cancer	(8)	2.7 ± 0.3	2.4 ± 0.3	2.5 ± 0.1	2.8 ± 0.2	3.08 ± 0.2

Statistical significance of differences between values of before and after operation shown * where $P < 0.05$ and ** where $P < 0.02$.

Table 3.4 Concentrations of plasma essential amino acids in patients undergoing surgical operation for hiatus hernia, (HH), oesophageal cancer (OC) and lung cancer (LC).

Values are means ± S.E.M. for the number of patients shown in parentheses.

Amino acid µmol/l plasma	Patient	Before operation	Immediately after operation	After operation (days) 2	7	14
Isoleucine	HH (9)	74 ± 6	58 ± 7	68 ± 10	85 ± 7	76 ± 9
	OC (11)	79 ± 4	79 ± 8	75 ± 10	86 ± 14	99 ± 10
	LC (8)	77 ± 8	69 ± 9	82 ± 11	86 ± 9	83 ± 5
Leucine	HH (9)	132 ± 9	118 ± 10	135 ± 16	147 ± 12	113 ± 14
	OC (11)	140 ± 14	139 ± 16	144 ± 22	156 ± 18	169 ± 18 †
	LC (8)	139 ± 9	118 ± 14	153 ± 15	160 ± 16	154 ± 6 ††
Valine	HH (9)	231 ± 12	177 ± 22	215 ± 20	225 ± 13	169 ± 16***
	OC (11)	223 ± 22	209 ± 21	212 ± 30	236 ± 32	269 ± 28 †††
	LC (8)	239 ± 26	193 ± 23	243 ± 31	284 ± 30	228 ± 14 †††
Methionine	HH (9)	28 ± 5	17 ± 3 †	35 ± 7	26 ± 5	23 ± 6
	OC (11)	31 ± 7	14 ± 2 †	29 ± 7	30 ± 5	30 ± 4
	LC (8)	20 ± 3	21 ± 2	31 ± 5	29 ± 4	18 ± 3
Theronine	HH (9)	159 ± 11	120 ± 14*	78 ± 7***	93 ± 11***	99 ± 12***
	OC (11)	158 ± 15	89 ± 9***	98 ± 11**	112 ± 18*	135 ± 23
	LC (8)	112 ± 7	93 ± 17	101 ± 11	134 ± 16	102 ± 5
Phenylalanine						
	HH (9)	62 ± 6	55 ± 9	81 ± 9*	71 ± 13	66 ± 5
	OC (11)	77 ± 7	57 ± 5	99 ± 11*	77 ± 9	69 ± 6
	LC (8)	80 ± 8	75 ± 12	112 ± 14**	76 ± 12	75 ± 7
Lysine	HH (9)	207 ± 15	170 ± 13	150 ± 11	161 ± 22	175 ± 20
	OC (11)	234 ± 26	176 ± 16*	173 ± 22	185 ± 17	258 ± 36 ††
	LC (8)	173 ± 23	163 ± 25	175 ± 24	220 ± 23	221 ± 23 †

Statistical significance of differences between values of before and after operation shown * where $P < 0.05$ ** where $P < 0.02$ and *** where $P < 0.01$. Statistical significance of differences between values for cancer patients and that of patients with hiatus hernia shown † where $P < 0.05$, †† where $P < 0.02$ and ††† where $P < 0.01$.

Table 3.5 Concentrations of plasma non-essential amino acids in patients undergoing surgery for hiatus hernia (HH), oesophageal cancer (OC) and lung cancer (LC)

Values are means ± S.E.M. for the number of patients shown in parentheses

Amino acid µmol/l	Patient	Before operation	Immediately after operation	After operation (days) 2	7	14
Glutamine	HH (9)	514 ± 23	398 ± 15***	389 ± 25***	437 ± 28**	372 ± 18***
	OC (11)	517 ± 31	348 ± 19***	346 ± 25***	407 ± 23**	397 ± 44*
	LC (8)	595 ± 42	440 ± 16*†	419 ± 43***	418 ± 31***	464 ± 26**††
Glutamic acid	HH (9)	57.1±8.5	41.06±6.4*	32.11±7.6**	56 ±6.9	50.40±10.5
	OC (11)	56.8±7.0	46.6 ±6.0*	30.0 ±4.2***	61.0±8.0	61±8.7
	LC (8)	57.3±7.5	95.0 ±10.2***†††	48±8.5*	84 ±8.5*†	68.4±8.6
Glycine	HH (9)	274 ± 26	182 ± 23**	150 ± 13***	218 ± 22	220 ± 23
	OC (11)	245 ± 21	156 ± 15***	157 ± 21***	175 ± 13	240 ± 29
	LC (8)	266 ± 28	217 ± 14	211 ± 19†	195 ± 22*	205 ± 32
Alanine	HH (9)	459 ± 36	349 ± 38***	243 ± 26***	265 ± 35***	361 ± 36*
	OC (11)	392 ± 40	248 ± 33***	242 ± 32**	223 ± 38**	289 ± 53*
	LC (8)	357 ± 32	385 ± 23	318 ± 44	232 ± 30	270 ± 17
Arginine	HH (9)	84 ± 3	62 ± 6***	46 ± 4***	60 ± 8*	48 ± 5***
	OC (11)	84 ± 8	61 ± 6*	47 ± 8***	62 ± 5*	74 ± 9
	LC (8)	61 ± 5†††	58 ± 2	52 ± 3	62 ± 10	50 ± 7
Histidine	HH (9)	81 ± 5	68 ± 7*	70 ± 7	62 ± 5**	46 ± 6***
	OC (11)	79 ± 7	67 ± 11*	64 ± 8	54 ± 4***	78 ± 18†
	LC (8)	69 ± 8	66 ± 9	72 ± 11	59 ± 5	53 ± 3
Serine	HH (9)	157 ± 25	131 ± 19*	130 ± 23*	159 ± 18	146 ± 18
	OC (11)	176 ± 23	129 ± 16*	95 ± 11***	118 ± 20*	156 ± 17
	LC (8)	179 ± 23	159 ± 21*	110 ± 18*	139 ± 18	132 ± 14

Statistical significance of differences between values of before and after operation shown * where $P < 0.05$, ** where $P < 0.02$ and *** where $P < 0.01$.

Statistical significance of differences between values for cancer patients and that of patients with hiatus hernia shown † where $P < 0.05$ †† where $P < 0.02$ and ††† where $P < 0.01$.

Table 3.6 Plasma urea-nitrogen concentration (mg/100 ml) in patients undergoing surgery for hiatus hernia, oesophageal cancer and lung cancer.

Values are means ± S.E.M. for the number of patients shown in parentheses.

		Before operation	Immediately after operation	Time after operation (days) 2	7	14
Hiatus hernia	(9)	19.22 ± 2.26	16.75 ± 2.47	16.78 ± 1.46	18.44 ± 3.38	12.04 ± 0.62*
Oesophageal cancer	(11)	17.50 ± 2.50	14.58 ± 2.7	19.08 ± 2.50	14.02 ± 1.50	13.37 ± 1.50
Lung cancer	(8)	13.47 ± 3.00	19.00 ± 1.80	25.00 ± 4.00*	27.00 ± 5.00*	21 ± 1.90**

Statistical significance of differences between values of before and after operation shown * where $P < 0.05$ and ** where $P < 0.01$.

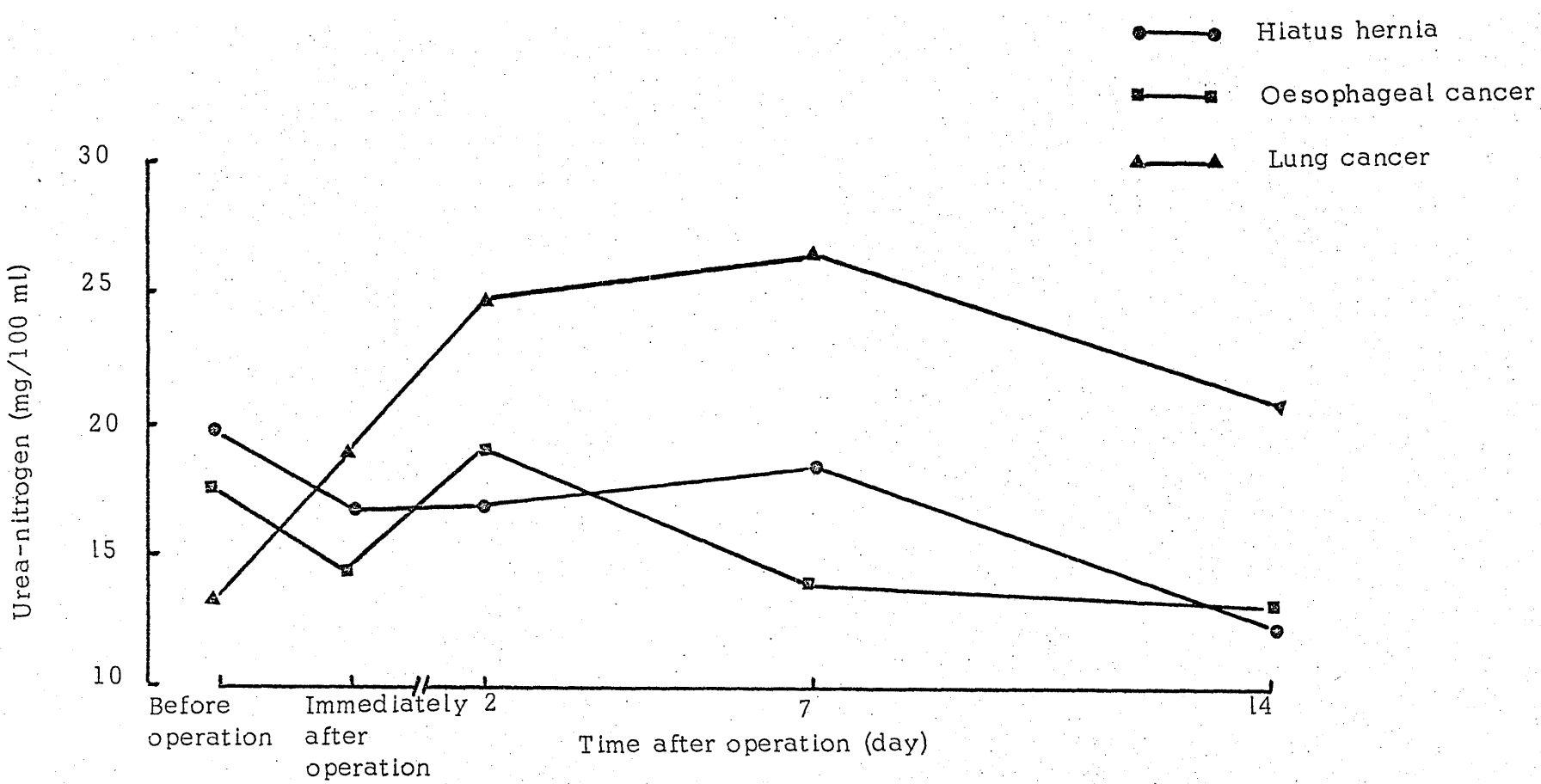


Fig. 3.1. Effect of operation on the plasma urea-nitrogen concentration in patients with hiatus hernia, oesophageal cancer and lung cancer.

Table 3.7 Plasma zinc concentrations (μmole/l) in patients undergoing surgery for hiatus hernia, oesophageal cancer and lung cancer.

Values are means ± S.E.M. for the number of patients shown in parentheses.

		Before operation	Immediately after operation	Time after operation (days) 2	7	14
Hiatus hernia	(10)	12.27 ± 0.83	11.20 ± 1.20	7.05 ± 0.69**	11.50 ± 0.58	15.76 ± 1.37
Oesophageal cancer	(11)	12.92 ± 0.72	11.77 ± 0.93	7.89 ± 0.71*	12.45 ± 0.54	13.89 ± 0.59
Lung cancer	(8)	12.24 ± 1.21	11.93 ± 0.60	7.65 ± 1.07**	11.83 ± 1.36	15.15 ± 0.96

Statistical significance of differences between values of before and after operation shown * where $P < 0.02$ and ** where $P < 0.01$.

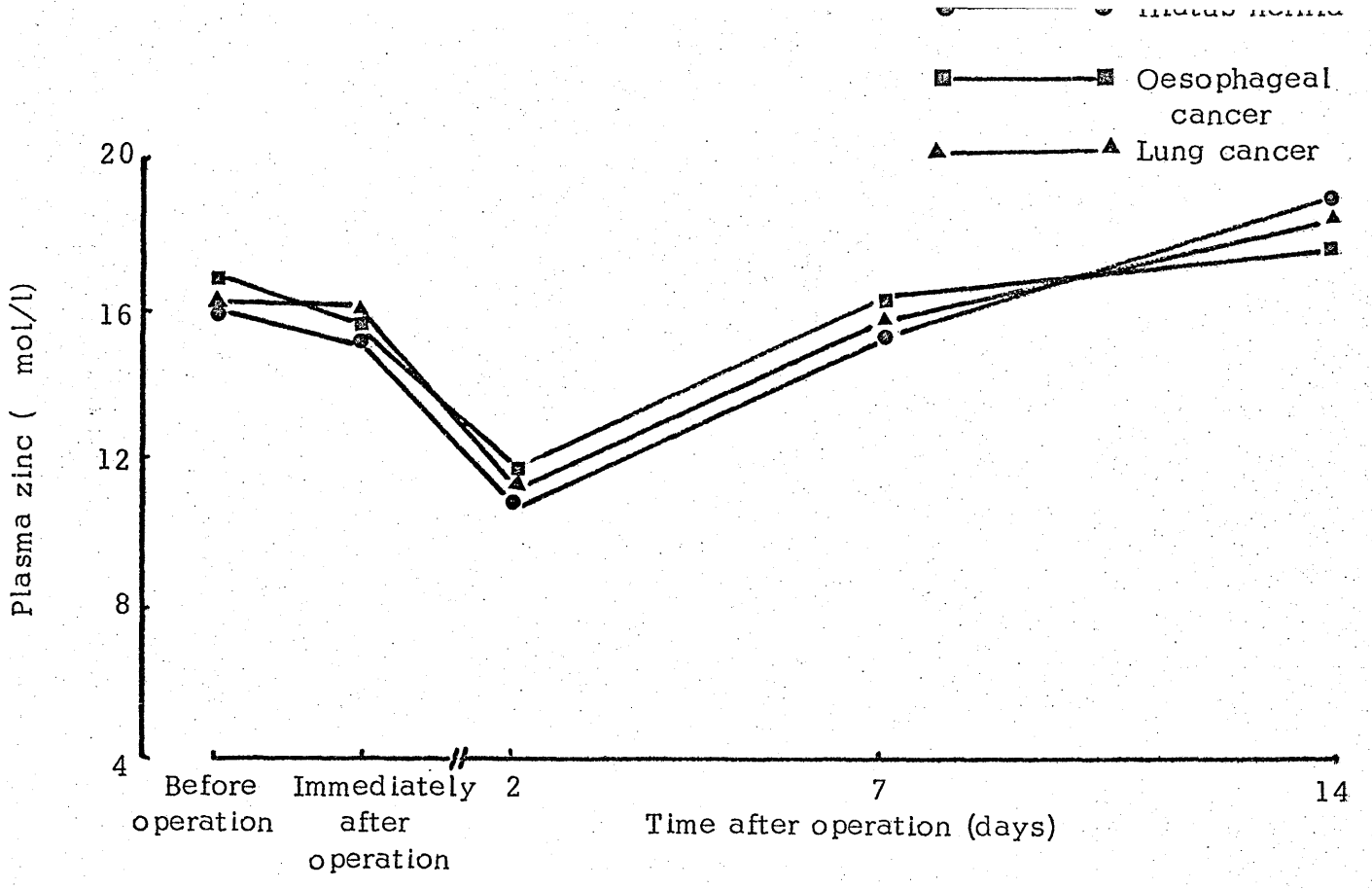


Figure 3.2a. Effect of operation on plasma zinc concentration in patients undergoing surgery for hiatus hernia, oesophageal cancer and lung cancer

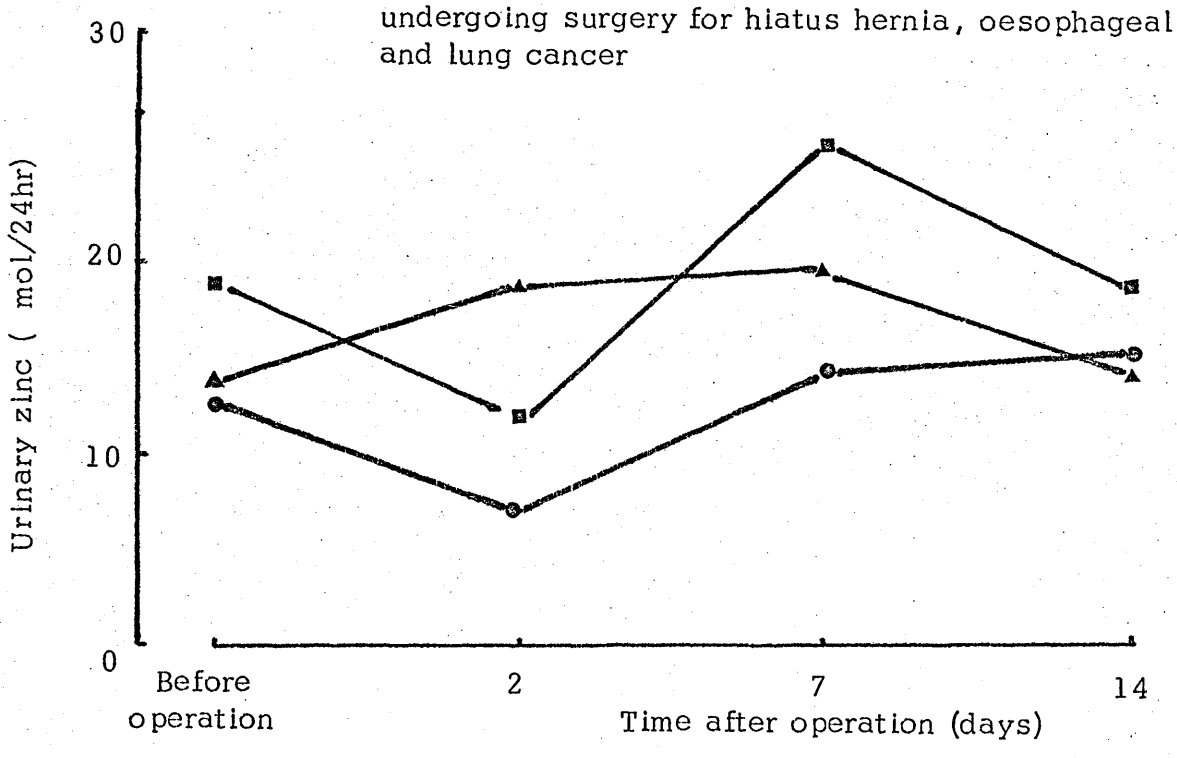


Figure 3.2b. Effect of operation on urine zinc excretion in patients undergoing surgery for hiatus hernia, oesophageal cancer and lung cancer

Table 3.8 Urinary excretions of zinc (μmole/24 hr) in patients undergoing surgery for hiatus hernia, oesophageal cancer and lung cancer.

Values are means ± S.E.M. for the number of patients shown in parentheses.

	Before operation	Time after operation (days) 2	7	14
Hiatus hernia	12.58 ± 5.6 (9)	6.98 ± 1.69 (9)*	14.62 ± 1.52 (6)	14.75 ± 2.30 (6)
Oesophageal cancer	19.95 ± 5.33 (7)	12.88 ± 4.47 (9)*	26.66 ± 8.73 (7)	19.93 ± 6.05 (7)
Lung cancer	14.83 ± 3.22 (6)	19.17 ± 7.62 (6)	20.48 ± 4.33 (6)	14.48 ± 5.58 (4)

Statistical significance of difference between values of before and after operation shown * where $p < 0.05$.

Table 3.9 Plasma copper concentrations (μ mole/l) in patients undergoing surgery for hiatus hernia, oesophageal cancer and lung cancer.

Values are means ± S.E.M. for the number of patients shown in parentheses

		Before operation	Immediately after operation	Time after operation (days) 2	7	14
Hiatus hernia	(10)	18.04 ± 0.76	18.96 ± 0.87	17.31 ± 1.69	22.94 ± 1.47**	24.60 ± 1.86*
Oesophageal cancer	(12)	25.10 ± 2.08†	20.89 ± 2.10	18.04 ± 1.57***	19.94 ± 1.76*	25.14 ± 1.87
Lung cancer	(8)	18.58 ± 2.49	16.08 ± 1.72	20.44 ± 2.17	18.98 ± 1.85	22.90 ± 1.91

Statistical significance of differences between values of before and after operation shown * where $P < 0.05$ ** where $P < 0.02$ and *** where $P < 0.01$.

Statistical significance of differences between values for cancer patients and that of patients with hiatus hernia shown † where $p < 0.01$.

Table 3.10 Urinary copper excretions (μmole/24 hr) in patients undergoing operation for hiatus hernia, oesophageal cancer and lung cancer.

Values are mean ± S.E.M. for the number of patients shown in parenthesis.

	Before operation	Time after operation (days) 2	7	14
Hiatus hernia	1.55 ± 0.22 (9)	2.08 ± 0.48 (9)	1.44 ± 0.34 (6)	1.30 ± 0.22 (6)
Oesophageal cancer	1.18 ± 0.27 (7)	1.80 ± 0.26 (9)	1.11 ± 0.12 (7)	1.38 ± 0.22 (7)
Lung cancer	1.36 ± 0.21 (6)	1.14 ± 0.19 (6)	1.22 ± 0.11 (6)	1.56 ± 0.15 (4)

Table 3.11 Urinary excretions of potassium (mmole/Kg.B.W./24hr) in patients undergoing surgical operation for hiatus hernia, oesophageal cancer and lung cancer

Values are means ± S.E.M. for the number of patients shown in parentheses

	Before operation	Time after operation (days) 2	7	14
Hiatus hernia	0.45 ± 0.12 (9)	1.43 ± 0.5 (9)*	0.26 ± 0.05 (6)*	0.44 ± 0.11 (6)
Oesophageal cancer	0.59 ± 0.09 (7)	0.91 ± 0.16 (9)*	0.55 ± 0.12 (7)	0.38 ± 0.03 (7)
Lung cancer	0.57 ± 0.22 (6)	0.56 ± 6.16 (6)	0.55 ± 0.10 (6)	0.43 ± 0.12 (4)

Statistical significance of differences between values of before and after operation shown * where $p < 0.05$.

Table 3.12 Urinary excretions of sodium (mmole/Kg.B.W./24 hr) in patients undergoing surgical operation for hiatus hernia, oesophageal cancer and lung cancer

Values are means ± S.E.M. for the number of patients shown in parentheses

	Before operation	Time after operation (days) 2	7	14
Hiatus hernia	1.81 ± 0.39 (9)	0.62 ± 0.20 (9)**	0.92 ± 0.16 (6)*	1.10 ± 0.4 (6)
Oesophageal cancer	1.21 ± 0.26 (7)	0.73 ± 0.17 (9)**	1.46 ± 0.36 (7)	1.43 ± 0.78 (7)
Lung cancer	1.64 ± 0.21 (6)	0.38 ± 0.16 (6)**	0.82 ± 0.2 (6)*	1.16 ± 0.40 (4)

Statistical significance of difference between values of before and after operation shown * where $P < 0.05$ and ** where $P < 0.01$.

Table 3.13 Urinary excretions of nitrogenous compounds (mg/Kg B.W./24 hr) in patients undergoing operation for hiatus hernia.

Values are means ± S.E.M. for the number of patients shown in parentheses

Compound	Before operation	Time after operation (days) 2	7	14
Total-nitrogen	140 ± 20 (5)	160 ± 30 (8)	90 ± 10 (6)*	120 ± 20 (5)
Urea-nitrogen	101 ± 13 (5)	110 ± 21 (8)	68 ± 6 (6)**	73 ± 12 (5)**
Amino-nitrogen	3.60 ± 0.89 (5)	4.81 ± 1.34 (8)	4.73 ± 0.89 (6)	3.10 ± 0.60 (5)
Creatinine-nitrogen	6.66 ± 0.97 (5)	5.49 ± 0.70 (8)	4.44 ± 0.47 (6)	4.38 ± 0.74 (5)*

Statistical significance of differences between values before and after operation shown * where $P < 0.05$ and ** where $P < 0.01$.

Table 3.14 Urinary excretions of nitrogenous compounds (mg/Kg.B.W./24 hr) in patients undergoing operation for lung cancer.

Values are means ± S.E.M. for the number of patients shown in parentheses

Compound	Before operation	Time after operation (days) 2	7	14
Total-nitrogen	143 ± 20 (7)	192 ± 30 (6)	142 ± 10 (6)	100 ± 20 (4)
Urea-nitrogen	108 ± 16 (7)	168 ± 20 (6)*	102 ± 11 (6)	73 ± 14 (4)
Amino-nitrogen	4.91 ± 0.77 (7)	11.38 ± 2.3 (6)**	13.33 ± 1.15 (6)****	3.20 ± 1.15 (4)
Creatinine-nitrogen	5.13 ± 0.33 (7)	5.70 ± 0.57 (7)	3.98 ± 0.17 (6)***	2.97 ± 0.20 (4)

Statistical significance of differences between values before and after operation shown *where $P < 0.05$ **where $P < 0.02$, ***where $P < 0.01$ and ****where $P < 0.001$.

Table 3.15 Urinary excretions of nitrogenous compounds (mg/Kg.B.W./24hr) in patients undergoing operation for oesophageal cancer.

Values are means ± S.E.M. for the number of patients shown in parentheses

Compound	Before operation	Time after operation (days) 2	7	14
Total-nitrogen	148 ± 20 (7)	141 ± 20 (10)	110 ± 30 (6)	74 ± 10 (5)**
Urea-nitrogen	110 ± 20 (7)	107 ± 16 (10)	73 ± 13 (6)	51 ± 13 (5)*
Amino-nitrogen	8.68 ± 1.29 (7)	6.58 ± 1.31 (10)	4.28 ± 1.14 (6)*	4.79 ± 0.98 (5)*
Creatinine-nitrogen	5.38 ± 0.82 (7)	4.96 ± 0.53 (10)	4.91 ± 1.00 (6)	4.32 ± 0.59 (5)

Statistical significance of differences between values before and after operation shown *where $P < 0.05$ and ** where $P < 0.01$.

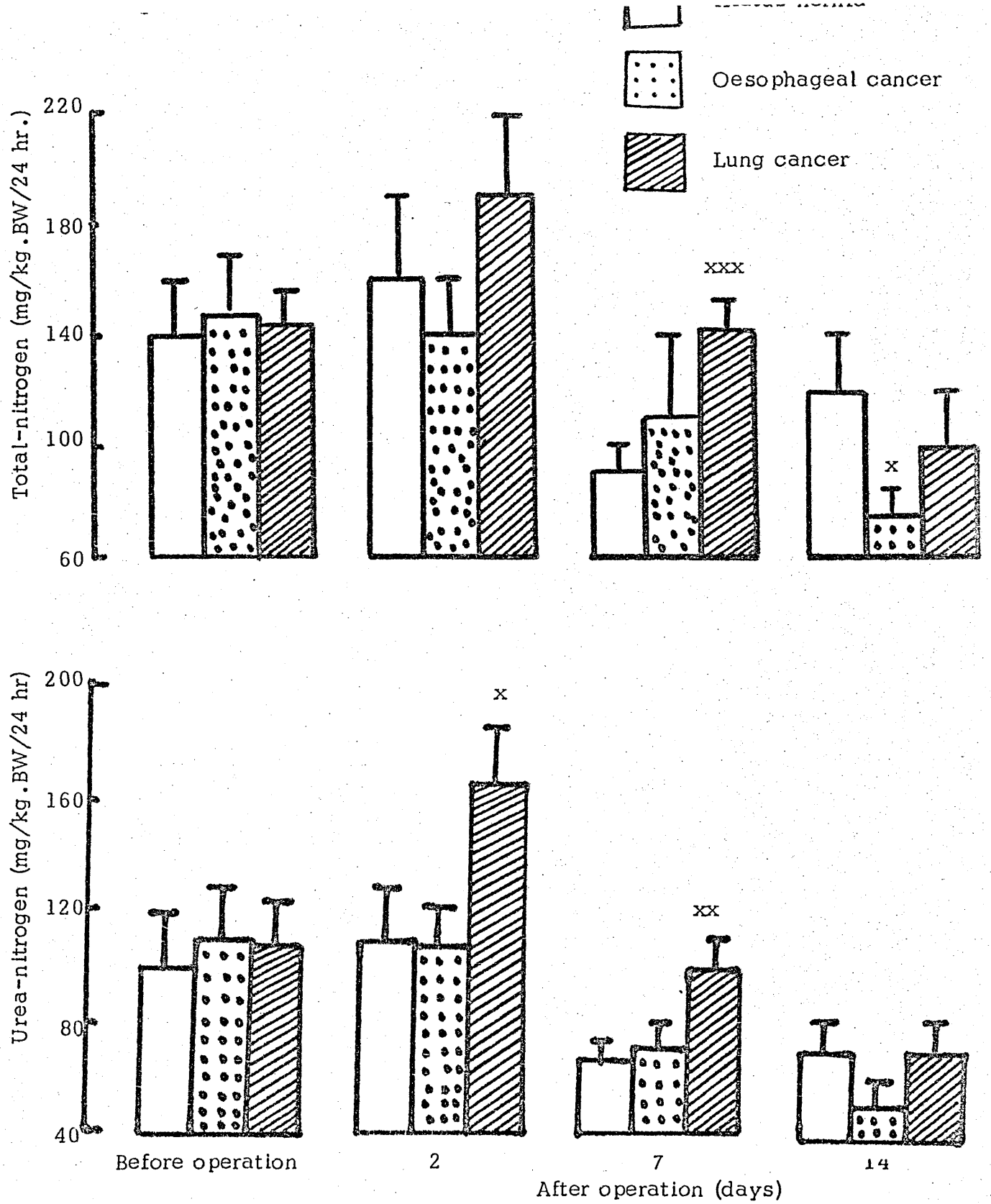


Figure 3.3 Effect of operation on the urinary excretion of total-nitrogen and urea-nitrogen in patients with hiatus hernia, oesophageal cancer and lung cancer. (Mean ± S.E.M.)

Statistical significance of differences between values for cancer patients and that of patients with hiatus hernia shown x when P 0.05, xx where P 0.02 and xxx where P 0.01.

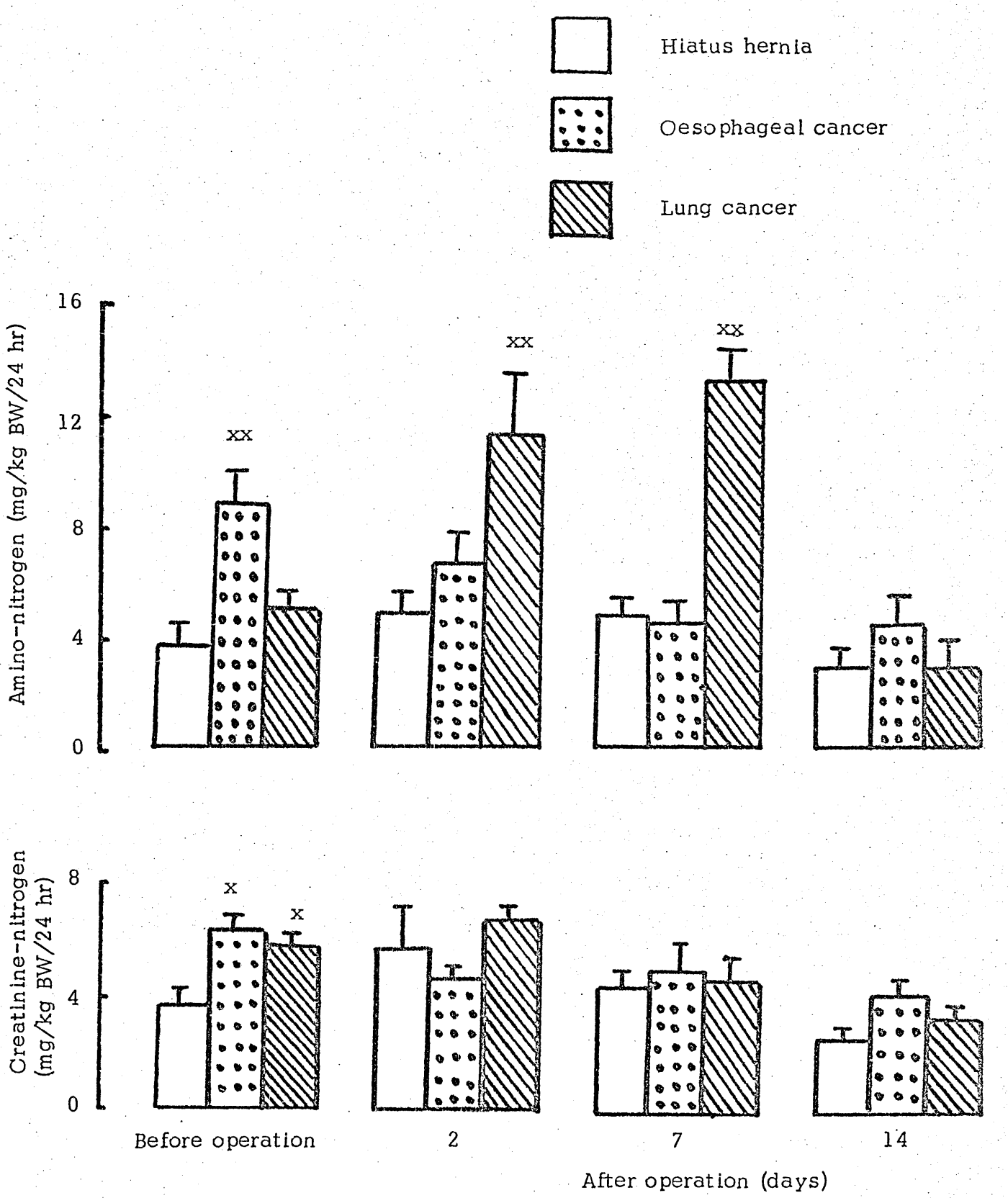


Figure 3.4 Effect of operation on the urinary excretion of amino-nitrogen and creatinine-nitrogen in patients with hiatus hernia, oesophageal cancer and lung cancer. (Mean ± S.E.M.)

Statistical significance of differences between values for cancer patients and that of patients with hiatus hernia shown x where P 0.05 and xx where P 0.01.

Table 3.16 Urinary activity of alkaline ribonuclease (Unit/mg creatinine) in patients undergoing surgical operation because of hiatus hernia, oesophageal cancer and lung cancer

Values are means ± S.E.M. for the number of samples analyzed shown in parentheses

	Before operation	Time after operation (days) 2	7	14
Hiatus hernia	4.38 ± 1.63 (9)	11.05 ± 4.22 (9)*	2.97 ± 0.93 (6)	4.43 ± 1.38 (6)
Oesophageal cancer	2.25 ± 0.4 (7)	5.53 ± 1.46 (9)*	5.09 ± 1.62 (7)	4.93 ± 1.60 (7)
Lung cancer	3.66 ± 1.03 (6)	6.13 ± 1.44 (6)*	6.07 ± 1.6 (6)*	5.66 ± 1.67 (4)

Statistical significance of differences between values of before and after operation shown * where $p < 0.05$.

3. DISCUSSION

Three groups of patients have been studied in this investigation. One group had a benign condition, hiatus hernia, and served as a control for patients with oesophageal cancer. Differences between these patients before operation are likely to be due to cancer and the consequent malnutrition resulting from dysphagia. Differences after operation are likely to result from the different operative procedures. Whereas this has a common feature in that it involves a thoracotomy, it differs in its severity, in degree of shock, blood loss and in the extent to which the gut is handled. There is also the problem that oesophagectomy whether with or without partial or total gastrectomy causes disturbances of intestinal function which are in part due to the necessary sacrifice of the vagus nerves at operation.

The third group of patients also had cancer, but at a site removed from the alimentary tract. Surgical treatment therefore did not involve interference with the tract, but like the other operations does necessitate a thoracotomy. Furthermore, some lung tumours produce hormones which may modify the metabolic response to surgery. The following discussions will therefore focus on the following aspects of the problems presented by these patients:- (a) The effects of cancer on metabolism including the differences between the effects of cancer at different sites (b) The effects of different types of surgical operation (c) The ways in which the metabolic response to surgery is modified by the pre-operative condition of the patient. All these aspects of the problem are closely linked together so that discussion of the various points in order will

not be possible.

Plasma proteins: Hypoalbuminaemia is common in patients with cancer (Mider et al., 1950; Steinfled, 1960; Mariani, 1976). In the present study low levels of albumin have been found pre-operatively in both the cancer groups studied, but particularly in patients with oesophageal cancer. In this latter group malnutrition is at least a contributing factor as it is well-known that low levels of albumin are found in patients with protein energy malnutrition (P.E.M.) (Dean and Schwartz, 1953). Increased catabolism of albumin as well as decreased hepatic synthesis are responsible for the low levels in P.E.M. However, in patients with certain types of cancer the condition may be further exacerbated by the loss of a protein-rich exudate into the gastrointestinal tract (Waldmann et al., 1977). This is unlikely to occur in patients with lung cancer, but may well occur in those with oesophageal cancer, particularly if the tumour extends into the cardia of the stomach.

The post-operative fall of plasma albumin observed in hiatus hernia and oesophageal cancer patients is in agreement with the findings of others that after trauma, the plasma albumin concentration falls reaching a minimum around the third to sixth day (Fleck, 1976). A fall in plasma albumin concentration may not, however, be an inevitable consequence of injury, for the concentration hardly changed in the patients with lung cancer who had a lobectomy or pneumonectomy. In addition, patients having lung operations normally resume their normal diet a few days after surgery whereas those having oesophagectomy or oesophagogastrectomy are unable to do so for one or two weeks. It seems, therefore, that malnutrition could be an important contributory factor to the observed

post-operative hypoalbuminaemia in oesophageal cancer patients. The post-operative fall in plasma albumin concentration in patients with hiatus hernia could be a direct result of surgical catabolism, but the surgical procedure used for the treatment of these patients is less severe than operations on the lung or the oesophagus. However, the fact that these patients are frequently overweight and receive a restricted calorie diet as part of their treatment, makes it possible to assume that the post-operative fall in albumin concentration in these patients is due to a low calorie intake rather than to surgical catabolism.

Hypoalbuminaemia could have a profound effect on the response of patients to surgery. This effect has been investigated in experimental animals and in man. Impaired wound healing (Thompson, Ravdin and Frank, 1938), increased susceptibility to haemorrhagic shock (Ravdin, McNamee, Kamholzand Rhoades, 1944) and increased incidence of post-operative infection (Rhoades and Alexander, 1955) have been reported to be associated with hypoalbuminaemia. More recently, impaired immune function has been reported in surgical patients with low plasma albumin levels (Law, Dudrick, and Abdou, 1973).

A moderate increase in plasma globulin concentration in cancer patients has been reported before (Mider et al., 1950). In the present investigation, the mean pre-operative concentrations of globulin in cancer patients were slightly higher than those in patients with hiatus hernia. Furthermore, whereas, the globulin levels in hiatus hernia and lung cancer patients remained unchanged, they fell slightly, but significantly, in oesophageal cancer patients and returned to their pre-operative level by 14 days after surgery. However, these post-

operative changes in plasma globulin fractions did not resemble those reported in fracture patients, in which the general trend was a slight increase in the globulin fraction with an increased fibrinogen level (Cuthbertson and Tompsett, 1935). In view of the fact that patients with oesophageal cancer had a lower plasma total protein level than the other two groups and also that they had a progressive decline in urinary nitrogen excretion it would appear that the post-operative fall in the concentration of plasma globulin as well as in that of albumin was a reflection of their generally poor nutritional status.

Plasma free amino acids: Although an increased level of plasma amino nitrogen has been shown to accompany tumour growth in experimental animals (Wu and Bauer, 1960) and cancer patients (Goldfeder, 1933 reported by Greenstein, 1954), very few studies have been conducted in which the pattern of plasma amino acids in a tumour bearing host has been investigated. Rouser, Kelly et al. (1962) did, however, report a distinct elevation of glutamic acid in patients with chronic leukaemia. In the present study, the pre-operative levels of all essential amino acids were found to be similar in both groups of cancer patients and in those with hiatus hernia. Of the non-essential amino acids, the concentrations of alanine and arginine were significantly lower in lung cancer patients than in the other two groups pre-operatively. After operation, however, the pattern of plasma amino acids in patients treated for lung carcinoma differed distinctly from those in the other two groups. Plasma branched chain amino acid concentrations were not affected by surgery, while those of alanine, glycine, threonine and arginine fell immediately after operation in oesophageal and hiatus hernia patients, but not in patients with lung cancer. Furthermore,

whereas the glutamic acid level rose after surgery in lung cancer patients, it fell in the other two groups. A post-operative fall of plasma amino acids has been reported in recent years (Schonhyder et al., 1974; Woolf et al., 1976; Dale et al., 1977). However, the mechanism by which surgical operation results in a lowered concentration of plasma amino acids has not been described. Schonhyder et al., (1974) have suggested that post-operative malnutrition is the main causative factor. This suggestion is not, however, supported by the fact that contrary to present findings malnutrition and protein deprivation result in a rise in non-essential amino acids and a fall in those of essential amino acids. The post-operative fall in the concentrations of plasma amino acids could also be attributed to the enhanced rate of gluconeogenesis known to follow surgery (Kinney et al., 1970). The present finding that in hiatus hernia and oesophageal cancer patients low levels of plasma alanine and glycine following immediately after operation were accompanied by elevated levels of glucose (see Chapter 4) provides direct evidence in support of this suggestion, as these two amino acids are known to serve as the main substrate for gluconeogenesis.
Moreover this hypothesis would also explain the failure of surgical trauma to affect plasma concentrations of branched chain amino acids, as the main site of metabolism for these amino acids is muscle, and therefore, they pass through the liver without being taken up for the purpose of glucose synthesis (Miller, 1962).

The post-operative changes in the concentration of alanine in patient G.L. require a separate discussion. This patient was overweight and had a high blood glucose (15.9 mM), alanine (500 μ mole/l) and

FFA (0.7 mmole/l), pre-operatively. After operation, in order to reduce her body weight she received a low calorie diet. Immediately after surgery, the blood glucose level did not change, the alanine concentration fell and the FFA level rose to a value which was twice the pre-operative value. By 2 days after operation plasma alanine rose to an extremely high level (16 mmol/l) and remained high until 7 days, and this was accompanied by very high level of glucose (19.4 mmole/l), and FFA (1.5 mmol/l). However plasma alanine, but not glucose or FFA, fell to a level comparable to the pre-operative value by 14 days after operation. These results would suggest that the patient was in a diabetic state before undergoing operation, and that her diabetic condition became more severe after surgery. Moreover, administration of a low calorie diet seems to have resulted in an increased breakdown of protein in the muscle, with the consequent release of high amounts of alanine into the circulation faster than it could be removed by the liver. This explanation is consistent with the observation that the blood urea nitrogen was also high after surgery in this patient. Thus indicating an enhanced rate of glucose production at the expense of plasma amino acid. The plasma urea nitrogen in other patients with hiatus hernia or oesophageal cancer did not change significantly but in lung cancer patients its level rose after operation, and this rise was not accompanied by a high blood glucose level.

The post-operative fall in the concentration of plasma glutamine and the early rise in the level of plasma phenylalanine observed in all patients, are in agreement with the findings of Vinnars et al., (1976), who also found a fall in the concentration of glutamine and a rise in the

level of plasma phenyl alanine following surgery. Moreover, the present results provide further evidence in support of Kinney's view (Kinney, 1977) that the decrease in glutamine observed in injured subjects is unique to surgical catabolism.

Plasma and urinary zinc and copper: Serum copper levels have been reported to be elevated in patients with Hodgkin's disease (Warren, Jelliffe et al., 1969) and in those with osteosarcoma (Fisher, Byer et al., 1976). The present results indicated that pre-operative levels of plasma copper in oesophageal cancer patients, but not in patients with lung cancer, were significantly higher than those in patients with hiatus hernia. Furthermore, the post-operative fall in the concentration of plasma copper in oesophageal cancer patients can be attributed to their response to surgical therapy, since it has been shown that in patients with osteao-sarcoma, the high serum copper level was related to disease activity, with a return to a normal level after treatment (Fisher et al., 1976). On the other hand, the mean levels of plasma copper in hiatus hernia and lung cancer patients showed a tendency to increase after surgery. This can be attributed to the effect of operation, as it has been reported that a mild hypercupraemia may develop after operation (Sass-Cartsak, 1965). Finally the present investigation suggests that the presence of a tumour probably has no effect on the rate of copper excretion in the urine. Moreover, surgical operation exerts no significant effect on the urinary copper excretion.

There is no general agreement as to whether the presence of a tumour in the body changes the concentration of zinc in the plasma or its

excretion in the urine. While, Wolff (1956), in a study on 45 patients with carcinoma of different aetiology and site, found decreased values for serum zinc., Davies et al. (1968) in an investigation carried out in 49 cancer patients found that only patients with carcinoma of the bronchus had a significantly lower plasma zinc as compared with controls. In the present investigation, the pre-operative levels of plasma zinc in cancer patients were similar to those in patients with hiatus hernia and all levels were within the normal range. As to the effect of surgical operation on plasma zinc, the present results suggest that surgery is followed by an early fall in the concentration of plasma zinc which does not appear to be related to the type or severity of the procedure. In addition, in oesophageal and hiatus hernia patients the fall in plasma zinc concentrations coincided with a fall in its urinary excretion which suggests that in these patients surgery resulted in an increased rate of zinc uptake by the tissues. However, in patients with lung cancer the operation was followed by a moderate rise in urinary zinc excretion. Since in these patients urinary amino nitrogen followed the same pattern, it can be postulated that the rise in zinc was the result of increased tissue breakdown.

Sodium and potassium excretion: The alterations in the rate of sodium and potassium excretion following surgery which were observed in the present investigation are essentially consistent with the findings of others. That is to say, that after operation there is a retention of sodium in the body and the excretion of sodium in the urine falls while that of potassium is increased due to tissue breakdown. However, in lung cancer patients the excretion of potassium remained unchanged after surgery. In so far as increased amounts of potassium are found

in the urine whenever the tissues are losing potassium such as occurs after injury, the absence of post-operative change observed in lung cancer patients may indicate that those patients were in a less severe catabolic state than were those in the other two groups. The fact that these patients had a higher plasma insulin (Chapter 4) post-operatively as compared with the other two groups, gives support to this idea, as it is known that potassium excretion is increased in diabetes (Davidson and Passmore, 1969).

Urinary excretion of nitrogenous compounds: The pre-operative excretions of total nitrogen and urea nitrogen were similar in patients with cancer and in those with hiatus hernia. These findings are consistent with those of White (1945) who showed that the total urinary nitrogen in tumour-bearing subjects was not increased and this was interpreted as being due to the action of the tumour in retaining nitrogen. Similarly, Bach and Maw (1953) studied the excretion of ammonia, urea and creatinine in rats bearing the Jensen sarcoma. They found that the levels of these compounds were not significantly different from those of the controls. However, the present investigation showed that before operation, cancer patients excreted more creatinine nitrogen per Kg body weight than patients with hiatus hernia. Since it is well known that the excretion of creatinine in males is slightly higher than in females, the present finding can be attributed to the sex difference rather than to the presence of a tumour for the majority of patients with hiatus hernia were females whereas the majority of those with cancer were males.

With regard to the effect of operation on the urinary excretion of nitrogenous compounds, the present results showed that apart from the

excretion of amino-nitrogen by lung cancer patients, which was found to be elevated on the 2nd and 7th days after surgery, the excretion of other urinary nitrogenous constituents was not usually increased and even showed a tendency to decrease after operation. This was specially evident in oesophageal cancer patients, in whom a progressive fall in the excretion of total nitrogen, urea-nitrogen and amino-nitrogen occurred following surgery. Moreover, the fact, that in these patients the post-operative fall in the excretion of urinary nitrogenous compounds was parallel to a decline in their plasma albumin concentration, would probably suggest that starvation was an important contributory effect, since total nitrogen in the urine reflects the nitrogen intake and falls by about 50% after 2 days of starvation (Folin, 1905). On the other hand, patients undergoing operation for lung carcinoma would appear to be in a better nutritional state when compared with either hiatus hernia or oesophageal cancer patients. Neither plasma albumin, nor the urinary excretion of nitrogenous compound in these patients fell markedly after operation. Moreover, the slight early post-operative increase in the excretion of urea nitrogen and amino nitrogen can be attributed to their response to surgical operation because of their better nutritional status. However, the results of the present investigation are in general terms consistent with the common view that urinary nitrogen response to surgical trauma is more marked in males and in well-nourished subjects.

Urinary ribonuclease activity: The pre-operative levels of urinary ribonuclease activity were similar in cancer and hiatus hernia patients. In all patients, the levels rose significantly by 2 days after operation but returned to pre-operative levels by 14 days after surgery. These

results provide further evidence that physical injury is associated with an increased excretion of ribonuclease in the urine, and agree with those of Barlow and Withear (1977) who showed that the activity of ribonuclease in the urine of children with burn injuries was increased. However, the mechanism of this phenomenon is yet to be explained. The clearance of plasma ribonuclease is believed to be, at least in part, due to renal excretion (Sigulem, Brosel et al., 1973) and the serum ribonuclease activity is increased when the glomerular filtration rate is decreased in man (Houck and Berman, 1958). On this evidence, it can be speculated that the increased urinary ribonuclease activity observed on the second post-operative day was the result of an increased glomerular filtration of this enzyme. On the other hand, the suggestion that measurement of ribonuclease activity in the urine of children can be used to detect malnutrition (Sigulem et al., 1973) seems not to be applicable in surgical patients, moreover, the observation that urinary ribonuclease activity was the same in all patients throughout the study, would indicate that the activity of this enzyme is not closely related to nutritional status in these groups of subjects, and therefore could not be regarded as a measure of nutritional status in surgical patients.

In conclusion, the observations presented suggest that the presence of a tumour in either of the two sites investigated, oesophagus and lung, has very little, if any, effect upon the general pattern of plasma free amino acids or upon the rate of urinary excretion of nitrogen-containing substances. Low plasma albumin concentrations were, however, found in patients with both kinds of cancer.

With regard to the effects of surgical operation on protein and amino

acid metabolism, the present findings suggest that different surgical procedures could have different effects, depending on their severity and nature. In general, it seems that operations such as lobectomy or pneumonectomy are associated with a higher degree of post-operative catabolism in comparison with oesophagectomy. It is suggested that this difference could be related to the handling of the gut, rather than to the severity of the operation.

CHAPTER 4

PLASMA CONCENTRATIONS OF GLUCOSE, FFA, INSULIN 11-HYDROXYCORTICOSTEROIDS AND URINARY EXCRETION OF KETONE BODIES IN PATIENTS UNDERGOING SURGERY

1. INTRODUCTION

Free fatty acids (FFA) and glucose are the major metabolic fuels supplied by the blood to peripheral tissues for energy production (Randle et al, 1963) and insulin plays a major role in the regulation of energy metabolism. This hormone increases the rate of glucose utilisation and controls the rate of FFA release from adipose tissue (Cahill, 1971). It is now well established that injury and operation induce a diabetic-type condition, associated with glucose intolerance (Thomsen, 1938; Hayes and Brandt, 1952; Drucker et al., 1953). Lipolysis has also been reported to be increased post-operatively (Schultis and Geser, 1970), leading to an elevated concentration of plasma FFA (Allison et al, 1968).

Following injury there are changes in the secretion of a number of hormones that affect carbohydrate metabolism. In this connection, Allison et al (1968) performed a glucose tolerance test in a patient with burn injury and found that in the acute phase there was a failure of insulin response to a glucose load, associated with marked glucose intolerance. Subsequently, there was persistent glucose intolerance associated with abnormally high insulin levels. Essentially similar results were obtained when the experiment was carried out in a patient undergoing laparatomy and exploration of the common bile duct (Allison et al, 1969). More recently Russell et al (1975) studied plasma insulin levels in twenty patients before and after operations such as

vagotomy, partial gastrectomy, mastectomy, colon resection and cholecystectomy. They found that the insulin level fell during, but rose after surgery and reached a maximum level by one day after operation.

Changes in the plasma levels of catabolic hormones following injury have also been investigated. Thus, Hume et al (1962) reported that during an abdominal operation the level of hydroxycorticosteroids rose to a amaximum level by 4 to 6 hours after operation. Similarly Johnston (1964) found that in a female patient undergoing abdomino-perineal resection of rectum, the plasma cortisol level rose and reached a maximum level by 4 hr after operation. An elevation of plasma glucagon level as a result of surgical stress has also been reported. Thus, Russell et al (1975) studying twenty patients undergoing different types of abdominal operation found that the plasma glucagon levels rose during operation, and declined during the closure of the wound.

However, in most of the studies conducted so far to investigate changes in glucose and hormonal metabolism following injury, the observations have been made on rather heterogenous groups of patients and few studies have been directed towards the elucidation of the effects of specific operative procedures for closely defined conditions. Moreover, studies in which plasma insulin or cortisol has been investigated following surgery, the observations have mainly been made during the "ebb" phase of injury, and therefore, a limited amount

of information is available about the plasma pattern of these hormones during the recovery period after surgery.

The present study was, therefore, undertaken on patients with the three conditions in which we were interested, hiatus hernia, oesophageal cancer and lung cancer involving thoracic surgery.

Patients and Methods

The patients investigated in this study were those described in Chapter 2. Plasma glucose was determined enzymatically as described by Werner and Wirlinger (1970). Plasma insulin was measured with a single antibody radioimmunoassay procedure, developed by Amersham Radiochemical Centre, and total 11-hydroxycorticosteroids by the method of Mattingly (1964). Plasma FFA levels and urinary concentrations of ketone bodies were measured as described by Duncombe (1964) and Natelson (1963) respectively.

2. RESULTS

Pre-operatively, the concentrations of plasma glucose (Table 4.1 and Fig. 4.1) were similar in all three groups of patients. The levels were significantly raised immediately after operation in patients with hiatus hernia and oesophageal cancer and subsequently fell so that by the 7th post-operative day they had fallen to their pre-operative levels. The glucose concentrations in patients with lung cancer were not significantly raised immediately after operation. On the second post-operative day however, they were significantly raised above the pre-operative value, but only to a value that was similar to that of the two groups on that day. Before operation the mean plasma insulin concentration (Table 4.2 and Fig. 4.2) was highest in patients with lung cancer, intermediate in those with oesophageal cancer and lowest in those with hiatus hernia, although the differences were not statistically significant. In all three groups the plasma insulin concentrations were not significantly changed immediately after surgery but were raised on the second day after operation. Both the rise, and the absolute concentration were greater in the lung cancer patients. In all patients the concentration had fallen by the 7th post-operative day. It then remained low in the patients with oesophageal cancer, but rose again in the other two groups. Again the rise was greater in the patients with lung cancer. Thus a rise in plasma insulin concentration followed the rise in blood sugar level in patients with hiatus hernia and oesophageal cancer and a larger rise was coincident

with a similar change in plasma glucose concentration in the patients with lung cancer.

The plasma concentration of 11-hydroxycorticosteroids (Table 4.3 and Fig. 4.3) was higher pre-operatively in patients with hiatus hernia than in either groups of cancer patients, and the immediate post-operative rise was also greater in these patients though a rise did occur in each group. By the second post-operative day the concentration of 11-hydroxycorticosteroids had fallen to values not significantly above the pre-operative level in patients with hiatus hernia and oesophageal cancer but they remained higher than the pre-operative level in patients with lung cancer. Plasma FFA concentrations (Table 4.4 and Fig. 4.4) rose significantly in all patients immediately after operation and by two days afterwards had fallen to their pre-operative levels.

Before surgery, the excretion of ketone bodies in the urine of oesophageal cancer patients was higher than that of the other two groups (Table 4.5). In all three groups the level of excretion was higher on the second post-operative day. It was lower on the 7th and 14th post-operative days in all patients but above the pre-operative level in the hiatus hernia patients and had fallen below the pre-operative value in oesophageal cancer patients. At 14 days the urinary excretion of ketone bodies was lower in the two groups of patients with cancer than in the patients with hiatus hernia.

In one female patient (GL) with hiatus hernia, a distinctly different pattern of change in the various measurements was observed from that described above. Thus, unlike other patients, this patient had high levels of plasma glucose, FFA, 11-hydroxycorticosteroids, alanine and urinary excretion of ketone bodies both before and after operation. Values for blood glucose and alanine concentrations in particular were very high following surgery.

Table 4.1 Concentrations of plasma glucose (mg/100 ml.) in patients undergoing surgery for hiatus hernia, oesophageal cancer and lung cancer.

Values are mean ± SEM for the number of patients shown in parentheses.

Patient	Before Operation	Immediately after operation	Time after operation (day)		
			2	7	14
Hiatus hernia (9)	76.5 ± 1.3	128.8 ± 13.3*	90.4 ± 7.9	79.2 ± 3.5	76.8 ± 8.0
Oesophageal cancer (11)	78.8 ± 4.9	118.5 ± 8.7**	91.7 ± 5.5	76.08 ± 4.7	76.6 ± 4.6
Lung cancer (8)	67.4 ± 5.2	76.8 ± 8.9	93.10 ± 8.7	68.59 ± 5.93	73.8 ± 6.7

Statistical significance of differences between before and after operation shown * where $p < 0.01$ and ** were $p < 0.001$.

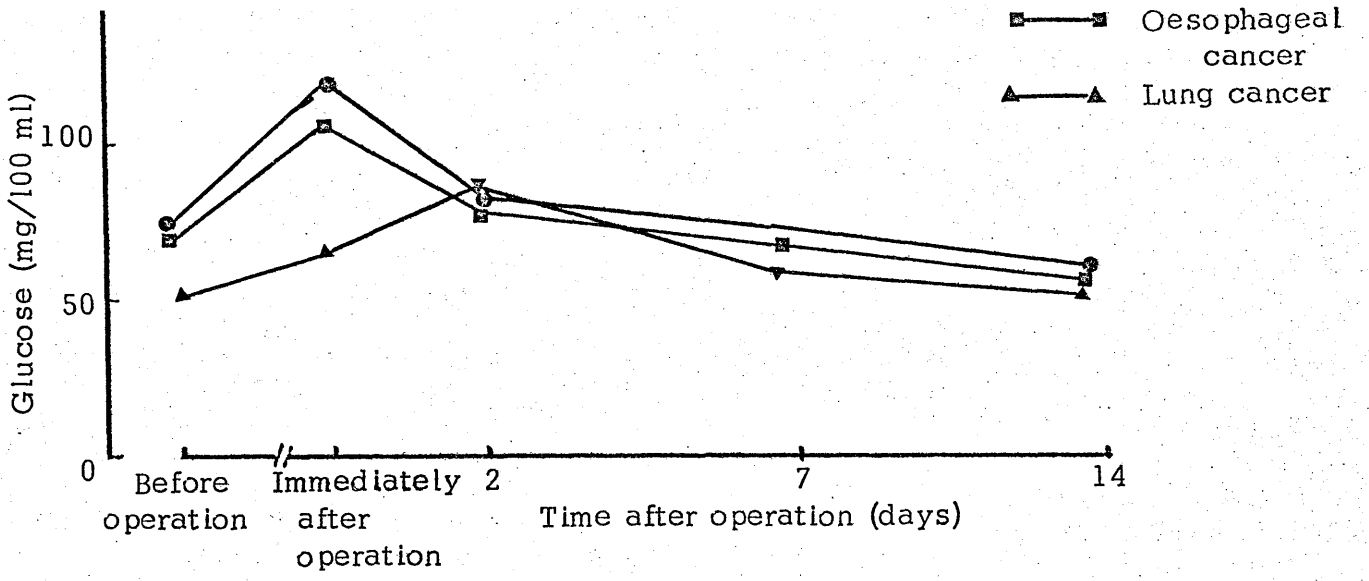


Figure 4.1. Effect of operation on the concentrations of plasma glucose in patients with hiatus hernia, oesophageal cancer and lung cancer

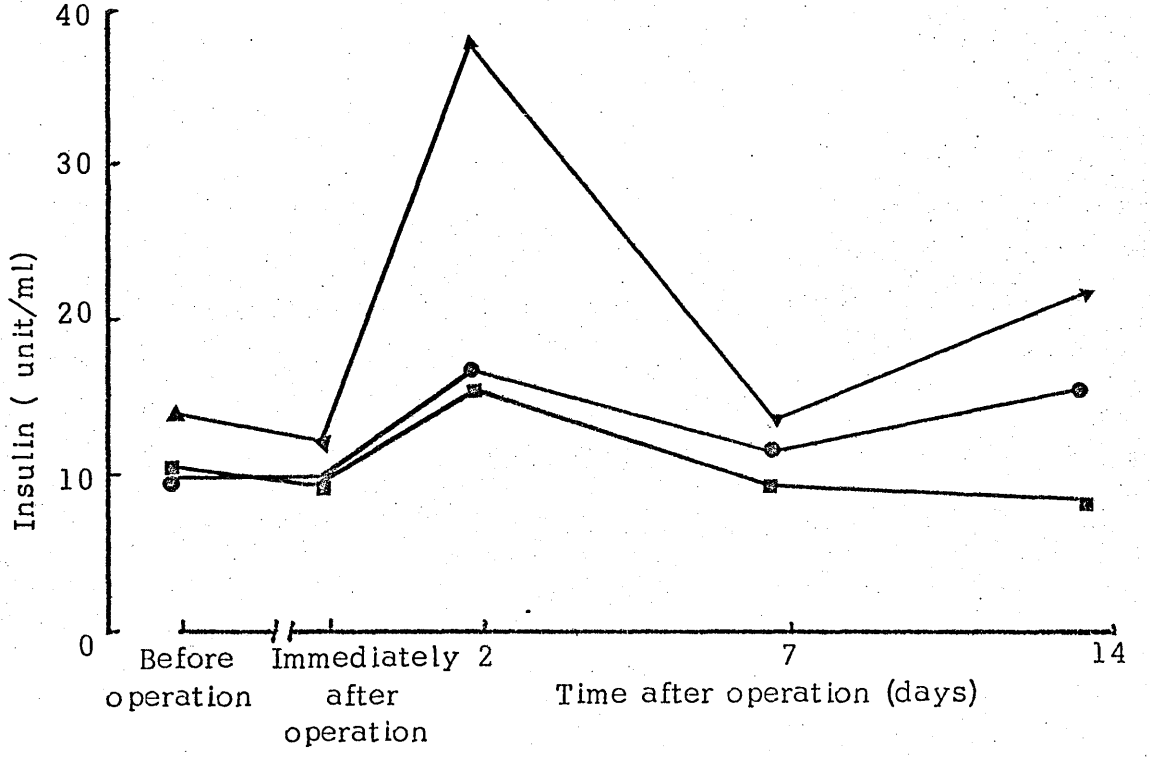


Figure 4.2. Effect of operation on the concentrations of plasma insulin in patients with hiatus hernia, oesophageal cancer and lung cancer

Table 4.2 Concentrations of plasma insulin (μunit/ml.) in patients undergoing surgery for hiatus hernia, oesophageal cancer and lung cancer.

Values are mean ± SEM for the number of patients shown in parentheses.

Patient	Before Operation	Immediately after operation	Time after operation (day)		
			2	7	14
Hiatus hernia (9)	9.9 ± 2.7	10.7 ± 2.5	16.4 ± 2.3*	12.0 ± 3.0	16.4 ± 3.7*
Oesophageal cancer (11)	11.2 ± 1.3	10.3 ± 1.3	17.3 ± 2.1*	9.3 ± 1.6	8.0 ± 2.2
Lung cancer (8)	15.0 ± 3.0	13.0 ± 3.0	39.0 ± 5.0**	13.0 ± 3.8	23.4 ± 4.4

Statistical significance of differences between before and after operation shown * where $p < .05$ and ** where $p < 0.02$

Table 4.3 Concentrations of plasma 11-hydroxycorticosteroids (μg/100ml) in patients undergoing surgery for hiatus hernia, oesophageal cancer and lung cancer.

Values are mean ± SEM for the number of patients shown in parentheses.

Patient	Before Operation	Immediately after operation	Time after operation (day)		
			2	7	14
Hiatus hernia (9)	19.1 ± 1.9	40.2 ± 5.4**	19.6 ± 2.7	19.5 ± 2.5	14.2 ± 1.1
Oesophageal cancer (11)	14.3 ± 2.2	28.0 ± 2.9**	16.7 ± 0.8	18.0 ± 2.8	18.3 ± 2.9
Lung cancer (8)	14.0 ± 2.2	31.4 ± 2.2***	25.0 ± 3.8*	17.7 ± 3.9	15.9 ± 2.7

Statistical significance of differences between before and after operation shown * where $p < 0.05$

** where $p < 0.01$ and *** where $p < 0.001$

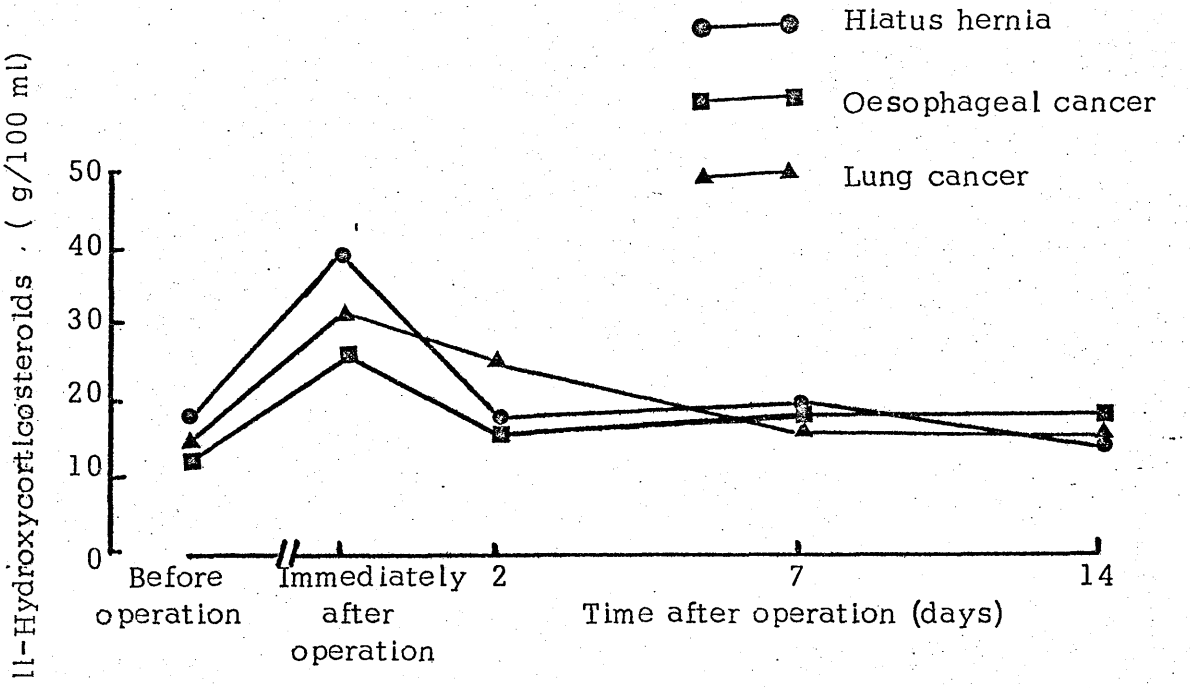


Figure 4.3. Effect of operation on the concentrations of plasma 11-Hydroxycorticosteroids in patients with hiatus hernia, oesophageal cancer and lung cancer

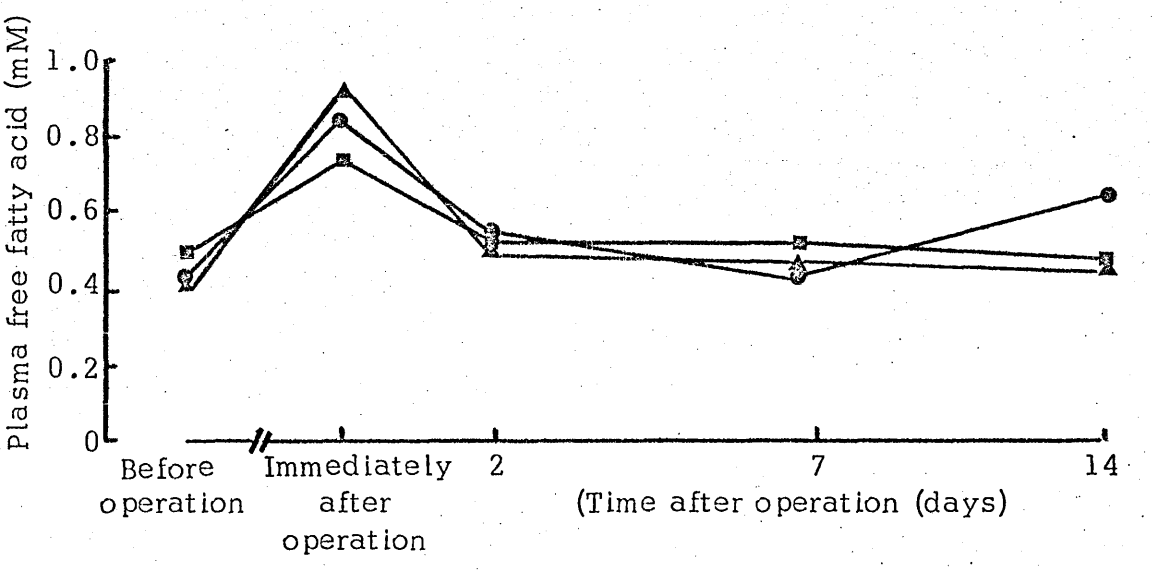


Figure 4.4. Effect of operation on the concentrations of plasma free fatty acid in patients with hiatus hernia, oesophageal cancer and lung cancer

Table 4.4 Concentrations of plasma FFA (mM) in patients undergoing surgery for hiatus hernia, oesophageal cancer and lung cancer.

Values are mean ± SEM for the number of patients shown in parentheses.

Patient	Before Operation	Immediately after operation	Time after operation (day)		
			2	7	14
Hiatus hernia (9)	0.44 ± 0.06	0.84 ± 0.17*	0.55 ± 0.10	0.43 ± 0.09	0.65 ± 0.14
Oesophageal cancer (11)	0.49 ± 0.03	0.74 ± 0.06**	0.54 ± 0.04	0.52 ± 0.03	0.47 ± 0.07
Lung cancer (8)	0.43 ± 0.03	0.92 ± 0.09***	0.53 ± 0.08	0.44 ± 0.04	0.45 ± 0.02

Statistical significance of differences between before and after operation shown * where $p < 0.05$ ** where $p < 0.01$ and *** where $p < 0.001$.

Table 4.5 Urinary excretions of ketone bodies (mg/Kg. B.W./24 hr) in patients undergoing surgery for hiatus hernia, oesophageal cancer and lung cancer.

Values are mean ± SEM for the number of patients shown in parentheses

Patient	Before operation	Time after operation (day)		
		2	7	14
Hiatus hernia	0.16 ± 0.01 (5)	0.81 ± 0.17 (8)***	0.3 ± 0.06 (6)*	0.4 ± 0.03 (4)***
Oesophageal cancer	0.32 ± 0.05 (6) †	1.04 ± 0.22 (9)**	0.31 ± 0.03 (5)	0.19 ± 0.04 (5)* ††
Lung cancer	0.18 ± 0.02 (5)	0.40 ± 0.10 (5)*	0.21 ± 0.04 (5)	0.17 ± 0.03 (4) †††

Statistical significance of differences between before and after operation shown * where $p < 0.05$ ** where $p < 0.02$ and *** where $p < 0.01$.

Statistical significance of differences between values for cancer patients and that of patients with hiatus hernia shown † where $p < 0.02$;†† where $p < 0.01$ and ††† where $p < 0.002$.

3. DISCUSSION

The first indication of the abnormality in the metabolism of carbohydrate in cancer patients came from the studies by Glicusman and Rowson (1956) who performed glucose tolerance tests (GTT) in a large group of patients with different kinds of malignant tumour at different sites. Abnormal GTT were found in 37% of the patients with cancer in contrast to 9% of patients with benign diseases. In another study, using intravenous GTT, Marks and Bishop (1957) reported that patients with carcinoma of cervix, breast and with lukaemia and lymphoma showed a decrease in the fractional rate of glucose disappearance despite normal fasting blood sugar. More recent measurements, however, using ^{14}C labeled glucose (Waterhouse and Kemperman, 1971) failed to find any significant difference in the rate of glucose utilisation between cancer patients and normal subjects.

In the present study the pre-operative fasting level of blood glucose was similar in cancer patients and in patients with hiatus hernia and the levels in all patients were within the normal range. The pre-operative concentrations of plasma insulin and 11-hydroxy-corticosteroids were also similar in all patients. The findings of normal hydroxycorticosteroid concentrations was perhaps surprising in view of the fact that many extra-pituitary tumours, especially those of the lung sometimes produce a large amount of ACTH with values as high as 10,000 pg/ml. (normal level 100 pg/ml.) being reported in patients with bronchial carcinomas (Laurence and Neville, 1976).

An increased excretion of ketone bodies in the urine has been reported in a number of pathological conditions mainly associated with abnormal carbohydrate metabolism (Bondy and Felig, 1974). In the present study it was found that before surgery, patients with oesophageal cancer excreted a larger amount of ketone bodies per Kg body weight than patients with hiatus hernia. However, since the rate of excretion in lung cancer patients was similar to that in patients with hiatus hernia, the elevated level of excretion of ketone bodies in patients with oesophageal cancer would seem to be an indirect, rather than a direct, effect of the malignant tumour. It may in fact be attributed to malnutrition in these patients as it is known that starvation leads to high production of ketone bodies (Foster, 1967).

As to the effect of injury and surgical operation on carbohydrate metabolism, it is now well established that accidental injury and surgery induce a diabetic like condition associated with glucose intolerance. However, the exact mechanism of this phenomenon has not yet been defined. Insulin suppression has been implicated in the hyperglycaemia occuring shortly after injury. The observation by Allison et al (1969) that during abdominal surgery the plasma level of insulin failed to rise in response to a glucose load would support this view. It has also been shown that during abdominal operation the plasma level of sinulin falls (Russell et al, 1975). In Allison's view (Allison, 1974) insulin release following injury is suppressed by the raised level of catecholamines observed after injury and this would seem to be supported by studies in rabbits and man (Coore and Randle, 1964; Porte, Graber et al, 1966).

On the other hand more recent studies in which the effect of injury on glucose utilisation was studies, have provided evidence that glucose oxidation is not impaired in the injured subject (Kinney et al, 1970). This observation and the fact that the level of blood glucose represents a balance between depletion by peripheral utilisation and input from hepatic glycogenolysis and gluconeogenesis, led Wilmore et al (1976) to suggest that the hyperglycaemia which follows injury is a result of increased glucose production and not impaired glucose disappearance.

In the present studies the concentrations of glucose, insulin and individual amino acids were measured in the same individuals at the same time and the results showed that the post-operative hyperglycaemia occurred at the same time as the concentrations of the glucogenic amino acids fell. These changes clearly are in support of the view of Wilmore et al (1976). They did, however, occur only in patients with hiatus hernia and oesophageal cancer. In lung cancer patients negative evidence in support of Wilmore et al (1976) view found that in these patients the level of blood glucose were unchanged immediately after surgery and so too were the concentrations of glucogenic amino acids. Moreover, it is increasingly apparent that the control of blood sugar concentration is a function not only of insulin and catecholamines but also of glucagon. The main physiological role of glucagon is to increase the output of glucose from the liver (Cherrington et al, 1972), and the level of this hormone in the plasma has been shown to increase during abdominal operation (Russell et al, 1975).

In gluconeogenesis, conversion of three carbon compounds to glucose is brought about by enzymatic reactions. One of the enzymes involved in these reactions is pyruvate carboxylase, and increased synthesis of this enzyme in the liver occurs in the presence of high levels of glucagon, catecholamines and glucocorticosteroids and of low levels of insulin that is precisely the hormonal environment present during the catabolic phase of injury (Wilmore et al, 1976). Moreover, thiamin pyrophosphate (TPP) is tightly bound to pyruvate carboxylase and also to transketolase (TK) and therefore changes in the concentration of TPP in the blood would be expected to reflect the activity of these enzymes. In the current study it was possible to measure the activity of TK and also the TPP effect (Brin, Tat et al, 1960) in four patients with hiatus hernia and three with oesophageal cancer and also in one patient with lung cancer (enzyme measurements were made by Miss Elizabeth A.Drury as part of the project report required for the B.Sc. Honours Degree in Human Biology at Surrery University). The results indicated that all three patients with oesophageal cancer and the patient with lung cancer had raised TPP effects (using 15% TPP effect value as indicative of thiamin deficiency (Dreyfus, 1962)) before surgery. This observation is in agreement with that of Basu, Dickerson, Reven and Williams (1974) showing a high incidence of thiamin deficiency amongst patients with advanced malignant disease.

Two of the patients with hiatus hernia also had TPP effects above the 15% level, thus indicating that they too were deficient in thiamin.

As regards the effect of surgery on thiamin status, it was found that immediately after operation there was a rise in the TPP effect in patients with hiatus hernia and oesophageal cancer (Fig. 4.5), whereas in the patient with lung cancer there was a fall in the TPP effect immediately after surgery. Studies by Govier and Greer (1941) and Govier (1943) in dogs and those by Levenson et al (1946) in human have also indicated a change in thiamin status following injury. Govier and Greer (1941) found that thiamin supplementation increases the survival time of drugs subjected to haemorrhogic shock.

This post-operative rise in the TPP effect could be related to an increased synthesis of pyruvate carboxylase caused by the increased secretions of glucagon and 11-hydroxycorticosteroids as a consequence of surgical stress with the result that less TPP was available for the synthesis of TK.

However, the reason for the present finding that lung operations such as pneumonectomy or lobectomy are not followed by an immediate rise in the level of blood glucose remains to be elucidated. It is possible that it is related to the operative procedure, for lung operations differ from those of the oesophagus in that they do not necessitate handling of the gastrointestinal tract and stimulation of the vagus nerve, which has been reported in man to lead to hyperglucagonaemia (Bloom et al, 1974).

As to the effects of surgical operation on the concentrations of

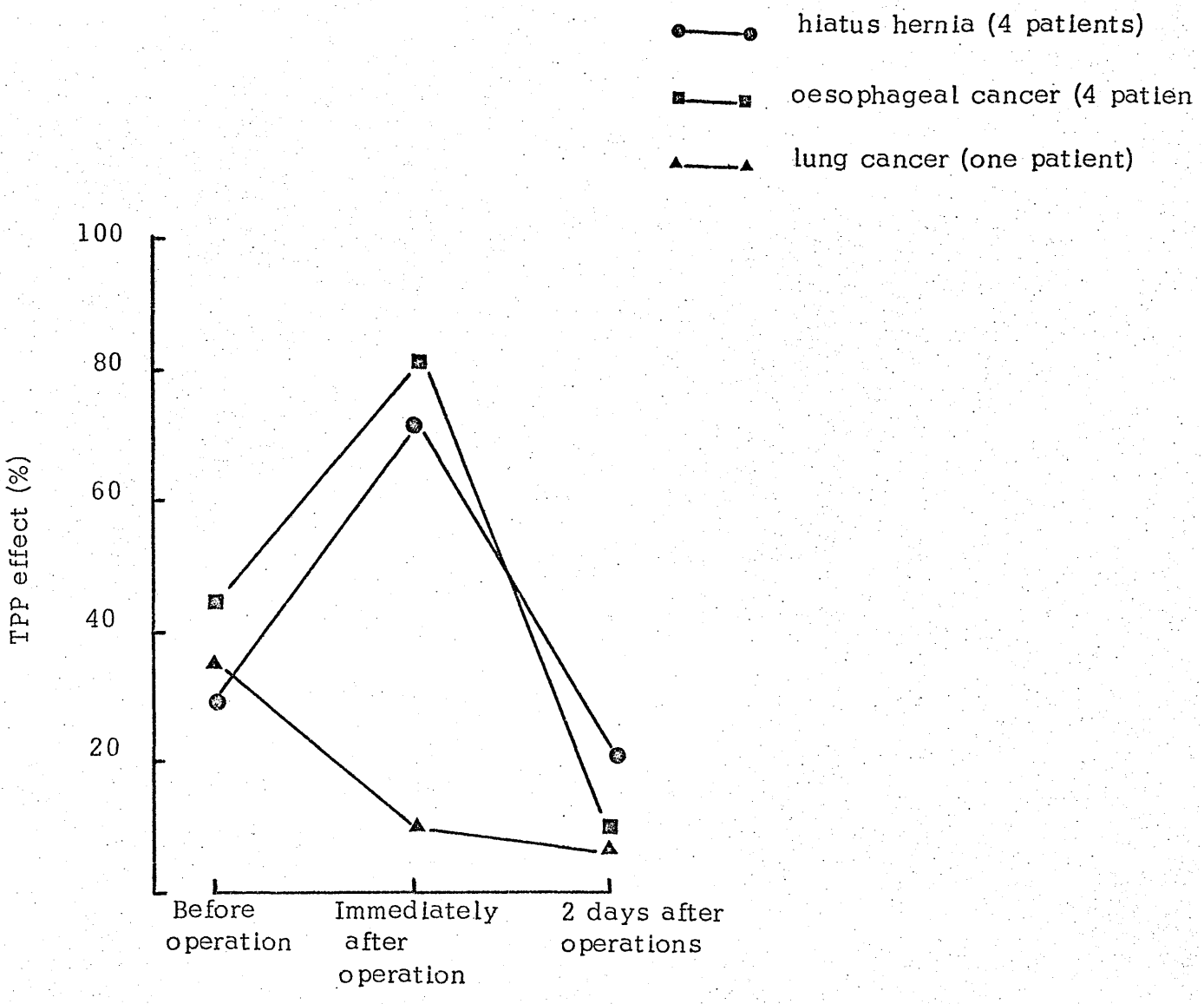


Fig. 4.5. Effect of surgery on TPP effect in patients with hiatus hernia, oesophageal cancer and lung cancer.

plasma corticosteroids, studies by Hume et al (1962) and Johnston (1964) have shown that the levels of these hormones are raised following abdominal surgery and that they reach a peak level in 2 to 4 hours. These findings have been confirmed in the present investigation as it was found that in all patients the levels of 11-hydroxycorticosteroids immediately after operation were significantly above the pre-operative values. However, whereas in hiatus hernia and oesophageal cancer patients the levels fell towards the pre-operative values by the second post-operative day, they remained raised in lung cancer patients and did not fall to their basal pre-operative values until the 7th post-operative day. Moreover, in these patients the small elevation in the blood sugar concentration seen on the second post-operative day could be attributed to the raised level of 11-hydroxycorticosteroids, as these hormones are known to enhance the rate of hepatic gluconeogenesis. Furthermore, the rise in the level of plasma FFA accompanied by a normal level of insulin which occurred immediately after operation in all patients, could be related to the elevated concentrations of 11-hydroxycorticosteroids, as these hormones by releasing the FFA are believed to furnish the signal molecules which provide the switching mechanism for decreasing glycolysis and shifting the balance of glucose breakdown and synthesis towards glucose production (Ashmore and Weber, 1968).

The finding that the level of ketone bodies rose after surgery, and at the time when insulin was at its highest level, provides further evidence in support of the idea that insulin resistance occurs after

injury. Moreover, the lower rate of excretion of ketone bodies found in lung cancer patients on the second post-operative day seems to be due to the higher level of plasma insulin found in these patients on that day.

Finally, the high levels of plasma glucose, FFA, 11-hydroxy-corticosteroids and urinary excretion of ketone bodies observed in patient GL throughout the period of study would probably indicate that this patient was suffering from diabetes pre-operatively and her state of diabetes became more severe after surgery.

CHAPTER 5

TRYPTOPHAN METABOLISM IN PATIENTS UNDERGOING SURGERY

1. INTRODUCTION

Tryptophan exists in plasma in two forms, namely in a "bound" form, reversibly associated with albumin, and as the "free" amino acid (McMenamy and Oncley, 1958). The free fraction can pass across the brain barrier and is converted to the brain neurotransmeter serotonin. It has been shown that the entry of tryptophan into the brain is controlled by the ratio of the concentration of tryptophan to the sum of the concentrations of the neutral amino acids, leucine, isoleucine, valine, tyrosine and phenylalanine, which compete with it for uptake into the brain (Fernstrom and Wartman, 1972).

In recent years there has been considerable interest in the metabolism of tryptophan in cancer patients. However, most of these studies have been done in patients with cancer of the bladder or breast (Rose, 1967; Yoshida, Brown and Bryan, 1970; Degeorge and Brown, 1970; Gailani, Murphy et al, 1973; Fahl, Rose et al, 1974). Furthermore, no studies could be found in which plasma free tryptophan has been investigated in patients suffering from malignant disease.

It seemed to be of particular interest to study the metabolism of this amino acid in patients with oesophageal cancer, since its concentration in the plasma is reduced markedly by a reduced food intake and particularly by a reduced protein intake (Dickerson and Pao, 1975).

This chapter describes the pattern of plasma free, and total tryptophan and the ratio of free tryptophan to other plasma neutral

amino acids in patients undergoing surgery for hiatus hernia, oesophageal cancer and lung cancer. Urinary excretory levels of 5 hydroxyindolacetic acid (5HIAA), the end product of the hydroxy indole pathway (Fig. 5.1) and the excretory levels of N^1-methyl-nicotinamide (NMN), the end product of the kynurenine pathway, have also been investigated in these patients.

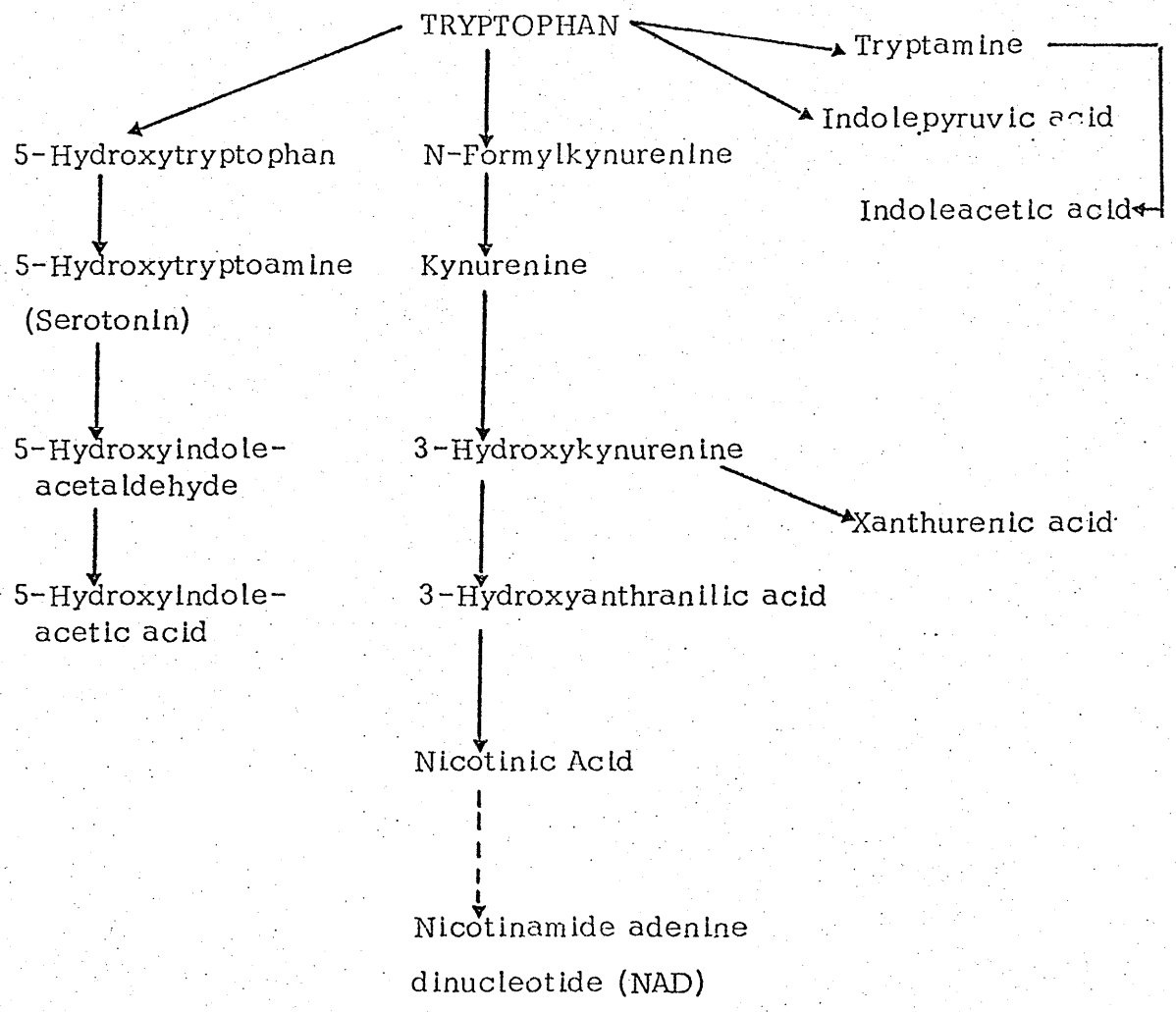


Fig. 5.1. Catabolic Pathways for tryptophan in man. The pathway from tryptophan to nicotinic acid and NAD represents the major degradative route.

2. RESULTS

The pre-operative concentrations of plasma total tryptophan were similar in cancer patients and patients with hiatus hernia (Table 5.1). In all patients the level fell immediately after operation and whereas in hiatus hernia and oesophageal cancer patients it returned to the pre-operative level by 7 and 14 days after operation respectively, it failed to do so in patients with lung cancer and remained low throughout the post-operative period.

The pre-operative levels of plasma free tryptophan (Table 5.2) in cancer patients were significantly lower than those in patients with hiatus hernia. In all patients the levels showed a tendency to rise immediately after operation and since the total tryptophan concentration was low at this time, the ratio of free to total tryptophan rose considerably (Fig. 5.2). Furthermore, in patients with hiatus hernia, but not in cancer patients, free tryptophan concentrations increased considerably, between 7 and 14 days after operation.

The ratio of free tryptophan to other neutral amino acids (Table 5.2) in cancer patients tended to be lower than those in hiatus hernia patients and on the fourteenth post-operative day the levels in cancer patients were about 30% of those in hiatus hernia patients. The pre-operative urinary excretion of NMN was similar in cancer patients and in patients with hiatus hernia (Table 5.3). After operation, the levels in cancer patients remained essentially unaltered except for a high value in lung cancer patients on the seventh post-operative

day. On this day the excretion in lung cancer patients was significantly higher than in the hiatus hernia patients whereas on the second and fourteenth day the values in hiatus hernia patients were higher than in lung cancer patients. Furthermore, in hiatus hernia and lung cancer patients, there was a positive correlation between plasma free tryptophan concentrations and the urinary excretion of NMN (Fig. 5.3 and 5.4). No such relationship was found in oesophageal cancer patients (Fig. 5.5).

The pre-operative concentrations of urinary 5HIAA in cancer patients were slightly higher than those in patients with hiatus hernia (Table 5.4) although the differences were not statistically significant. At 2 days after operation the level in oesophageal cancer patients, but not in the other two groups was higher than before operation and returned to the pre-operative value by 14 days after. Between 7 and 14 days after surgery, the levels in hiatus hernia patients but not in the cancer patients, fell markedly and were significantly lower than those in cancer patients at the fourteenth day.

Table 5.1.

The concentration of total tryptophan in the plasma (μM) in patients undergoing surgical operation for hiatus hernia, oesophageal cancer and lung cancer.

values are mean ± SEM Number of patients shown in parentheses

Patient	Before Operation	Immediately after Operation	Time after Operation (day) 2	7	14
Hiatus hernia(10)	54.1 ± 5.3	34.2 ± 4.0***	41.6 ± 4.2**	45.2 ± 6.4	61.2 ± 10.8
Oesophageal cancer (11)	51.8 ± 3.3	34.7 ± 2.9***	39.3 ± 4.6*	39.3 ± 3.7*	49.2 ± 9.7
Lung cancer (8)	51.5 ± 2.9	34.4 ± 3.0***	46.6 ± 4.6	38.5 ± 1.8***	40.2 ± 3.2***

Statistical significance of differences between values of before and after operation are shown * where $p < 0.05$ ** where $p < 0.01$ and *** where $p < 0.01$.

The concentration of free tryptophan in the plasma and its ratio to the sum of the plasma concentrations of neutral amino acids (tyrosine, valine, isoleucine and leucine) in patients undergoing surgical operations for hiatus hernia, oesophageal cancer and lung cancer.

values are means ± S.E.M. Number of patients shown in parentheses.

Patient	Amino acid μmol/l plasma	Before Operation	During Operation	After operation (days) 2	7	14
HH (7)	Tryptophan	11.3 ± 0.8	13.8 ± 2.0	10.2 ± 1.8	9.8 ± 1.2	20.2 ± 2.5††
	Tyr + val + iso + leu	532 ± 28	413 ± 62	500 ± 57	527 ± 33	428 ± 18
	Ratio x 100	2.1 ± 0.1	3.5 ± 0.7	2.1 ± 0.2	1.9 ± 0.2	4.7 ± 0.5††
OC (8)	Tryptophan	7.3 ± 2.9	10.4 ± 1.4	9.5 ± 1.1	14 ± 1.2	8.4 ± 1.5 **
	Tyr + val + iso + leu	546 ± 59	507 ± 53	515 ± 79	606 ± 90	607 ± 72
	Ratio x 100	1.4 ± 0.3	2.3 ± 0.4	2.2 ± 0.4	2.5 ± 0.4	1.5 ± 0.4**
LC (6)	Tryptophan	6.7 ± 0.6 ***	8.5 ± 0.5 *	9.2 ± 0.7†	9 ± 0.9†	7.6 ± 0.4 **
	Tyr + val + iso	555 ± 47	465 ± 48	532 ± 27	645 ± 76	489 ± 37
	Ratio x 100	1.2 ± 0.1 ***	1.7 ± 0.1	1.7 ± 0.1	1.4 ± 0.2	1.6 ± 0.2 **

Statistical significance of differences between cancer patients and patients with hiatus hernia shown * where $p < 0.05$, ** where $p < 0.01$ and *** $p < 0.001$.

Statistical significance of differences between before and after operation shown † where $p < 0.05$, †† where $p < 0.01$ and ††† where $p < 0.001$.

HH - Hiatus Hernia OC - Oesophageal Cancer LC - Lung Cancer

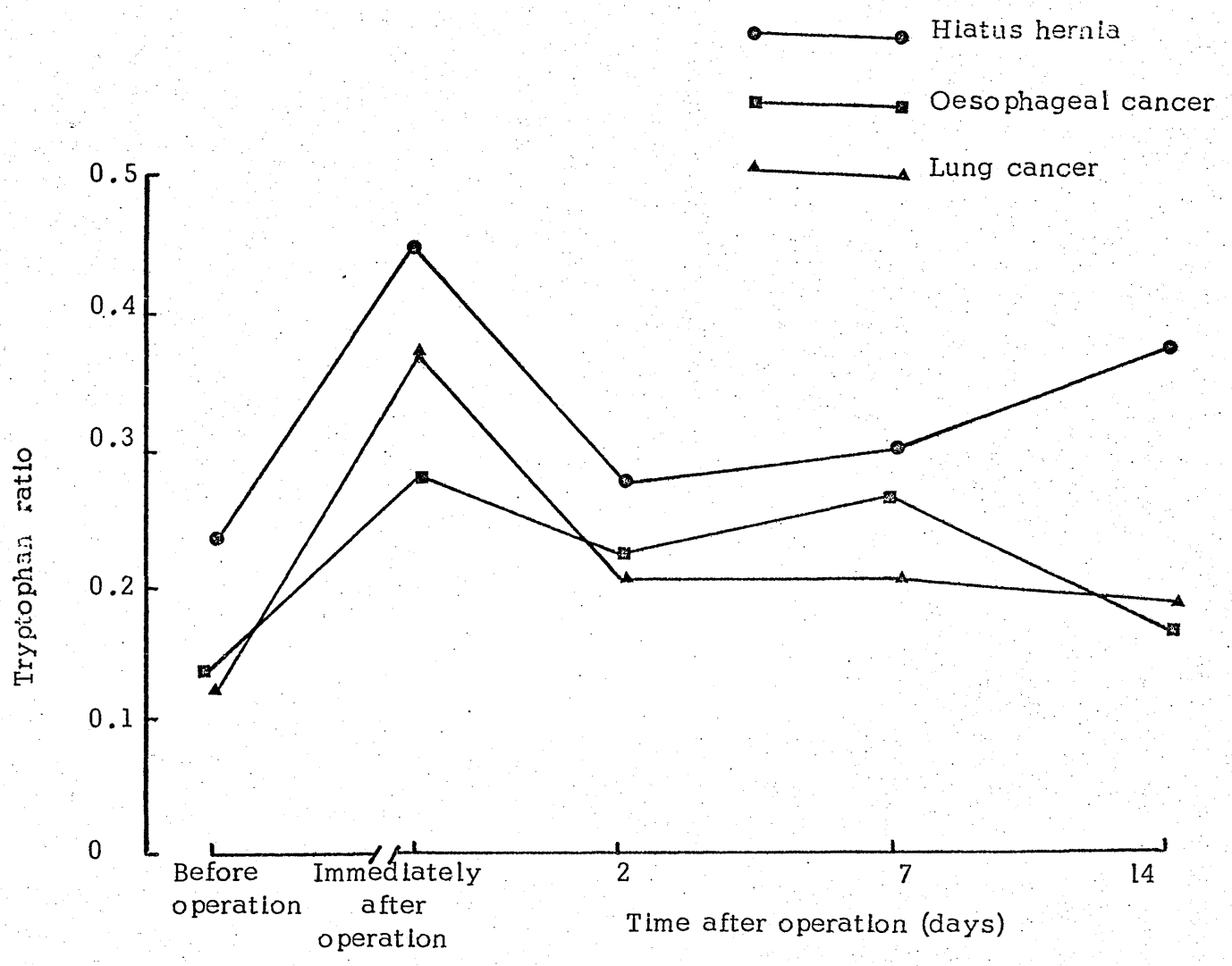


Fig. 5.2. Effect of operation on the ratio of free tryptophan to total tryptophan in patients with hiatus hernia, oesophageal cancer and lung cancer.

Table 5.3. Urinary excretion of N^1 methylnicotinamide (mg/g creatinine) in patients undergoing surgical operation for hiatus hernia, oesophageal cancer and lung cancer.

values are mean ± SEM Number of patients shown in parentheses

Patient	Before Operation	Time after operation (day) 2	7	14
Hiatus hernia	3.6 ± 0.6 (9)	4.8 ± 1.8 (9)	3.7 ± 0.9 (6)	13.1 ± 2.6 (6)**
Oesophageal cancer	5.3 ± 0.8 (7)	8.8 ± 2.8 (9)	4.5 ± 0.8 (7)	7.6 ± 1.7 (7)
Lung cancer	4.3 ± 0.9 (6)	2.7 ± 0.4 (6)	9.2 ± 2.2 (6)* †	5.6 ± 1.2 (4) †

Statistical significance of differences between values of before and after operation shown * where $p < 0.05$ and ** where $p < 0.01$.

Statistical significance of differences between values for cancer patients and that of patients with hiatus hernia shown † where $p < 0.05$.

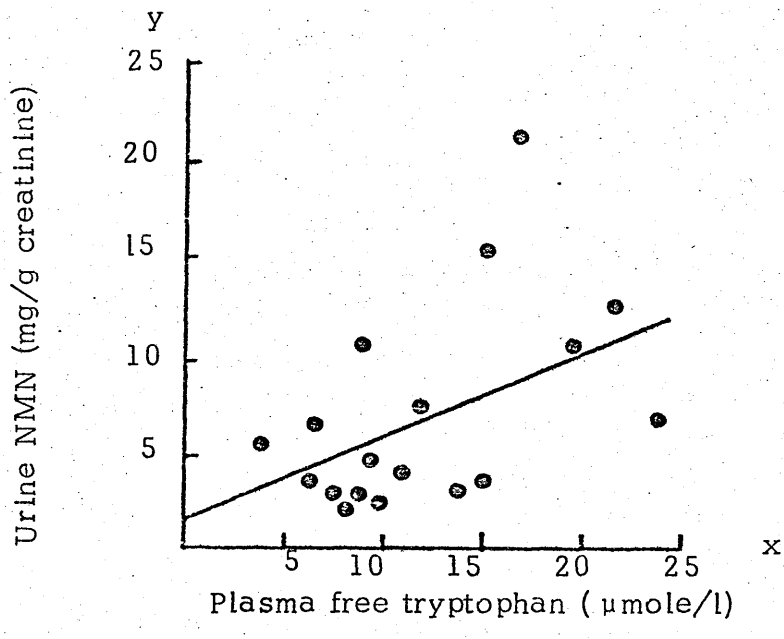


Fig. 5.3. Variation of urine NMN with plasma free tryptophan in patients undergoing surgery for hiatus hernia.

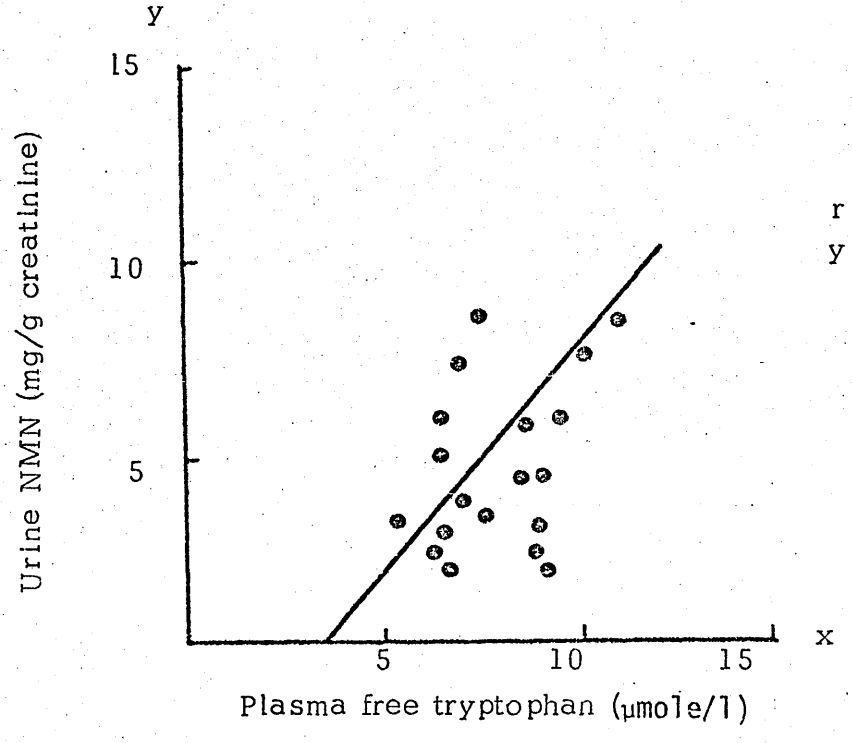


Fig. 5.4. Variation of urine NMN with plasma free tryptophan in patients undergoing surgery for lung cancer.

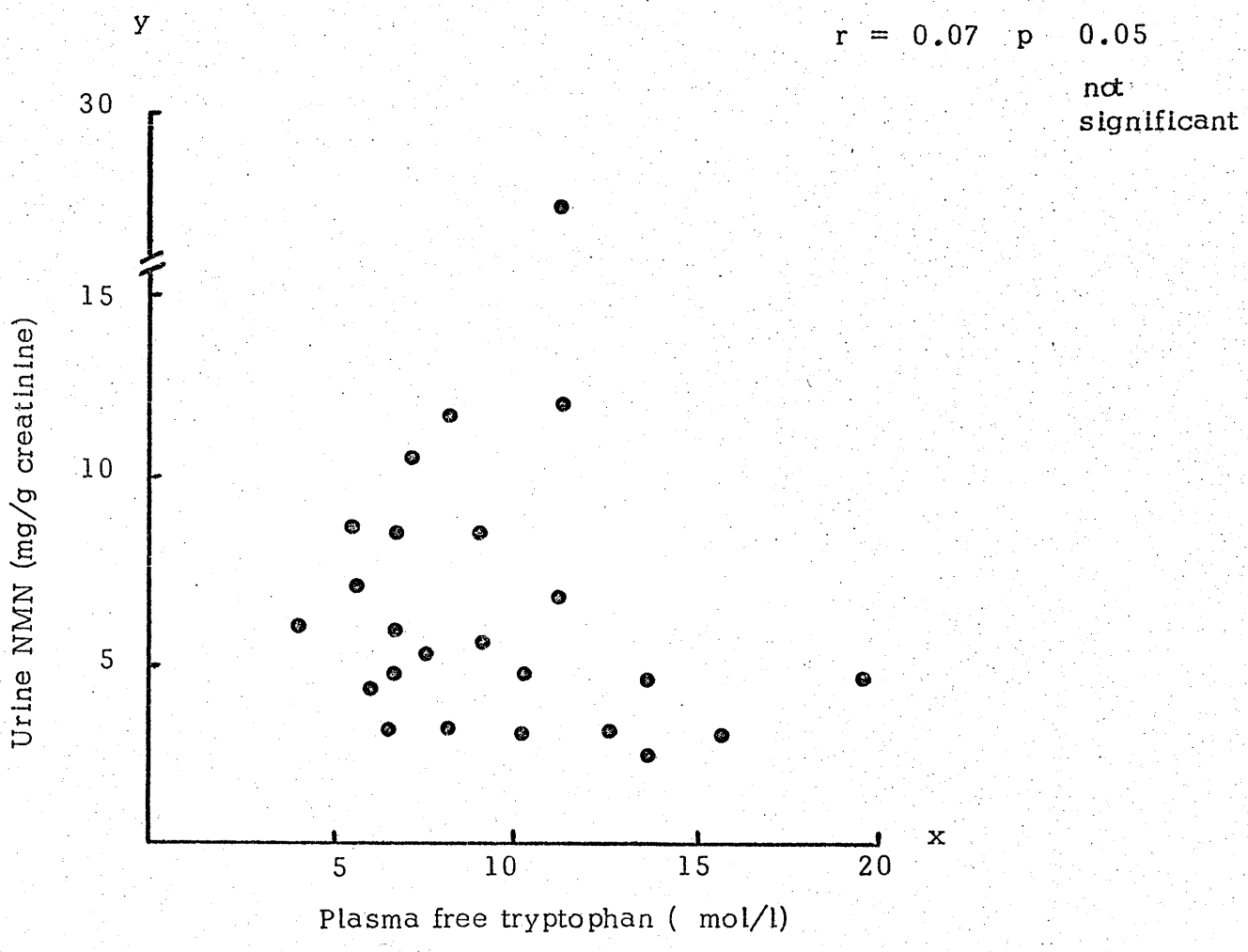


Fig. 5.5. Variation of urine NMN with plasma free tryptophan in patients undergoing surgery for oesophageal cancer.

Table 5.4.

Urinary excretion of 5-Hydroxyindolacetic acid (mg/g creatinine) in patients undergoing surgical operation for hiatus hernia, oesophageal cancer and lung cancer.

Values are mean ± SEM Number of patients shown in parentheses

Patient	Before operation	Time after operation (day) 2	7	14
Hiatus hernia	2.4 ± 0.4 (8)	2.6 ± 0.6 (9)	2.0 ± 0.7 (9)	0.9 ± 0.2 (5)**
Oesophageal cancer	3.8 ± 0.7 (8)	6.1 ± 0.8 (9)*†	4.7 ± 1.0 (6)	3.7 ± 1.0 (5) ††
Lung cancer	3.2 ± 0.4 (6)	4.2 ± 0.3 (6)	3.1 ± 0.5 (7)	4.5 ± 1.5 (4)

Statistical significance of differences between values of before and after operation shown * where $p < 0.05$ and ** where $p < 0.01$

Statistical significance of differences between values for cancer patients and that of patients with hiatus hernia shown † where $p < 0.05$ and †† where $p < 0.01$.

3. DISCUSSION

The pre-operative concentration of plasma free tryptophan but not that of total tryptophan was lower in cancer patients than in patients with hiatus hernia. Amongst the factors which affect the albumin-binding of tryptophan in plasma, insulin has been shown to increase binding (Lipsett, Madras, Wartman and Munro, 1973). However, in the present study the pre-operative levels of insulin in cancer patients were comparable to those in patients with hiatus hernia and the low free tryptophan concentrations found in these patients could thus not be attributed to insulin. On the other hand, it has been shown that in patients with malignant tumours a greater proportion of tryptophan is metabolised by way of the hydroxyindole pathway (Sjoedsma, Weissbach and Udenfriend, 1956). On this evidence it is conceivable that in these patients more free tryptophan is taken up by the tumour to be used for 5HIAA synthesis. This possibility is strengthened by the finding that the urinary excretion of 5HIAA tended to be higher in cancer patients than in patients with hiatus hernia.

As far as the effect of surgery on plasma tryptophan is concerned, the present results show that immediately after surgery, tryptophan is released from binding sites on albumin and this together with the reduction in total tryptophan at this time resulted in a marked rise in the ratio of free to bound tryptophan. Studies by Curzon, Friedel

and Knott (1973) in the rat and those by Curzon, Friedel et al, (1974) in man, have shown that plasma free fatty acids (FFA) reduce the binding affinity of albumin for tryptophan, so that increases in plasma FFA concentrations are accompanied by a rise in the fraction of tryptophan in the free state. More recently Gentil, Lader et al (1977) reported that subcutaneous injection of adrenaline into normal subjects caused large increases of plasma FFA and free tryptophan, associated with a fall in the concentration of plasma total tryptophan.

In the light of these observations, it would appear that the immediate post-operative changes of plasma tryptophan are the result of elevated levels of plasma FFA caused by increased secretion of cathecholamines. The possibility that plasma FFA plays such a role is strengthened by the finding that low levels of plasma total tryptophan observed immediately after surgery were accompanied by high concentrations of plasma FFA (Fig. 5.6) and cathecholamine secretions are also known to increase after injury (Johnston, 1974). The mechanism by which plasma total tryptophan is lowered is yet to be found. Curzon and Knott (1975) have suggested that the fall in plasma total tryptophan caused by an increased level of plasma FFA results from a shift of the newly freed tryptophan into intracellular compartments other than the brain, because rapid transient changes of plasma FFA which occur as a result of brief stress do not appear to cause comparable brain tryptophan changes. Consistent with this view, the present results show that the immediate post-operative fall in the concentration of total tryptophan is also accompanied by

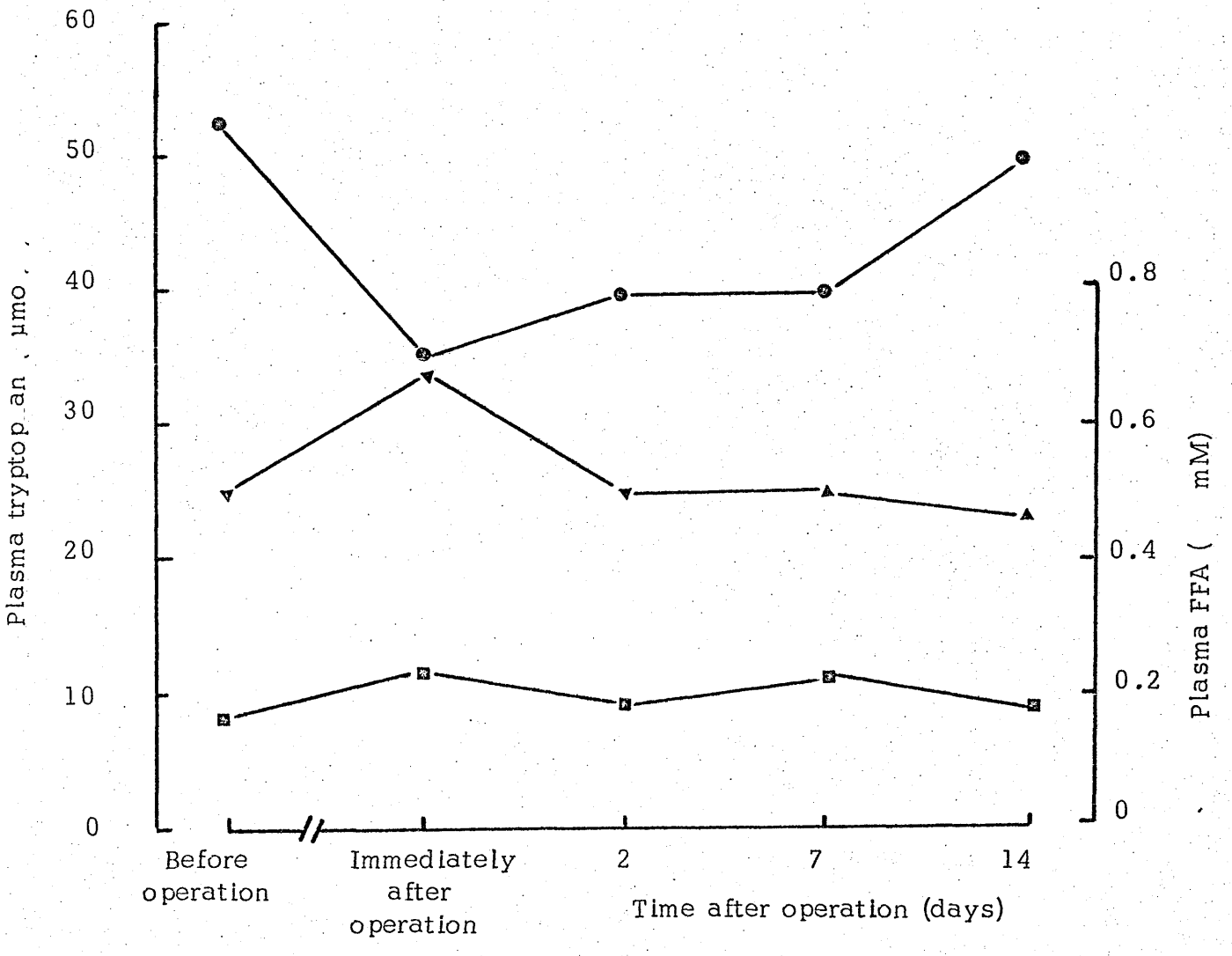


Fig. 5.6. Effect of operation on the concentrations of plasma total tryptophan ⊙—⊙ free tryptophan ■—■ and FFA ▲—▲ in patients with oesophageal cancer.

high level of blood glucose (Chapter 4), and this may suggest that plasma tryptophan is taken up by the liver in the process of gluconeogenesis to be used for glucose synthesis. On the other hand, the finding that in lung cancer patients the immediate post-operative fall in plasma total tryptophan was not accompanied by elevated blood glucose is not compatible with this view.

The ratio of plasma free tryptophan to plasma neutral amino acids was lower in cancer patients than in patients with hiatus hernia. It has been shown that in the rat there is a direct relationship between plasma free tryptophan and the level of this amino acid in the brain (Knott and Curzon, 1972; Tagliamonte, Biggio, Vargiu and Gessa, 1973). It has also been reported that increased level of plasma tryptophan is associated with an increased rate of serotonin synthesis in the brain (Fernstrum and Wurtman, 1971). These workers have also found that serotonin synthesis in the brain is not controlled by plasma tryptophan alone, but by the ratio of this amino acid to the sum of other plasma neutralmino acids that compete with it for uptake into the brain (Fernstrum and Wurtman, 1972). Based on these observations, it is therefore, tempting to suggest that the cancer patients studied in the present investigation had a low concentration of serotonin in their brains. Moreover, in so far as the concentrations of plasma amino acids that compete with tryptophan uptake into the brain remained unchanged following surgery while that of tryptophan fell, this suggests that immediately after operation the rate of tryptophan uptake by the brain falls and that this leads to decreased synthesis of brain serotonin.

Between 7 and 14 days after operation, the level of plasma free tryptophan in patients with hiatus hernia, but not in cancer patients, rose considerably. This high level of plasma tryptophan would appear to be the cause of the high urinary excretion of NMN observed in these patients on the same day as there was a significant positive correlation between plasma free tryptophan and urinary excretion of NMN in these patients. Some correlation was also found in lung cancer patients, but no correlation could be found in oesophageal cancer patients.

In view of the fact that under normal circumstances, tryptophan is mainly metabolised through the kynurenine pathway leading to the excretion of NMN and only a small percentage is metabolised through the indolacetic acid pathway, it appears that while the pathway of tryptophan metabolism remains unaltered in hiatus hernia and lung cancer patients, it may be changed in patients with oesophageal cancer. Abnormal tryptophan metabolism associated with increased secretion of a number of tryptophan intermediary metabolites has been reported in patients with bladder cancer (Rose, 1967). It has also been reported that the rate of urinary excretion of NMN was lower and that of 5HIAA was higher in cancer patients as compared with normal subjects (Basu, Raven, Bates and Williams, 1973). Rose (1967) suggested that the increased levels of tryptophan metabolites observed in patients with bladder cancer was due to the induced activity of β-glucoronidase which hydrolyses 3-hydroxyanthranilic acid glucosiduronate in the urine. On the other hand, several enzymes of the kynurenine pathway

involved in the conversion of tryptophan to NMN are pyridoxal phosphate dependent. Since patients with oesophageal cancer are often malnourished as a result of low food intake, it is tempting to suggest that perhaps in these patients the activities of enzymes responsible for the conversion of tryptophan to NMN are impaired due to sub-normal intake of pyridoxine.

CHAPTER 6

URINARY EXCRETIONS OF CYCLIC NUCLEOTIDES IN PATIENTS UNDERGOING SURGERY

1. INTRODUCTION

Since the discovery of cyclic 3'5' adenosine monophosphate (cyclic AMP) by Sutherland, Rall et al in 1958, many studies have been undertaken to investigate its role and biological significance in cellular metabolism.

It was soon established that cyclic AMP acts as an intracellular mediator for adrenaline and glucagon stimulation of glycogenolysis in liver cells (Sutherland and Rall, 1960). Since then it has become clear that cyclic AMP functions as an intracellular messenger which communicates the stimuli of a great many hormones in mammalian and non-mammalian animal tissues (Robison et al, 1971).

Cyclic 3'5'-guanosine monophosphate (cyclic GMP) was first identified in urine by Ashman and colleagues in 1963 following the administration of ^{32}P inorganic phosphate to rats. Unlike cyclic AMP cyclic GMP was not identified as part of a hormone stimulated pathway, and when it was first discovered, nothing was known of its significance in cellular metabolism. However, in more recent years cyclic GMP has become the subject of considerable interest as an alternative "second messenger" to cyclic AMP for the action of some hormones. There is now evidence that cholinergic and α-adrenergic stimulation and the action of certain peptide hormones such as cholecystokinin, histamine and possibly insulin are mediated by an increase in the intracellular level of cyclic GMP rather than of cyclic AMP (Nature, 1973).

In an attempt to establish clinical applications for cyclic nucleotide analyses both cyclic AMP and cyclic GMP have been measured in physiological fluids under a variety of different pathological conditions. Chase, Melson and Aurbach (1969) were the first to report an alteration in cyclic nucleotide metabolism in human disease. Patients with pseudohypoparathyroidism were found to have less than a four-fold increase in their urinary cyclic AMP excretion after the intravenous administration of parathyroid hormone. Normal subjects and patients with hyperparathyroidism all gave responses which were greater than those of the pseudohypoparathyroidism group.

Urinary cyclic AMP excretion has been reported to be increased in mania and decreased in depressive illness (Abdulla and Hamadah, 1970; Paul, Cramer and Goodwin, 1972).

The metabolism of cyclic nucleotides in malignancy has also been investigated in recent years. Criss and Murad (1976) have reported an increased urinary excretion of cyclic GMP in rats bearing transplantable liver and kidney tumours, and Neethling and Shanley (1976) found that the excretion of cyclic GMP in three patients with primary hepatoma was significantly higher than that in 24 normal subjects.

An increase in the concentration of cyclic AMP in the plasma and in urine has been reported to follow injury. Thus Gill, Prudhoe et al (1975) in a study involving 7 general surgical patients found a

consistent rise in the level of plasma cyclic AMP during operation with the levels falling back almost to normal within six hours. In another study involving 150 patients with severe accidental trauma, Vitek et al (1976) observed an increased urinary excretion of cyclic AMP during the first 24 hours and continuing for approximately 5 days after injury. However, to date no study could be found in the medical literature in which the urinary output of cyclic GMP was investigated after surgery. Such a study was undertaken during the course of the work described in this Thesis.

Patients and Methods

The patients used in this study were those described in Chapter 2.

Urinary concentrations of cyclic AMP and cyclic GMP were measured by Dr. Peter Wood of the Division of Chemical Pathology, Southampton General Hospital, using a radioimmunoassay technique.

2. RESULTS

There was no significant difference between the excretion of the cyclic nucleotides per g creatinine in the urine from the three groups before surgery (Tables 6.1 and 6.2). After operation, the cyclic GMP levels showed a tendency to be higher in the cancer patients than in the hiatus hernia patients, although this trend only achieved statistical significance for oesophageal cancer patients 14 days after operation. Post-operative cyclic AMP excretions in cancer patients were not significantly different from those in hiatus hernia although again there was a trend towards higher levels in the oesophageal cancer group 2 and 14 days after surgery.

When pre- and post-operation cyclic nucleotide excretions were compared (Fig. 6.1), statistically significant increases were observed in the levels of cyclic GMP but not cyclic AMP in both groups of cancer patients on the second and seventh post-operative day. This was in contrast to the levels in hiatus hernia patients which showed an only slight and not statistically significant rise on the same days.

Table 6.1.

The concentration of cyclic AMP (μmole/g creatinine) in the urine of patients undergoing surgery for hiatus hernia, oesophageal cancer and lung cancer.

Values are mean $\pm$ SEM for the number of patients shown in parantheses

Patients	Before Operation	Time after operation (day) 2	7	14
Hiatus hernia	2.8 $\pm$ 0.2 (10)	3.1 $\pm$ 0.5 (10)	2.6 $\pm$ 0.4 (10)	2.5 $\pm$ 0.6 (7)
Oesophageal cancer	2.8 $\pm$ 0.5 (9)	4.3 $\pm$ 1.0 (9)	2.9 $\pm$ 0.5 (9)	3.6 $\pm$ 0.9 (5)
Lung cancer	2.8 $\pm$ 0.5 (7)	2.8 $\pm$ 0.2 (7)	2.8 $\pm$ 0.3 (6)	3.1 $\pm$ 0.1 (4)

Table 6.2.

Concentration of cyclic GMP (μ mole/g creatinine) in the urine of patients undergoing surgery for hiatus hernia, oesophageal cancer and lung cancer.

Values are mean ± SEM of the number of patients shown in parantheses

Patient	Before Operation	Time after operation (day) 2	7	14
Hiatus hernia	0.38 - 0.1 (10)	0.79 - 0.3 (10)	0.73 - 0.2 (10)	0.26 -
Oesophageal cancer	0.55 - 0.1 (9)	1.47 - 0.2 (9)***	1.05 - 0.1 **	0.88 - 0.1 (5) †
Lung cancer	0.54 - 0.1 (7)	0.96 - 0.2 (6)*	1.15 - 0.3***	0.7 - 0.3 (4) †

Statistical significance of differences between values of before and after operation show * where $p < 0.05$, ** where $p < 0.02$ and *** where $p < 0.01$.

Statistical significance between values for cancer patients and that of patients with hiatus hernia shown † where $p < 0.01$.

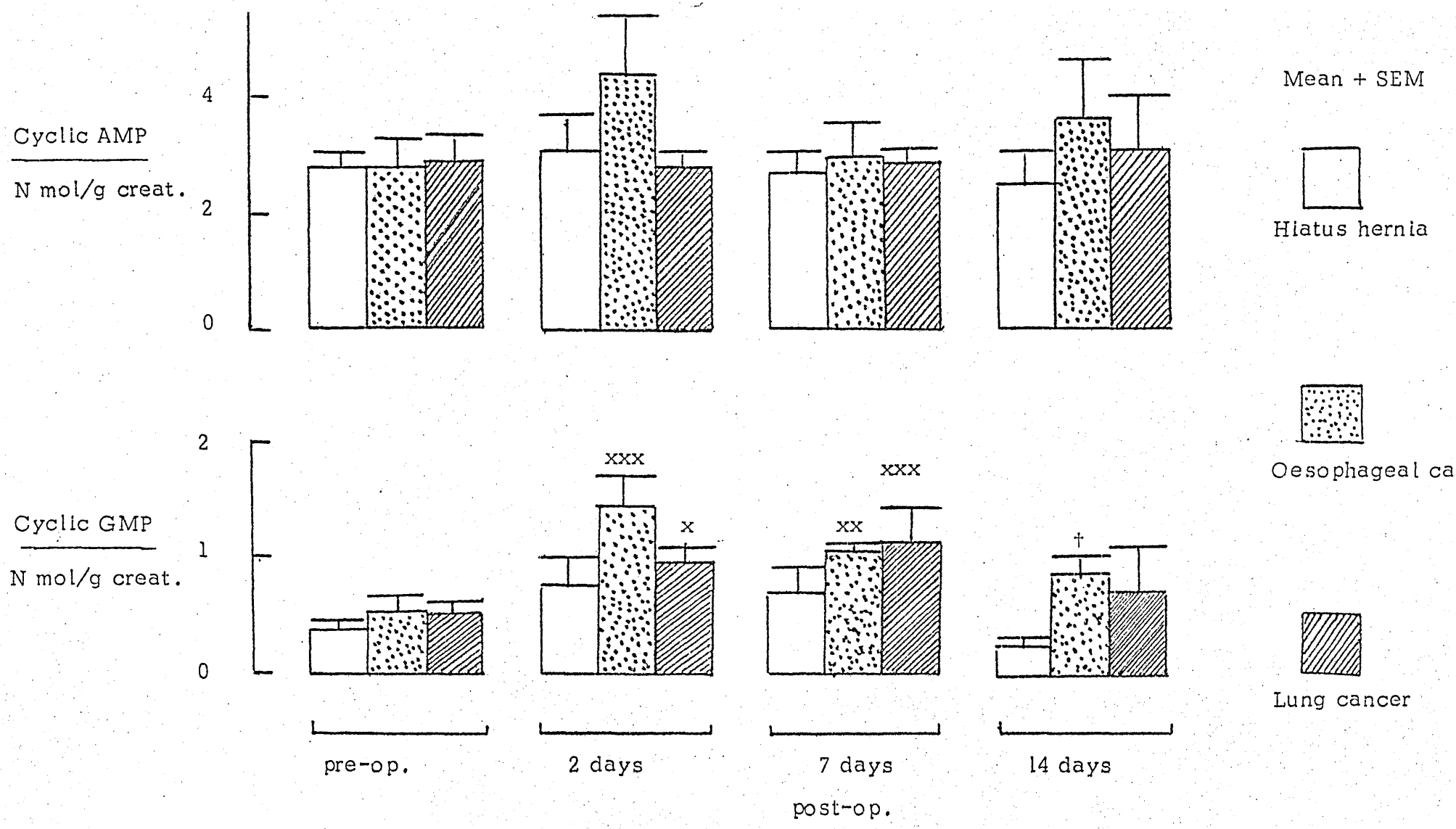


Fig. 6.1. Pre- and post-operative cyclic nucleotide excretion in patients with hiatus hernia, oesophageal cancer and lung cancer. Statistical significance of differences between values of before and after operation shown x where $p < 0.05$, xx where $p < 0.02$ and xxx where $p < 0.01$. Statistical significance of difference between values for cancer patients and that of patients with hiatus hernia shown † where p

3. DISCUSSION

The urinary excretion of cyclic GMP in normal subjects is of the order of 1 mol/day or 0.5 mol/g creatinine, and is about one fifth of the normal cyclic AMP output (Steiner et al, 1972). It has also been shown that in a normal subject about one half of the basal urinary cyclic AMP excretion is derived from the glomerular filtration of plasma and the remainder is contributed mainly by the proximal nephron under the influence of PTH (Broadus et al, 1970).

The results presented in this study showed that the rate of excretion of cyclic nucleotides in all patients was within the normal range pre-operatively, and the values found in patients with tumours of the oesophagus or lung were not different from those found in the control (hiatus hernia) group before surgery. These findings thus agree with those of Bershtein et al (1976) who reported no significant differences in the excretion of cyclic AMP in patients with breast or lung cancer and in control subjects. To date, there are no reports in the literature of cyclic GMP excretion in patients with lung or oesophageal tumours. In contrast to the report of increased urinary cyclic GMP excretion in primary hepatoma patients (Neethling and Shanley, 1976) the results of this investigation suggest that the presence of oesophageal or lung cancer is not associated with altered excretion of cyclic nucleotides.

The excretion of cyclic GMP increased after surgery and was higher

in patients with malignancy than in patients with non-malignant disease. In view of the fact that surgery for cancer of the oesophagus or lung involves procedures that are more prolonged and severe than the operation for the repair of hiatus hernia, the higher cyclic GMP output observed after surgery in cancer patients may reflect a greater degree of surgical stress in these patients. This view is reinforced by the finding that on the second post-operative day the level of 11-hydroxycorticosteroids in lung cancer patients was significantly above the basal pre-operative value (Chapter 4). However, plasma 11-hydroxycorticosteroid levels were not significantly increased in the oesophageal cancer group on the second or subsequent days after operation. Gill et al (1975) reported that in one patient prolonged elevation of cyclic AMP levels were associated with a continued elevation of plasma cortisol.

Vitek et al (1976) measured the excretion of cyclic AMP daily after surgery and found a rise on the first and fifth days only. It would appear, therefore, that the excretion is intermittent and that this may account for the fact that no such rise was found in the present study in which measurements were made on days 2, 7 and 14 after surgery. The fact that cyclic GMP excretion was elevated on these days indicates that the post-operative excretion of this cyclic nucleotide may be more prolonged or may occur later, than rises in cyclic AMP excretion.

CHAPTER 7

EFFECTS OF SURGERY ON PLASMA PANCREATIC GLUCAGON

1. INTRODUCTION

Glucagon has a hyperglycaemic action resulting primarily from stimulation of hepatic glycogenolysis. In addition, studies with perfused liver have demonstrated that glucagon also enhances gluconeogenesis as indicated by increased incorporation of amino acids into glucose (Garcia, et al, 1966).

Observations in intact man also support a glucogenic effect as evidenced by increased hepatic uptake of amino acids (Kibler, Taylor and Mayers, 1964) and a fall in serum amino acid levels (Marliss et al., 1970).

The development of a radioimmuno assay technique for the measurement of plasma pancreatic glucagon has made it possible to study the glucagon response to stress due to different pathological condition. An increased plasma level of glucagon has been reported in patients with burn injuries (Wilmore et al, 1974b) and in patients with bone fracture (Meguid et al, 1973).

Plasma glucagon changes in patients undergoing surgical operation have also been studied. In this connection, Russell et al (1975) reported that plasma glucagon rose during abdominal surgery. In contrast, Giddings et al (1975) studying a heterogenous group of patients undergoing different kinds of operation found no change in glucagon concentration during operation. The discrepancy between the findings in these two studies seems to be related to the surgical

procedures involved. This view is strengthened by the finding in the earlier part of the current studies (Chapter 4) that lobectomy and pneumonestomy unlike oesophagectomy and oesophagagastrectomy did not give rise to hyperglycaemia immediately after surgery. It was postulated that this difference in blood glucose response to operation was related to the surgical procedure employed for lung operations differ from those of the oesophagus in that they do not necessitate handling of the gastrointestinal tract and stimulation of the vagous nerve which has been reported to lead to hyperglycaemia (Bloom et al, 1974).

In order to examine this hypothesis the present study was undertaken and plasma glucagon response to surgery in the two different groups of patients, namely oesophageal cancer and lung cancer, was investigated.

Patients and Method

Eight patients, four with oesophageal cancer and four with lung cancer, who were admitted to the Milford Chest Hospital for surgical treatment were studied for 14 days. Clinical details of the patients studied are presented in Table 7.1. The timing and intervals of the blood sample collection were the same as described earlier in this thesis (Chapter 2.). To avoid the degredation of glucagon, blood (10 ml) was drawn into the heparinized tube, into which 0.5 ml of trasylol, a proteinase inhibitor, had been added. Plasma was quickly separated

Table 7.1.

Patients and surgical treatment - post-operative glucagon study

Patient	Sex	Age (years)	Body Weight (Kg)	Diagnosis	Treatment
			OESOPHAGEAL CANCER		
BT	male	59	64	Carcinoma of oesophagus	Oesophagectomy
AC	"	65	55	" "	"
SJ	"	68	56	" "	Oesophago-gastrecton
WW	female	57	62	" "	Oesophagectomy
			LUNG CANCER		
HA	male	56	77	Adenocarcinoma of the lung	Lower lobectomy
RH	"	70	62	Epidermoid carcinoma of the lung	Radical pneumonecton
FM	female	64	57	Carcinoma of the left main branches	Radical pneumonecton
GD	male	64	70	Carcinoma of the lung	Middle and lower lobe

by centrifugation in 4°C and kept frozen until analysed. The glucagon determinations were done by Mr. D. Rsiolakis of the Biochemistry Department, University of Surrey, using a radio-immunoassay technique.

2. RESULTS

There was no significant difference in the concentration of plasma glucagon between patients with oesophageal cancer and those with lung cancer before surgery (Table 7.2). The glucagon level rose significantly in oesophageal cancer patients immediately after operation and was still elevated on the second day. The mean level on the seventh and fourteenth post-operative days in oesophageal cancer patients were less than the mean basal level, though the differences were not statistically significant. The level of plasma glucagon in the lung cancer patients did not rise immediately after surgery and in fact, the post-operative levels tended again to be less than the mean basal value.

Table 7.2.

Plasma concentration of pancreatic glucagon (pg/ml) in patients undergoing surgery for oesophageal cancer and lung cancer.

Values are mean ± SEM for the four patients of each kind

Patient	Before Operation	Immediately after Operation	After operation (day)		
			2	7	14
Oesophageal cancer	150.5 ± 16.3	316.5 ± 90.2*	277 ± 8.3**	116 ± 21	99.0 ± 24.2
Lung cancer	180.0 ± 20.2	177.5 ± 58.4	139 ± 47.7	137.3 ± 23.5	151.5 ± 41.2

Statistical significance of differences between values of before and after operation are shown * where $p < 0.05$ and ** where $p < 0.001$.

3. DISCUSSION

After an overnight fast, the normal levels of plasma pancreatic glucagon as measured by radioimmunoassay have been reported to vary from 100 pg/ml, (Unger, 1971) to 250 pg/ml (Heding, 1971). Using these values, it would appear that tumours of oesophagus or lung are probably not associated with hyperglucagonaemia.

The observation that lung operations did not lead to an immediate rise in plasma glucagon provides direct evidence in support of the suggestion made earlier in this thesis (Chapter 4), that the failure of plasma glucose and glucogenic amino acid to change after surgery in lung cancer patients might be because the vagus nerve stimulation of which produces hyperglucagonaemia is not handled during the operation. Moreover, the present study which indicates that different surgical procedures can have a different effect on plasma glucagon, would seem to explain the discrepancy between the findings of Russell et al (1975) who found hyperglucagonaemia in patients undergoing abdominal surgery and those of Giddings et al (1975) who failed to find hyperglucagonaemia during different kinds of surgery. Stimulation of the vagus nerve does not, however, provide a reason why patients with fractures and thermal injuries develop hyperglucagonaemia. It would seem that in these patients another factor is responsible.

The observation that the mean level of plasma glucagon on the seventh and fourteenth post-operative day was less than the mean

basal level in the both groups of patients, are. in agreement with the finding of Russell (1975) who also found lower glucagon level on the eighthpost-operative day than the mean basal level. These workers have suggested the apprehension before the operation raises the plasma glucagon level.

CHAPTER 8

EFFECTS OF POST-OPERATIVE PARENTERAL NUTRITION ON THE LEVELS OF PLASMA PROTEINS AND AMINO ACIDS

1. INTRODUCTION

Changes in the metabolism of plasma protein and amino acids following surgical operation have been discussed in the preceding chapters. Ther results indicated that the levels of plasma protein and of some free amino acids fall immediately after operations such as oesophagectomy, oesophago-gastrectomy or those employed for the treatment of hiatus hernia. While the exact mechanism by which plasma amino acid levels fall after operation is not known, there is now strong evidence which suggests that malnutrition plays an important role in causing the hypoalbaminaemia observed after surgery.

In general, the average patient will be able to take food and fluid orally in one to three days after operation. However, for some surgical patients such as those operated upon for the removal of cancer of the oesophagus, a longer time is required for the resumption of normal oral feeding. Under these circumstances, therefore, it might become necessary to feed the patient intravenously.

A daily intake of about 0.5g/Kg body weight (35g for 70Kg man) is adequate for an active healthy adult provided his calorie intake is adequate. In the presence of infection and trauma there is an increased breakdown of protein within the body associated with elevated urinary nitrogen excretion, and unless the extra protein and calorie is made available to the patient, catabolism of body protein occurs.

Dudrick and Rhoads (1972) have estimated that after trauma an adult requires 40 to 70 K cal. per Kg body weight (2,800 to 4,900 K cal. for a 70 Kg man). These needs cannot be met by the 5% dextrose very often given as the sole nutritional support post-operatively. Each 1000 ml of 5 per cent dextrose provides 200 K cal. and on this basis 14 litres of dextrose solution would be required to meet the minimum requirement. Indeed in many medical circumstances the infusion of such large amounts of fluid would be contraindicated. A possible alternative has been the provision of more concentrated solutions which are markedly hypertonic and which almost inevitably give rise to phlebitis and/or thrombosis of peripheral veins (Allen and Lea, 1969). However, over the past recent years, great advances have been achieved in the introduction of new intravenous solutions and techniques of their administration which has made it possible to employ complete parenteral nutrition for a relatively long time and with minimum risk to the patients health. As Lea (1974) has pointed out, there is now ample evidence of the feasibility and value of parenteral nutrition in the management of a wide range of upper gastro-intestinal disorders with an improvement in nitrogen balance and a reduction in post-operative complication.

Nevertheless, in spite of these observations and clinical evidence indicating beneficial effects of parenteral nutrition in the practice of surgery, administration of 5 per cent solution of glucose alone, which from the nutritional point of view is totally inadequate, continues to be widely used (Lee, 1974). Moreover, in contrast to the well

documented effects of intravenous nutrition preparation on nitrogen balance, to date, little is known about possible effects on the post-operative pattern of plasma free amino acids.

The aim of present study was to investigate the effects of post-operative parenteral nutrition on the levels of plasma proteins, amino acids, including free tryptophan (not bound to the serum albumin) and urinary excretion of total nitrogen in patients treated surgically for carcinoma of the oesophagus.

Patients and Methods

It was originally planned to monitor the plasma levels of albumin, globulin and amino acids for 14 days in 8 patients undergoing surgery for carcinoma of the oesophagus and four of them were to receive parenteral nutrition for 5 days post-operatively and four to act as controls with no post-operative parenteral nutrition. However, after collection of 40 blood and 16 urine specimens from 8 patients over a period of 4 months it was realised that out of the eight patients studied, only one had received complete parenteral nutrition for five days commencing immediately after operation. The others had received either no post-operative nutrition at all or had been given parenteral nutrition for only one day between 1 to 4 days after surgery. Because of these difficulties the results obtained from the patient in whom complete parenteral nutrition had been administered for 5 consecutive post-operative days and those of another patient who had received parenteral nutrition only on the second post-operative day, were analysed and compared with

those obtained from another patient undergoing a similar type of operation who had received no parenteral nutrition throughout the study. Clinical details of the three patients studied are set out in Table 8.1. One female (LW) undergoing oesophago-gastrectomy and two males (GB and AC) undergoing oesophagectomy were studied. Table 8.2 shows the amounts and composition of the intravenous preparations used. These were given to LW on the second day and to AC in each of the first five days after surgery.

Analytical techniques used for the measurement of plasma protein, amino acids and urinary excretion of total-nitrogen were those described in Chapter 2.

2. RESULTS

The plasma albumin concentration (Table 8.3) in patient GB who received no post-operative intravenous nutrition and also in patient LW who had parenteral nutrition on the second post-operative day only, fell following surgery and in both patients was lower 14 days after operation than before operation. In contrast, the plasma albumin level in patient AC who had received intravenous nutrition for five post-operative days rose from 1.7 g/100 ml pre-operatively to 4.1 g/100 ml by 14 days after surgery. The plasma globulin concentrations were comparable in all three patients and were slightly raised on the 14th post-operative day (Table 8.4).

The plasma free amino acid concentrations (Table 8.5) in the patient with no post-operative intravenous nutrition (GB) and in the patient who had received parenteral nutrition only on the second post-operative day (LW), fell after operation and the pattern of change was essentially similar to that described earlier (Chapter 3). On the other hand, the post-operative plasma amino acid changes in patient (AC) who received parenteral nutrition for 5 days, showed a pattern which was different from that observed in the other two patients. The levels of all the amino acids shown rose immediately after operation and remained raised until the 7th post-operative day. By 14 days after surgery, the concentrations of alanine, arginine, leucine and valine had fallen below the basal pre-operative levels, while those of glutamine, glycine, histidine, isoleucine, tyrosine and phenylalanine were higher than their basal value. Plasma total

tryptophan concentration (Table 8.6) fell in each patient immediately after surgery, though again the fall in patient AC was much less than that in the other two patients. Moreover, while the level in patient AC was elevated on days 2, 7 and 14 after surgery, they were depressed in the other two patients on the same days. Plasma free tryptophan (Table 8.6) concentration rose in all three patients immediately after surgery and were higher than the pre-operative value throughout the post-operative period.

Pre-operative level of urinary total nitrogen excretion (Fig. 8.1) was higher in patient AC than in the other two patients. The levels in GB and LW rose on the second post-operative day and the rise was greater in the patient (GB) who received no intravenous nutrition at the time. There was little change in the excretion of nitrogen by patient AC who received intravenous nutrition for 5 days. Of the two patients receiving intravenous nutrition, LW developed a small positive crude nitrogen balance (+ 0.7g) and the other, AC had a negligible negative balance (-0.448g). These results were in contrast to those in patient GB who received no intravenous nutrition and who had a large negative balance (16.7g).

Table 8.1.

Patients and surgical treatment - post-operative parenteral nutrition study

Patient	Sex	Age (years)	Body weight (Kg)	Diagnosis	Treatment	Intravenous nutrition
GB	Male	49	60	Carcinoma of oesophagus	Resection by right thoracotomy	None
LW	Female	57	62	"	Oesophago-gastrectomy	Day 2 only
AC	Male	60	45	"	Resection by right thoracotomy	Days 1 to 5

Table 8.2.

Composition of intravenous preparations used in the parenteral nutrition study

Name	Constituents	Amount used per day (ml)	Energy equivalent (K cal)	Nitrogen (g)
Vamin glucose	Synthetic l-amino acid + glucose	1000	650	9.4
Aminosol with fructose and ethanol	Casein hydrolysate + ethanol + fructose	500	450	2.12
Dextrose 20%	D-glucose	500	400	-
Intralipid 20%	Soybean oil + glycerol	500	1000	-
Intralipid 10%	Soybean oil + glycerol	500	550	-
Total		3000	3050	11.52

Table 8.3.

Plasma albumin concentration (g/100 ml) in three patients undergoing oesophagectomy or oesophago-gastrectomy with or without post-operative parenteral nutrition.

Patients	Before operation	Immediately after operation	After operation (day)		
			2	7	14
GB*	4.0	3.7	3.27	2.8	3.1
LW**	4.3	4.5	3.6	3.0	3.4
AC***	1.7	1.9	2.8	3.5	4.1

* received no parenteral nutrition throughout the study

** received parenteral nutrition on day 2 only

*** received parenteral nutrition on days 1 to 5.

Table 8.4.

Plasma globuline concentration (g/100 ml) in three patients undergoing oesophagectomy or oesophago-gastrectomy with or without post-operative parenteral nutrition.

Patient	Before operation	Immediately after operation	After operation (day)		
			2	7	14
GB*	1.76	1.78	1.74	2.27	1.82
LW**	2.4	1.44	2.0	2.60	2.88
AC***	1.76	1.92	1.90	2.30	2.00

* Received no parenteral nutrition throughout the study

** Received parenteral nutrition on day 2 only

*** Received parenteral nutrition on days 1 to 5

Plasma concentrations of free amino acids in three patients undergoing oesophagectomy or oesophago-gastrectomy with or without post-operative parenteral nutrition

Amino acid μM	Patient	Before operation	Immediately after operation	After operation (days) 2	7	14
Glutamine	GB	778	581	520	655	425
	LW	625	325	250	270	260
	AC	306	676	262	550	533
Glycine	GB	305	277	106	229	106
	LW	225	125	84	140	200
	AC	96	233	153	159	201
Alanine	GB	433	346	422	387	128
	LW	200	112	64	100	120
	AC	191	373	370	188	183
Arginine	GB	58	30	55	53	40
	LW	72	56	28	32	45
	AC	70	80	140	86	67
Histidine	GB	129	93	112	89	74
	LW	65	58	40	53	54
	AC	51	89	118	60	61
Isoleucine	GB	77	64	33	48	77
	LW	32	24	40	56	58
	AC	38	134	332	107	49
Leucine	GB	179	133	89	119	119
	LW	100	48	56	92	110
	AC	97	232	549	180	82
Valine	GB	339	180	149	204	140
	LW	78	108	96	100	85
	AC	128	283	345	202	122
Tyrosine	GB	80	48	68	89	43
	LW	40	20	40	46	43
	AC	38	62	67	64	56
Phenylalanimm	GB	60	58	68	53	42
	LW	36	20	50	42	48
	AC	38	80	189	85	60

Patient GB received no parenteral nutrition throughout the study, patient LW received parenteral nutrition on day 2 only and patient AC received parenteral nutrition on days 1 to 5.

Table 8.6.

Plasma concentrations of total and free tryptophan (μ mol/l) in three patients undergoing oesophagectomy or oesophago-gastrectomy with or without post-operative parenteral nutrition.

Patients	Before Operation		Immediately after operation		After operation (days)					
					2		7		14	
	Total	Free	Total	Free	Total	Free	Total	Free	Total	Free
GB*	70	13	50	15	52	14	48	12	46	11
LW**	50	7	27	10	36	7	29	6	17	4
AC***	47	10	43	13	106	38	51	19	52	13

* Received no parenteral nutrition throughout the study

** Received parenteral nutrition on day 2 only

*** Received parenteral nutrition on days 1 to 5.

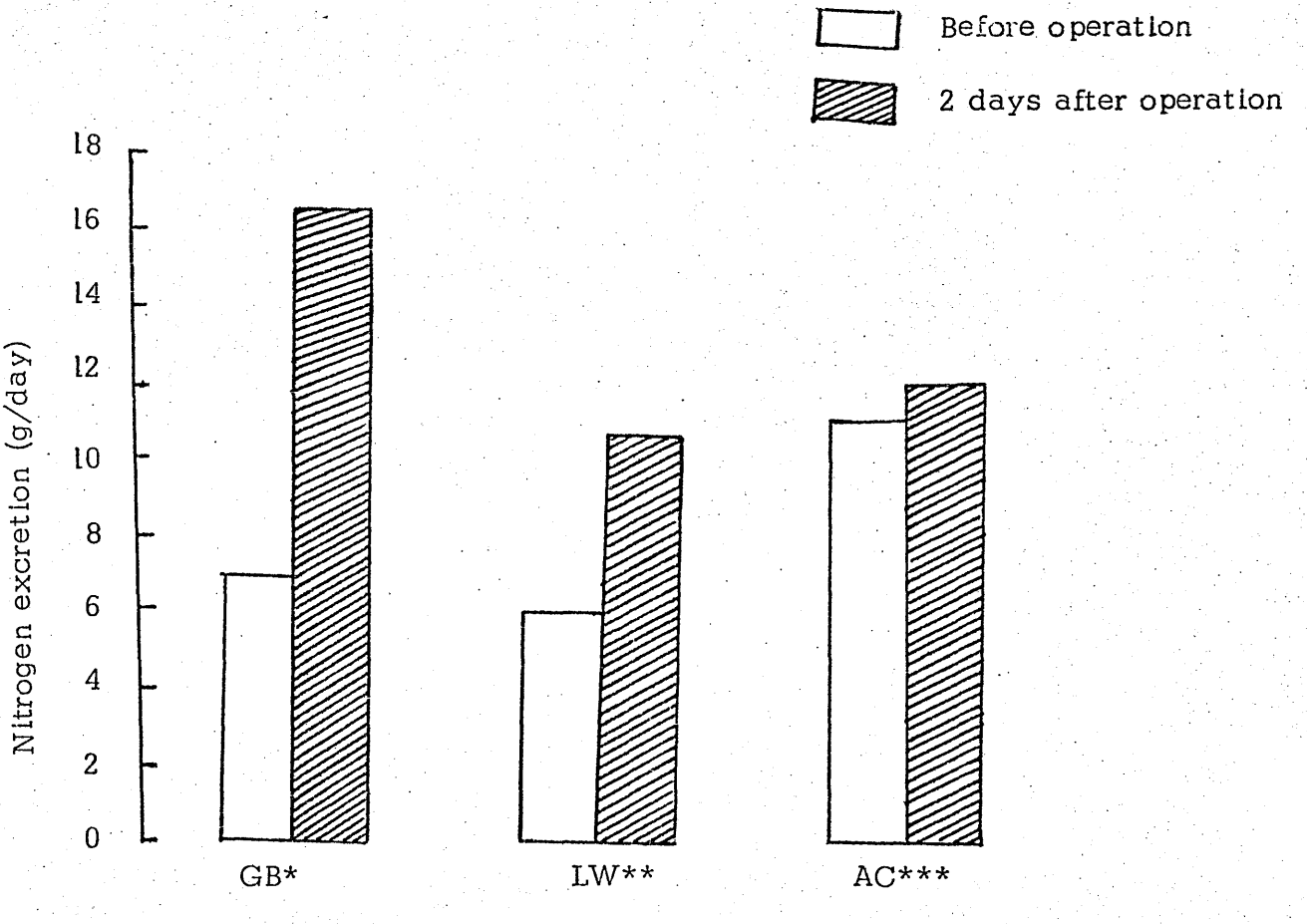


Fig. 8.1. Urinary excretion of total-nitrogen in three patients undergoing surgery for the carcinoma of oesophagus.

* Received no parenteral nutrition throughout the study

** Received parenteral nutrition on day 2 only

***Received parenteral nutrition on days 1 to 5.

3. DISCUSSION

This study has shown that a daily intravenous administration of 0.26 g N and 69 K cal/Kg body weight for two consecutive days immediately after surgery to a 60 year old male patient (AC) almost held the urinary nitrogen losses at 2 days after surgery to the pre-operative level (11.20 g and 11.96 g N, before and 2 days after operation respectively). Considering that on the second day of operation he received a total amount of 11.52 g N intravenously it indicated that the crude negative nitrogen balance for that day was only -0.44g, which might be considered negligible. On the other hand in a 57 year old female patient (LW) who had received 11.5g N on the second post-operative day, only the level of urinary nitrogen excretion was higher than before (6.0g and 10.8g, before and 2 days after operation respectively), the crude balance of nitrogen was slightly positive (11.5 - 10.8 = + 0.7g). These post-operative levels of urinary nitrogen excretion were much lower than that observed in another patient (GB) on whom a similar operation had been performed and to whom no intravenous nutrition had been administered. The level of urinary nitrogen excretion was greatly increased after operation (from 6.8g/day pre-operatively to 16.7g on the second post-operative day). Although this patient was younger than either of the other two patients, and therefore his higher response to operation could be partly attributed to the age difference, still

these results in general suggest that post-operative parenteral nutrition reduces the excessive losses of nitrogen. Reduction in the post-operative loss of nitrogen has also been reported by other workers. Thus, Johnston et al (1966) gave 0.18g N and 45 K cal/Kg body weight daily, intravenously, to a group of 5 male patients and found that losses of nitrogen were greatly reduced immediately after surgery although they were not abolished. No increase in the urinary nitrogen excretion was observed in this study.

The present study has also indicated that in a patient who had a low plasma albumin concentration pre-operatively intravenous feeding of high calorie and nitrogen preparations resulted in a rise in the plasma albumin concentration (from 1.7g/100 ml before operation to 4.1g/100 ml by 14 days after surgery). This finding is consistent with that of Allen and Lea (1969) commenting that in hypoalbuminaemic patients, parenteral nutrition was followed by a rise in the serum albumin level. It is, however, interesting to note that animal experiments have shown that intravenous infusion of casein hydrolysate and fat emulsion are followed by a reduction in serum albumin. For example Holm (1962) studied plasma protein levels in dogs after the administration of a casein hydrolysate and found that when it was given intravenously, there was a substantial reduction in the albumin concentration, with an equivalent rise in the globulin concentration. Such a response could have been partly due to infection. Similarly, Singleton, Benerito et al, (1960) found that in the dogs, administration of cotton-seed oil was followed by a reduction in the level of plasma albumin.

However, in both the experiments cited above, normal animals were used and to date, there is no report of the effect of parenteral nutrition on plasma protein in animals with low plasma protein values. With regard to the human studies, apart from the observations by Allen and Lea (1969) to which reference has been made, in the numerous publications on the effects of parenteral nutrition, no specific mention is made of the changes in serum proteins.

Measurement of plasma free amino acids concentrations before and after surgery in patients receiving parenteral nutrition indicated that infusion of amino acid and fat solutions not only prevented the fall in plasma amino acid concentrations after operation, but also raised the levels of the amino acids, and the concentrations of plasma glutamine, glycine, histidine, isoleucine, tyrosine and phenylalamine remained elevated even nine days after the intravenous nutrition had been stopped. These findings are in general agreement with those recently reported by Furst et al (1978), who showed that post-operative parenteral nutrition in patients with colon carcinoma resulted in a rise in the levels of amino acids in the plasma. Moreover, in the present study it was found that post-operative parenteral nutrition caused a rise in the concentration of free tryptophan in the plasma. No such finding has previously been reported. However, the present study which showed that raised levels of plasma amino acids were accompanied by a rise in the concentration of plasma albumin, would probably suggest that the fall in the concentration of plasma amino acids which occurs after operation is

undesirable and could be prevented by intravenous feeding of amino acid preparations.

CHAPTER 9

GENERAL DISCUSSION AND CONCLUSIONS

(i) Metabolic effects of tumours

A considerable amount of information has been accumulated over the past thirty years which indicate that the presence of a tumour profoundly affects the metabolism of the host. A significant numbers of patients with various cancers suffer from the cachexia syndrome which is associated with weakness, anaemia, loss of body fat and protein (Calman, 1977). There is also an increased metabolic rate (Theologides,1972) and energy requirement, which has been suggested that in the face of anorexia and reduced food intake, this appears to be a factor in the weight loss observed so frequently in these patients (Shils, 1976). However, some patients with active disease who are losing weight have been found to be in nitrogen equilibrium or even in positive nitrogen balance. On this evidence, Wiseman and Ghadially (1958) postulated that the enhanced ability of the neoplastic cell to concentrate amino acids causes a continued deprivation of the cells of the tissues of the host of the precursors of their proteins, as a result of which these normal cells eventually suffer a lack of one or more essential amino acids, protein synthesis ceases, and tissue protein breakdown occurs. An alternative mechanism is that the tumour, by producing low molecular weight metabolites changes the activity of the enzymes involved in the usual biochemical reactions and increases the metabolic activity in the host. This increased metabolic activity results in an increased energy expenditure and the release of intermediate metabolites then enter the metabolic pool

of the host and are trapped and used primarily by the tumour which is geared mainly to growth (Theologides, 1977).

Further studies with isotopically labeled amino acids in experimental animals have demonstrated the marked capacity of tumour tissue for protein synthesis and the amino acids tend to stay in the tumour in contrast to their turnover in certain normal tissues (Henderson and Lepage, 1959). These observations have given rise to the concept of the tumour as a "nitrogen trap", in other words, incorporation of amino acid into tumour is essentially a one-way passage from metabolic pool to the tumour (Mider, 1951).

The nitrogenous constituents of blood have been investigated in the tumour-bearing host. In this connection, Babson (1954) found a progressive rise in the level of nitrogen in the blood of tumour-bearing animals throughout the period of tumour growth. Similarly Wu and Bauer (1960) reported that large increases in blood amino acid concentration accompanied tumour growth and the magnitude of the increase was dependent upon the size of the tumour. On the other hand, in another investigation in which plasma amino acids were studied in patients with chronic lukaemia, Rouser et al (1962) using a paper chromatography technique, found that apart from a distinct elevation of plasma glutamic acid concentration the levels of other amino acid determines were normal.

In the current investigation the levels of plasma amino acids

(Tables 3.4 and 3.5) were studied in two groups of cancer patients, namely those with oesophageal and lung cancer before and after surgery by the standard method of ion exchange chromatography and they were compared with those obtained in another group of patients who were treated for a non-malignant disease of the oesophagus, hiatus hernia. The results indicated that before surgery, of all amino acids studied, the levels of alanine and arginine in patients with lung cancer and of free tryptophan (the fraction not bound to serum albumin) in both groups of cancer patients (Table 5. showed a tendency to be lower than in patients with hiatus hernia. The importance of these findings is not known at this stage. However, in so far as plasma free tryptophan has some role in controlling the level of brain tryptophan and the synthesis of the neurotransmitter, serotonin which in turn is believed to play a major role in controlling behaviour it is possible to suggest that the low free tryptophan in cancer patients might bring about behavioural changes.

The early studies by Mider et al (1950) on the effect of malignant tumours of different kinds in plasma protein, showed that the general trend was a fall in plasma albumin combined with a rise in the globulin fraction. Consistent with these observations, in the current investigations it was found that the mean level of plasma albumin in both groups of cancer patients was low (Table 3.1, 3.2 and 3.3) as compared with the suggested value for normal subjects (Jelliffe, 1966).

The exact mechanism by which malignant tumours lower plasma proteins is not known. However, it has been shown that tumours can use plasma albumin to a greater extent than normal tissues (Goodlad, 1964). Furthermore, studies by Allison, Wannemacher, Parmer and Gomez (1961) which indicated that the fall in serum albumin observed in rats bearing the Walker 256 carcinoma could be prevented by feeding diets of high protein content, would probably suggest that the minimum protein requirement is increased in malignancy. On the other hand, when a malignant tumour is present in the upper part of gastrointestinal tract, low plasma albumin could develop from malnutrition associated with impaired food intake secondary to obstruction. Indeed, the observation that plasma albumin was lower in patients with oesophageal cancer than in lung cancer patients (Table 3.1) underlines the contribution of malnutrition to the low serum albumin observed in these patients. Protein-losing entropathy has also been implicated as a contributory factor to hypoalbuminaemia observed in patients with tumours of the gastro-intestinal tract (Waldman et al, 1977).

As far as the effect of a tumour on the urinary pattern of nitrogen-containing constituents is concerned, it is generally believed that the total urinary nitrogen in the tumour-bearing subject or animal is not increased and may even be decreased due to the action of the tumour in retaining nitrogen (Goodlad, 1964). It has also been reported that the rate of excretion of ammonia, urea, creatinine and

creatin nitrogen remained unchanged during tumour growth in rats bearing the Jensen Sarcoma(Bach and Maw, 1953). In the current investigation also, there was no significant difference in the total nitrogen and urea nitrogen excretion between cancer patients and patients with hiatus hernia before surgery (Fig. 3.3). Moreover, the observation that before surgery, patients with oesophageal cancer, and not those with lung cancer, excreted more amino .nitrogen per Kg body weight in their urine than hiatus hernia patients (Fig. 3.4) indicates, that while total nitrogen excretion in the urine is not affected by the presence of tumour (Goodland, 1964), excretion of amino nitrogen can be increased in certain types of malignancy. The finding that before operation cancer patients excreted more creatinine nitrogen per Kg body weight than patients with hiatus hernia could, in fact, be attributed to a sex difference rather than to the presence of a tumour for the majority of patients with hiatus hernia were females whereas the majority of those with cancer were males.

Carbohydrate metabolism has been studied in cancer patients. In this connection Glicksman and Rawson (1956) were amongst the first to report a high incidence (37%) of abnormal GTT in cancer patients compared with an incidence of 9% in patients with benign diseases. Later work by Marks and Bishop (1957) using intravenous GTT indicated a decreased fractional rate of glucose disappearance in cancer patients despite normal fasting blood sugar. More recently Brennan and Goodgame (1977) have reported that of six patients

suffering from different kinds of malignancy, two had an abnormal GTT. Conversely, Waterhouse and Kemperman (1971) using ^{14}C glucose were unable to find any decrease in glucose utilization in fasting cancer patients.

In the present study, there was no apparent difference in the level of blood glucose between oesophageal or lung cancer patients and patients with hiatus hernia (Table 4.1). However, the mean level of plasma insulin in lung cancer patients tended to be higher than that in the other two groups (Table 4.2). This tendency towards an elevation of plasma insulin in patients with lung cancer is in agreement with the finding of Laurence and Neville (1976) who commented that occasionally, bronchial carcinoids and carcinomas synthesise and release insulin.

11-Hydroxycorticosteroids are closely associated with carbohydrate metabolism and carcinomas are likely to produce large amounts of intermediates in the biosynthesis of cortisol (Bondy, 1974). In the present study, it was found that the pre-operative fasting levels of plasma 11-hydroxycorticosteroids in cancer patients were not significantly different from those in patients with hiatus hernia (Table 4.3). Moreover, the levels in all patients were within the normal range of 6.5 - 26.3 g per 100 ml plasma reported by Chattoraj (1976). There are no reports in the literature of plasma 11-hydroxycorticosteroids concentration in patients with oesophageal tumours or hiatus hernia. The findings of normal 11-hydroxycorticosteroids in lung cancer patients was perhaps surprising in view of the fact that many

extra-pituitary tumours, especially those of the lung sometimes produce large amounts of ACTH (Laurence and Neville, 1976).

The pre-operative levels of plasma FFA were similar in cancer patients to those in patients with hiatus hernia (Table 4.4), but the rate of excretion of urinary ketone bodies was higher in oesophageal cancer patients than in patients with hiatus hernia. However, since the rate of excretion in lung cancer patients was comparable to that in hiatus hernia patients, the elevated level of excretion of ketone bodies in patients with oesophageal cancer was probably related to malnutrition rather than to a direct effect of the malignant tumour as these patients are frequently malnourished and it is known that starvation leads to a high production of ketone bodies (Foster, 1967).

The urinary excretion of 5HIAA and NMN, two end products of tryptophan metabolism has been investigated in cancer patients. The urinary level of 5HIAA is considerably increased in patients with the carcinoid syndrome. Boyland, Gasson and Williams (1956) reported that the urinary excretion of 5HIAA and serotomin (5HTP) in patients with cancer of larynx and bronchus was lower than that in the patients with the carcinoid syndrome but higher than in cancer-free patients. More recently Basu et al (1973) in a study involving 58 patients with advanced cancer of different types and at different sites, found an increased urinary excretion of 5HIAA with a decreased level of NMN relative to the corresponding values found in normal healthy subjects or patients withb enign diseases. Conversely, Levine (1974) has

concluded that apart from the carcinoid syndrome in which urinary excretion of 5HIAA is considerably increased, other types of malignancy do not usually lead to a raised excretion of urinary 5HIAA. In the present investigation it was found that before surgery, the level of 5HIAA in the urine of cancer patients was slightly higher than patients with hiatus hernia (Table 5. although the difference was not statistically significant, thus confirming the view of Levine (1974). Furthermore, the pre-operative level of NMN in the urine of cancer patients was not significantly different from that of patients with hiatus hernia (Table 5.) There are no reports in the literature in which the urinary level of NMN in oesophageal or lung cancer patients has been investigated.

Urinary activity of alkaline ribonuclease has been reported to increase in patients with burn injuries (Barlow and Withear, 1977) and malnutrition (Sigulem et al, 1973). The observation that its activity in the urine of oesophageal cancer or lung cancer (Table 3.16) patients was similar to that in the urine of patients with hiatus hernia would seem to suggest that the urinary activity of this enzyme remains unaltered in cancer patients.

Serum copper concentrations have been reported to be elevated in patients with Hodgkin's disease (Warren et al, 1969) and in those with osteosarcoma (Fisher et al, 1976). The present results provide further evidence to indicate that the plasma copper level is also raised in

oesophageal cancer, for its level in these patients was significantly higher than that in patients with hiatus hernia, pre-operatively (Table 3.9). However, the urinary excretion of copper was similar in lung cancer and hiatus hernia patients (Table 3.10).

Changes in the concentration of plasma zinc in cancer patients has also been investigated. However, there is no general agreement as to whether the presence of a tumour in the body changes the concentration of zinc in the plasma or its excretion in the urine. While Wolff (1956) found decreased values for serum zinc in cancer patients, Davies et al (1968) in an investigation carried out on 49 cancer patients reported that only patients with carcinoma of the bronchus had lower plasma zinc levels than the controls. Similarly, in the present investigation no significant difference could be found in the level of plasma zinc or in the urinary excretion of zinc between cancer patients and those with hiatus hernia (Tables 3.7 and 3.8).

These observations would seem to suggest that measurements of plasma or urinary excretion of zinc cannot be regarded as a reliable indication of the presence of a malignant tumour in the body.

Urinary levels of cyclic GMP or cyclic AMP in patients with tumours of the lung or oesophagus were no different from those in patients with hiatus hernia before surgery (Fig. 6.1). These findings are in agreement with those of Bershtein et al (1976) who found no significant differences in cyclic AMP excretion in patients with breast

or lung cancer and control subjects. There are no reports in the literature of cyclic GMP excretion in patients with lung or oesophageal tumour. In contrast to the report of increased urinary cyclic GMP excretion in primary hepatoma patients (Neethling and Shanley, 1976) the results of this investigation suggest that the presence of oesophageal or lung cancer is not associated with cyclic nucleotide excretion. It seems probable that increased urinary cyclic GMP output may only be associated with certain types of tumour and not with malignancy in general.

(ii) Metabolic effects of surgery

The observation that immediately after operation the levels of most amino acids in the plasma fell in patients with oesophageal cancer and hiatus hernia patients agrees with the findings of Dale et al (1977) who also observed that immediately after an abdominal operation of moderate or extensive nature, most plasma amino acids decreased. Moreover, the finding that the post-operative patterns of changes in the levels of plasma amino acid were essentially similar in oesophageal cancer and hiatus hernia patients (Table 3.4 and 3.5) would provide further evidence in support of the view of Dale et al (1977) suggesting that the changes in plasma amino acid levels are not associated with severity of the operation, since the surgical procedure employed for the resection of oesophagus is more severe than that used for the surgical treatment of hiatus hernia. On the other hand, the present results

indicated that the post-operative changes of plasma amino acids in patients with lung cancer did not follow the same pattern as for oesophageal cancer or hiatus hernia patients. This would seem to suggest that the post-operative fall of plasma amino acids is not an inevitable consequence of surgery. Nevertheless it seems to be related to the presence or absence of hyperglycaemia. The finding that the level of plasma glutamine fell immediately in all three groups of patients and remained low throughout the post-operative period, provides further evidence in support of Kinney's view (Kinney, 1977) that the decrease in glutamine observed in injured subjects is unique to surgical catabolism and may offer an important way to separate the influence of trauma from that of starvation. Furthermore, the finding that plasma phenylalanine rose by 2 days after surgery in all patients is in agreement with that of Vinnars et al (1976) who also found a rise in the concentration of plasma phenylalanine after surgery. The cause of this early post-operative rise in plasma phenylalamine is not known. It has, however, been suggested that it might indicate a transient liver dysfunctions (Dale et al, 1977).

The concentration of plasma total tryptophan fell immediately after surgery and since the level of plasma free tryptophan rose at the same time this led to a great increase in the ratio of free total tryptophan (Fig. 5.2). These changes in the concentration of plasma total and free tryptophan would seem to be due to the rise in the concentration of plasma FFA which occurred in all three groups of

patients for FFA have been shown to decrease the binding affinity of albumin for tryptophan (Curzon et al, 1973). The mechanism by which plasma total tryptophan is lowered is not known. Curzon and Knott (1975) have suggested that the fall of plasma total tryptophan caused by an increased level of plasma FFA results from a 'shift of the newly freed tryptophan into intracellular compartments other than the brain as rapid transient changes of plasma FFA did not cause comparable brain tryptophan changes. This suggestion receives further support from the finding that the fall in plasma tryptophan concentration observed in oesophageal cancer and hiatus hernia patients immediately after surgery was associated with a rise in the blood glucose level (Table 4.1) indicating that perhaps tryptophan has been taken up by the liver in the process of gluconeogenesis for glucose synthesis. It seems, however, that an alternative explanation might be found for the changes in plasma tryptophan in lung cancer patients.

The post-operative fall of plasma albumin observed in hiatus hernia and oesophageal cancer patients (Table 3.1) is consistent with the findings of others that after trauma, the plasma albumin concentration falls, reaching a minimum around the third to sixth day (Fleck, 1976). However, the present findings which showed that the concentration of plasma albumin in patients having lung operations showed a little change after surgery would seem to indicate that the fall in plasma albumin level is again not an inevitable consequence of surgery. Moreover, this post-operative difference in plasma albumin level between lung

cancer patients and patients with oesophageal cancer can be explained by the fact that patients having lung operations normally resume their normal diet a few days after surgery whereas those having oesophagectomy or oesophagogastrectomy are unable to do so for one or two weeks, and therefore post-operative malnutrition could have contributed in the observed hypoalbuminaemia in these patients. This suggestion is supported by the findings in the patient given post-operative intravenous nutrition (Chapter 8).

Post-operative malnutrition seems also to be the cause of the fall in plasma albumin observed after surgery in patients with hiatus hernia for these patients are frequently overweight and receive a restricted calorie intake as part of their treatment.

A moderate increase in the level of plasma globulin is believed to occur after injury (Cuthbertson, 1964). In the present studies, however, no significant change could be found in the concentration of total globulin in lung cancer or hiatus hernia patients after operation (Table 3.3). In fact, the plasma globulin level fell slightly in oesophageal cancer patients in the early post-operative period. In view of the fact that patients with oesophageal cancer had a lower plasma total protein than the other two groups, and also that they had a progressive decline in total urinary nitrogen excretion it appeared that the post-operative fall in the concentration of plasma globulin was a reflection of their generally poor nutritional status.

With regard to the effect of operation on the urinary excretion of nitrogenous compounds, the results of the present investigation has indicated that apart from the excretion of amino-nitrogen by lung cancer patients which was elevated on the second and seventh post-operative days, the excretion of other urinary nitrogenous constituents was not usually increased. In fact, in oesophageal cancer patients a progressive fall in the excretion of urinary nitrogenous compounds occurred throughout the post-operative period, which reflected their poor nutritional status. Johnston (1967) reported essentially the same decline in the daily nitrogen loss in 4 [illegible] poorly nourished patients after abdominal operation.

In so far as the loss of urinary nitrogen after severe injury is maximum in young, well nourished male patients, the poor urinary nitrogen response to surgical poperation observed in patients with hiatus hernia was perhaps not surprising, for the majority of these patients were females. This conclusion is strengthened by the observation that the urinary nitrogen response to surgery in the male lung cancer patients, was higher than hiatus hernia patients.

As to the effect of surgery on blood sugar concentration, the present study has indicated that immediately after operation, the blood sugar level rose in hiatus hernia and oesophageal cancer patients, but not in patients with lung cancer (Table 4.1). Since the rise in blood sugar level in these patients was associated with a fall in the levels of plasma glucogenic amino acids, it appeared that hyperglycaemia

was the result of increased glucose synthesis from plasma amino acid. These findings provide direct evidence in support of the view of Wilmor et al (1976) that hyperglycaemia occurring after injury is the result of increased glucose production, not glucose disappearance. Moreover, the observation that in hiatus hernia and oesophageal cancer patients, hyperglycaemia occurred in the presence of normal plasma insulin would seem to suggest that perhaps in the initial phase of injury insulin has a little part to play. Furthermore, it is increasingly apparent that the control of blood sugar is a function not only of insulin but also of glucagon. The main physiological role of glucagon is to increase output of glucose from the liver (Cherrington et al, 1972), while insulin is thought to be associated with the regulation of peripheral glucose disposal (Unger and Orci, 1975). Therefore, it is possible to suggest that glucagon might have a major contributory effect to the observed hyperglycaemia immediately after operation. The values for glucagon found in our patients support this suggestion.

The immediate rise in plasma corticosteroids observed after injury (Johnston, 1964) has been confirmed in the present investigation, as it was found that in all three groups of patients the levels of 11-hydroxycorticosteroids were significantly above the pre-operative level immediately after surgery (Fig. 4.3). Furthermore, elevated levels of 11-hydroxycorticosteroids would seem to have led to the rise in the concentration of FFA in the plasma, for the plasma FFA level increased in parallel to the rise in 11-hydroxycorticosteroid

immediately after operation (Fig. 4.4).

The observation that plasma insulin rose in all patients on the second post-operative day and that not in proportion to the blood glucose level and associated with high excretion of ketone bodies in the urine (Table 4.5), provides further evidence in support of the idea that insulin resistance occurs after injury (Allison, 1974).

As for the effects of surgery on copper and zinc metabolism, the results of the current investigation suggest that the post-operative changes of plasma copper are related to the underlying disease, for its level in oesophageal cancer on the second and seventh post-operative day fell, whereas it hardly changed in hiatus hernia and lung cancer patients (Table 3.9). Moreover, the results show that perhaps surgery, regardless of its nature or severity, has no apparent effect on the urinary excretion of copper, for its level did not change in any of these three groups of patients post-operatively (Table 3.10).

Post-operative changes in plasma zinc were more consistent than those in copper, and fell in all patients on the second post-operative day and returned to the basal pre-operative level by 7 days after surgery (Table 3.7). Furthermore, the observation that in hiatus hernia and oesophageal cancer the fall in plasma zinc was coincident with a fall in urinary excretion would seem to suggest that surgery could lead to an increased uptake of zinc by the tissues.

Surgery itself seems not to change the urinary excretion of NMN or 5HIAA (Tables 5.3 and 5.4). However, the finding that in oesophageal cancer patients there was no correlation between plasma tryptophan and its urinary metabolite NMN (Fig. 5.5) implies that perhaps in these patients the normal pathway of tryptophan metabolism (Fig. 5.1) is impaired. Abnormalities in tryptophan metabolism have been reported in patients with breast cancer (Rose, 1967) and also in patients with bladder cancer (Gailani et al, 1973). There are no reports in the literature concerning tryptophan metabolism in patients with lung or oesophageal tumours.

The present study has shown that surgical operation is followed by a transient rise in the activity of urinary alkaline ribonuclease for its activity rose significantly in all three groups of patients only on second post-operative day (Table 3.16). Sigulem et al (1973) have suggested that measurement of this enzyme in the urine of children can be used to detect malnutrition. However, the current investigations suggests that urinary activity of alkaline ribonuclease is not closely related to nutritional status in these patients and, therefore, could not be regarded as a measure of nutritional status in surgical patients.

Cyclic GMP excretion increased after surgery, and was higher in patients with malignancy than in patients with non-malignant disease (Fig. 6.1). Higher cyclic GMP output after the removal of a tumour

may reflect a greater incidence of post-surgical complications with an associated stress to the patients.

(iii) Effects of post-operative parenteral nutrition

The present study has shown that 5 days intravenous nutrition in a malnourished male patient suffering from carcinoma of oesophagus resulted in a great improvement in plasma albumin. Post-operative parenteral nutrition in this patient also reduced urinary nitrogen losses almost completely on the second post-operative day and are in agreement with the work of others (Johnston et al, 1966). Moreover, the present study has also shown that the post-operative fall of plasma amino acids which occurs in oesophageal cancer patients could be avoided by intravenous feeding.

CONCLUSIONS

The studies described in this thesis have added a further fact to our understanding of the metabolic effects of surgery. The effects evidently depend not only on the usually accepted factors such as state of nutrition, age of the patient and severity of operation but also upon the nature of the surgical procedure. The findings suggest that the response may be modified by the handling of organs and tissues during operation. They further demonstrate the close relationship between carbohydrate and amino acid metabolism and the release of certain hormones. Of the latter, studies of the effects of injury on the secretion of glucagon and the intestinal hormones, such as gastric inhibitory peptide (GIP) would seem to be of importance. A greater understanding of the detailed metabolic response to different kinds of surgical procedures may well point the way to more carefully controlled, individually prescribed nutritional management. In this respect the findings on individual amino acids and the preliminary work on thiamin seem worthy of further investigation.

REFERENCES

Abbott, W.E. and Albertson, K. (1963). Ann. New York Acad Sci., 39, 941

Abdulla, Y.H. and Hamadah, K.H. (1970). Lancet, i, 378

Ackerman, L.V., del Regato, J.A. (1970). "Cancer: Diagnosis, Treatment and Prognosis" eds. Ackerman, L.V. and del Regato, p. 408, Mosby Company, St. Louis,

Adams, W.E., Perkins, J.F. Jr., Harrison, R.W., Buhler, W. and Long, E.T. (1957). Dis. Chest., 32, 380.

Adibi, S.A. (1971). Am. J. Physiol., 221, 829.

Adkins, P.C. (1972). In "Textbook of Surgery" ed. Sabiston, D.C., p. 760. Saunders Philadelphia.

Aguilar-Parada, E., Eisentraaut, A.M., Unger, R.H. (1969). Diabetes, 18, 717.

Ahlbom, H.E. (1936). Bri. Med. J. 2, 331.

Alberti, K.G.M.M., Batstone, G.F. and Johnston, D.G. (1977). In "Nutritional Aspects of Care in the Critically Ill" eds. Richards, J.R. and Kinney, J.M. p. 225, Churchill, London

Allen, P.C. and Lee, H.A. (1969). "A Clinical Guide to Intravenous Nutrition". Blackwell Scientific Publication, Oxford and Edinburgh

Allison, S.P. (1974). In "Parenteral Nutrition in Acute Metabolic Illness" ed. Lea, H.A. p. 167, Academic Press, New York

Allison, S.P., Hinton, P. and Chamberlain, M.J. (1968) Lancet, 2, 1113.

Allison, S.P., Prowse, K., and Chamberlain, M.J. (1967) Lancet, 1, 478.

Allison, S.P., Tomlin, P.J. and Chamberlain, M.J. (1969) Br. J. Anaesth., 41, 588.

Allison, J.B., Wannemacher, R.W., Jr., Parmer, L.P. and Gomez, R. (1961) J. Nutr. 74, 176.

Aoki, T.T., Brennam, M.F., Muller, W.A. and Cahill, G.F. Jr. (1974) In "Protein Nutrition" ed. Brown, H. p. 180, Carles C. Thomas, Springfield, Illinois, U.S.A.

Ashly, D.J.B. (1967) Br. J. Cancer, 21, 243.

Ashman, D.F., Lipton, R., Melicow, M.M. and Price, T.D. (1963) Biochem. Biophys. Res. Commun., 11, 330.

Ashmore, J. and Weber, G. (1968) In "Carbohydrate Metabolism" eds. Dickens, F., Randle, P.J. and Whelan, W.J. p. 355, Academic Press, New York.

Azzopardi, J.G. and Williams, E.D. (1968) Cancer, 22, 274.

Babson, A.L. (1954) Cancer Res., 14, 89

Bach, S.J. and Maw, G.A. (1953) Biochim. Biophys. Acta., 11, 69

Ballantyne, F.C. and Fleck, A. (1973a). Clin. Chim. Acta., 44, 341

Ballantyne, F.C. and Fleck, A. (1973b). Clin. Chim. Acta., 46, 139

Barlow, G.B. and Withear, S.H. (1977) Clinica. Chimica. Acta, 78, 331.

Baron, D.N. (1961) Br. J. Surgery, 48, 344

Bartter, F.C. (1958) Proc. Roy. Soc. Med., 51, 201

Basu, T.K., Raven, R.W., Bates, C. and Williams, C. (1973) Europ. J. Cancer, vol. 9, 527

Basu, T.K., Dickerson, J.W.T., Raven, R.W. and Williams, D.C. (1974). Int. J. Vit. Nutr. Res., 44, 53.

Bauer, J. (1872). Z. Biol. 8, 567.

Baumann, P., Duruz, E. and Heimann, H. (1974). Clin. Chimica. Acta., 51, 35.

Bergstrom, J. (1962). J. Clin. Lab. Invest. Suppl. 68.

Bergstrom, J., Furst, P., Noree, L.O. and Vinnars, T. (1974) J. appl. physiol., 36, 693

Bershtein, L.M., Semiglazov, V.F. and Valdina, E.A. (1976) Vopr. Onkol., 22, (Part 8), 30.

Bignal, J.R., and Martin, M. (1972). Lancet, 2, 60.

Bistrian, R.B., Blackburn, G.L., Hollwell, H., Heddle, R. (1974) (J. Am. Med. Ass., 230, 858.

Bistrain, R.B., Blackburn, G.L., Sherman, M., Scrimshaw, N.S. (1975). Surgery, 141, 512

Black, D.A.K. (1967). "Essentials of Fluid Balance", ed. Black, D.A.K., Blackwell, Oxford.

Black, D.A.K., McCance, R.A. and Young, W.W. (1944) J. Physiol. 102, 406.

Blomstadt, D. (1965). Acta. Chir. Scand. 130, 424.

Bloom, S.R., Vaughan, N.J.A. and Russell, R.C.G. (1974) Lancet, ii, 546.

Bocker, D. (1963) National Library of Medicine (extensive bibliography).

Bondy, P.K. (1974) In "Diseases of Metabolism", eds. Bondy, P.K. and Rosenberg, L.E., p. 1152, Saunders, Philadelphia.

Bondy, P.K., Felig, P. (1974) In "Diseases of Metabolism" eds. Bondy, P.K. and Rosenberg, L.E., Saunders, Philadelphia.

Boyland, E. and Williams, D.C. (1956) Biochem. J., 64, 578.

Brennan, M.F. and Goodgame, J.T. (1977). In "Nutritional Aspects of Care in the Critically Ill" eds. Richards, J.R. and Kinney, J.M. p. 523, Churchill, London.

Brin, M., Tai, M., Ostashever, A.S. and Kalinsky, H. (1960) J. Nutr., 71, 273.

Broadus, A.E., Kaminsky, N., Northcatt, R.C., Hardman, J.G., Sutherland, E.W. and Liddle, G.W. (1970) J. Clin. Invest., 49, 2237

Broughton, A., Anderson, I.R.M. and Bowden, C.H. (1968) Lancet, 2, 1156.

Browne, J.S.L. (1944). In "Metabolic Aspects of Convalescence" Trans. 6th Conf., p. 67, Josiah Macy. Jr. Foundation, New York.

Burns, T.W., Langley, P.T. and Robison, G.A. (1971) Ann. N.Y. Acad. Sci., 185, 115.

Burrell, R.J.W. (1962) J. Nat. Cancer Inst., 28, 495.

Burrell, R.J.W., Roach, W.A. and Shadwell, A. (1966) J. Nat. Cancer. Inst., 36, 201.

Burt, R.L. (1954). J. Lab. Clin. Med., 44, 702.

Butterworth, C.E. Jr. (1974). J.A.M.A. (editorial), 230, 879.

Cahill, G.F., Jr. (1971) Diabetes, 20, 785.

Cairnie, A.B., Campbell, R.M., Pullar, J.D. and Cuthbertson, D.P. (1957) Br. J. exp. path., 38, 504.

Caldwell, F.T., Jr. (1962). Ann. Surg., 155, 119.

Caldwell, F.T. (1970). In "Energy Metabolism in Trauma" eds. Porter, R. and Knight, S. p. 23, Churchill, London

Calloway, D.H., Grassman, M.I., Bowman, J. and Calhoum, W.K. (1955). Surgery, 37, 935.

Calman, K.C. (1977). In "Nutritional Aspects of Care in the Critically Ill" eds. Richards, J.R. and Kinney, J.M. p. 513, Churchill, Livingstone, London

Campbell, R.M. and Cuthbertson, D.P. (1967). Quart, J. exp. physiol., 52, 114.

Cannon, W.B. (1929) "Bodily changes in Pain, Hunger, Fear and Rage", ed. Cannon, p. 49, McGrath Inc. Maryland.

Carlson, L.A. (1970). In "Energy Metabolism in Trauma" eds. Porter, R. and Knight, J. p. 155, Churchill, London

Carlson, L.A. and Hallberg, D. (1963) Acta. Physiol. Scand., 59, 52.

Carpenter, K.J. and Kodicek, E. (1950). Biochem. J., 46, 426.

Chalmers, M.I. and Munro, H.N. (1945). Br. J. Exptl. Path., 26, 396

Chase, L.R., Melson, G.L. and Aurbach, G.D. (1969). J. Clin. Invest., 48, 1832.

Chattoraij, S.C. (1976). In "Clinical Chemistry", ed. Tietz, N.W. p. 737, Saunders, Philadelphia.

Christensen, N.J., Alberti, K.G.M.M. and Brandsborg, O. (1975) European. J. of Clin. Invest., 5, 415.

Churchill, E.D., Sweet, R.H., Scannell, J.G. and Wilkins, E.W. Jr. (1958). J. Thorac. Surg., 36, 301.

Clark, R.G. (1967) Br. J. Surg., Lister Centenary Number

Coller, F.A., Campbell, K.N., Vaugham, H.H., Iob, V.L. and Moyer, C.A. (1944). Surgery, 119, 533.

Contractor, S.F. (1966). Biochem. Pharmaco., 15, 1701.

Cooper, C.E. and Nelson, D.H. (1962). J. Clin. Invest., 41, 1599

Coore, H.G. and Randle, P.J. (1964) Biochem. J., 93, 66.

Crane, C.W., Picou, D., Smith, R. and Waterlow, J.C. (1977) Br. J. Surg., 64, 129.

Criss, W.E. and Murad, F. (1976). Cancer Res., 36(5), 1714.

Crockson, R.A., Payne, C.J., Ratcliff, A.P. and Soothill, J.F. (1966). Clin. Chim. Acata., 14, 435.

Curreri, A.R. (1962). Cancer. Chemother. Rep., 16, 123.

Curreri, A.R., Ansfield, F.J., McIver, F.A., Wiseman, H.A. and Heidelberger, C. (1958). Cancer Res., 18, 478.

Curzon, G., Friedel, J. and Knott, P.J. (1973). Nature (London), 243, 198.

Curzon, G. and Knott, P.J. (1975). Brit. J. Pharmaco., 54, 389.

Curzon, G., Friedel, J., Kantamaneni, B.D., Greenwood, M.H. and Lader, M.H. (1974). Clinical. Science and Molecular Medicine, 47, 415.

Cuthbertson, D.P. (1930). Biochem. J., 24, 1244.

Cuthbertson, D.P. (1932). Quart. J. Med., 1, 233.

Cuthbertson, D.P. (1942). Lancet, 1, 433.

Cuthbertson, D.P. (1964). In "Mammalian Protein Metabolism" ed. Munro, H.N. and Allison, J.B., p. 373, Academic Press, New York

Cuthbertson, D.P., Smith, C.M. and Tilstone, W.J. (1968). Br. J. Surg., 55, 513.

Cuthbertson, D.P. and Tilstone, W.J. (1969) Adv. Clin. Chem., 12, 9.

Cuthbertson, D.P. and Tompsett, S.L. (1935). Br. J. exptl. Path., 16, 491

Dale, G., Young, F., Latner, A.L., Goode, A., Tweedle, D. and Johnston, I.D.A. (1977). Surg., 81, 295

Davidson, S. and Passmore, R. (1969). "Human Nutrition and Dietetics" p. 611, Livingstone, London.

Davidson, S., Passmore, R., Brock, J.F. and Truswell, A.S. (1975) "Human Nutrition and Dietetics", 6th ed., Churchill, London.

Davies, J.W.L. (1976). In "Plasma Protein Turnover", eds. Bianchi, R., Mariani, G. and McFarlane, A.S. p. 404, McMillan Press, London, Basingstoke.

Davies, H.E.F., Jepson, R.P. and Black, D.A.K. (1956) Clin. Sci., 15, 61.

Davies, J.W.L. and Lilgedahl, S.O. (1976). In "Metabolism and the Response to Injury" eds. Wilkinson, A.W. and Cuthbertson, D. p. 300, Pitman Medical, Tunbridge Wells, Kent.

Davies, J.W.L., Liljedahl, S.O. and Reizenstein, O. (1970) Injury, 1, 178.

Davies, I.J., Mussa, M. and Dormandy, T.L. (1968) J. Clin. Pathol., 21, 359.

Dean, R.F.A. and Schwartz, R. (1953). Br. J. Nutr., 7, 131.

Degeorge, F.V. and Brown, R.R. (1970) Cancer, 767

Denkla, W.D. and Dewey, H.K. (1967). J. Lab. Clin. Med., 69, 160.

Denoix, P.F. and Gelle, X. (1955). Cancer, 42, 247.

Dickinson, J.C., Rosenblum, H. and Hamilton, P.B. (1965) Paediatrics, 36, 2.

Dickerson, J.W.T. and Pao, S.K. (1975). J. Neurochem., 25, 559.

Dreyfus, P.M. (1962). New Eng. J. Med. 267, 596.

Drucker, W.R., Miller, M., Craig, J.W., Jeffries, W.M., Levey, S. and Abott, W.T. (1953). Surgery Forum 3, 548.

Dudrick, S.J. and Rhoads, J.E. (1972). In "Text book of Surgery" ed. Sabiton, D., p. 147, Saunders, Philadelphia.

Duckworth, J. and Warnock, G.M. (1942). Nutr. Abstr. Rev., 12, 167.

Duke, J.H.J.R., Jorgensen, S.B., Long, C.L., and Kinney, J.M. (1970). Surgery, 68, 168.

Duncombe, W.G. (1964). Clin. Chemical. Acta., 9, 122.

Ellis, F.H. (1972) In "Textbook of Surgery" ed. Sabiston, D.C. Jr. p. 718, Saunders, Philadelphia.

Engel, F.L., Winton, M.G. and Long, C.N.H. (1943). J. Exptl. Med., 77, 397.

Espiner, E.Q. (1966). J. Endocr., 35, 29.

Evans, E.I. and Butterfield, W.J.H. (1951). Ann. Surg., 134, 588.

Exton, J.H. (1972). Metabolism, 21, 945.

Exton, J.H., Lewis, S.B., Robison, G.A. and Park, C.R. (1971). Ann. N.Y. Acad. Sci, 185, 85.

Exton, J.H. and Park, C.R. (1968). J. Biol. Chem., 243, 4189.

Fahl, W.E., Rose, D.P., Liskowski, L. and Brown, R.R. (1974) Cancer 34, 1691.

Felig, P. and Wahren, J. (1971). J. Clin. Invest., 50, 1702.

Fell, G.S. and Burns, R.R. (1976). In "Metabolism and the Response to Injury", eds. Wilkinson, A.W. and Cuthbertson, D.P., p. 307, Pitman, Medical, London.

Fell, G.S. and Canning, E. (1971). Proc. Nutr. Soc., 30, 40A.

Fernstrom, J.D. and Wurtman, R.J. (1971). Science, 174, 1023.

Fernstrom, J.D. and Wurtman, R.J. (1972) Science, 178, 414.

Fisher, J.E. (1977). In "Nutritional Aspects of Care in the Critically Ill" eds. Richards, J.R. and Kinney, J.M. p. 471, Churchill, Livingstone.

Fisher, G.L., Byer, V.S., Shifrine, M., Levin, A.S. (1976). Cancer, 37, 356.

Flear, C.T.G. and Clarke, R. (1955). Clin. Sci. 14, 575

Fleck, A. (1976). In "Metabolism and the Response to Injury" eds. Wilkinson, A.W. and Cuthbertson, D.P. p. 44, Pitman Medical Inc., Tunbridge Wells, Kent.

Folin, O. (1905). Am. J. Physiol., 13, 117.

Foster, D.W. (1967). J. Clin. Invest., 46, 1283.

Franklin, R.H., Burn, J.I. and Lynch, G. (1964). Br. J. Surg., 51, 178.

Fredholm, B.B. (1970). Acta. Physiol. Scand. Suppl. 354, 5.

Furst, P., Bergstrom, J., Vinnars, E., Schildt, B. and Holstrom, B. (1978). In "Advances in Parenteral Nutrition" ed. Johnston, I.D.A. p. 107, MTP press, London.

Gailani, S., Murphy, G., Kenny, G., Nussbaum, A. and Silvernail, P. (1973). Cancer Research, 33, 1071.

Garcia, A., Williamson, J.R., and Cahill. (1966). Diabetes, 15, 188.

Gentill, V., Lader, M.H., Kantamaneni, B.D. and Curzon, G. (1977) Clinical Science and Molecular Medicine, 53, 227.

Gerlach, K., Morowitz, D.A. and Kirsner, J.B. (1970). Gastroent, 59, 567.

Gerst, P.H., Porter, M.R. and Fishman, R.A. (1964). Ann. Surg., 159, 402.

Giddings, A.E.B. (1974). Br. J. Surg., 61, 787.

Giddings, A., O'Connor, K.J., Rowlands, B.J., Mangnull, D. and Clark, R.G. (1975). Brit. Med. J. 1, 570.

Gill, G.V., Prudhoe, K., Cook, D.B. and Latner, A.L. (1975). Brit. J. Surg., 62, 441.

Glicksman, A.S. and Rawson, R.W. (1956) Cancer, 9, 1127.

Goldenberg, I.S., Lutwak, L., Rosenbaum, P.L. and Hayes, M.A. (1956). Surg. Gynecol. Obstet., 102, 129.

Goldfeder, A. (1933). Z. Krebsfarsch. 40, 394.

Goodlad, G.A.J. (1964). In "Mammalian Protein Metabolism" eds. Munro, H.N. and Allison, J.B. p. 415, Academic Press, New York.

Gornal, A.G., Bardawill, C.J. and David, M.M. (1949). J. Biol. Chem., 177, 751.

Govier, W.M. (1943). J. Pharmacol. Exp. Therap., 77, 40.

Govier, W.M. and Greer, C.M. (1941). J. Pharmacol. Exp. Therap., 72, 317.

Graham, E.A. (1933). J. Amer. Med. Assoc. 101, 1371.

Green, H.N., Stoner, H.B., Whiteley, H. and Eglin, D. (1949). Clin. Sci., 8, 65.

Greenstein, J.P. (1954). "Biochemistry of Cancer" ed. Greenstein, J.P. p. 556, Academic Press Inc., New York.

Greenwood, Mayor (1926). British Ministry of Health Reports on Public Health and Medical Subjects, No. 33, London.

Hagarty, G. (1960). Med. J. Aust., 47(2): 241.

Halme, A., Pekkarinen, A. and Turnon, M. (1957). Acta. Endocrinol. 24, Supp. 32.

Hallberg, D. (1965). Acta Physiol. Scand., 65, 153.

Harland, W.A., Orr, J.S. and Richards, J.R. (1972). Scot. Med. J., 17, 92.

Harvey, R.F. (1971). Lancet, 1, 208.

Hawk, P.B. and Gies, W.J. (1904). Amer. J. Physiol., 11, 171.

Hayes, M.A. and Brandt, R.L. (1952). Surgery, 32, 819.

Heckman, B.A. and Walsh, J.H. (1967). New Eng. J. Med., 276, 1082.

Heding, L.G. (1971). Diabetologia, 7, 10.

Henderson, J.F. and Le Page, G.A. (1959). Cancer Res. 19, 887.

Herbert, J.D., Coulson, R.A. and Hernandez, T. (1966). Comp. Biochem. Physiol., 17, 583.

Higginson, J. and Oettle, A.G. (1960). J. Nat. Cancer Inst., 24, 589

Hill, G.L., Blackett, R.L., Pikford, I., Burkenshaw, L., Young, G.A., Warren, J.V., Scharah, C.J. and Morgan, D.B. (1977). Lancet, 1, 689

Hinton, P., Allison, S.P., Littlejohn, S. and Lloyd, J. (1971). Lancet, 1, 767.

Holm, I. (1962). Acta. Chir. Scand. Suppl. 325, 108.

Houck, J.C. and Berman, L.B. (1958). J. Appl. Physiol., 12, 473.

Howard, J.E., Bigham, R.S. Jr., Eisenberg, H., Wagner, D. and Bailey, E. (1946). Bull. Johns Hopkins Hosp., 78, 282.

Howard, J.E., Bigham, R.S. Jr., Winternitz, J., Parson, W. and Eisenburg, H. (1944). Bull. Johns. Hopkins Hosp., 75, 209.

Howard, J.E., Parson, W., Stein, K.E., Eisenburg, H. and Reidt, V. (1944). Bull. Johns. Hopkins Hosp., 75, 156.

Howard, J.E., Wintermitz, J., Parson, W., Bigham, R.S. Jr., and Eisenberg, H. (1944). Bull. Johns Hopkins Hosp. 75, 209.

Hueper, W.C. (1959). Acta. Un. Int. Cancer, 15, 424.

Hume, D.M., Bell, C.C., and Barter, F. (1962). Surg., 52, 174.

Hunter, (1794) "A Treatise on the Blood, Inflammation and Gunshot Wounds", Nicol, London.

Ingel, D.J. (1952) J. Endoer., 8, 23.

James, A.G. (1966). Cancer Prognosis Manual., New York, Am. Cancer Society.

Jeejeebhoy, K.N., Anderson, G.H., Nakhooda, A.F., Greenberg, G.R., Sanderson, I. and Marliss, E.B. (1976). J. Clin. Invest., 57, 125.

Jones, J.E., Manalo, R. and Flink, E.B. (1967). J. Clin. Nut., 20, 632.

Johnston, I.D.A. (1964). Ann. Royal Coll. Surgery, 35, 270.

Johnston, I.D.A. and Bell, T.K. (1965) Proc. Roy. Soc. Med., 58, 1017.

Johnston, I.D.A. (1967) Bri. J. Surg., 54, 438.

Johnston, I.D.A. (1972). Adv. Clin. Chem. 15, 255.

Johnston, I.D.A. (1974). In "Parenteral Nutrition in Acute Metabolic Illness", ed. Lea, M., p. 214, Academic Press Inc., N.Y.

Johnston, I.D.A., Marino, J.D. and Stevens, J.Z. (1966) Br. J. Surg. 53, 885.

Jurgensen (1885) quoted by Hawk and Gies.

Khachadurian, A., Knox, W.E., Cullen, A.M. (1960) J. Lab. Clin. Med. 56, 321.

Kibler, R.F., Taylor, W.J., and Myers, J.D. (1964) J. Clin. Invest., 43, 904.

Kinney, J.M. (1959) Metabolism, 8, 809

Kinney, J.M. (1977) In "Nutrition Aspects of Care in the Critically Ill" eds. Richards, J.R. and Kinney, J.M. p. 95, Churchill Livingstone, London.

Kirby, R. and Johnston, I.D.A. (1971). Br. J. Surg., 58, 305.

Kinney, J.M., Duke, J.M., Long, C.L. and Gump, F.E. (1970) J. Clin. Pathol., 23, Suppl. 4, 65.

Kinney, J.M., Long, C.L. and Duke, J.H. (1970). In "Energy Metabolism in Trauma" eds. Porter, R. and Knight, J. p. 103, Churchill, London.

Knott, P.J. and Curzon, G. (1972). Nature (London), 239, 452.

Koj, A. (1970). In "Energy Metabolism in Trauma" eds. Porter, R. and Knight J. p. 79, Churchill, London.

Kouwenaar, W. (1950). Symposium on geographical pathology and demography of cancer- sponsored by the W.H.O. (cited by Akerman and del Regato, 1970)

Krebs, E.G., Delange, R.J., Ken, R.G. and Riley, W.D. (1966) Pharmacol. Rev., 18, 163.

Laurence, D.J.R. and Neville, A.M. (1976). In "Scientific Foundations of Oncology" eds. Symington, T. and Carter, R.L. p. 597, William Heinemann Medical Books Ltd., London.

Law, D.K., Dudrick, S.J. and Abdou, N.I. (1973) Ann of Internal Medicine, 79, 545

Lea, H.A. (1974). In "Parenteral Nutrition in Acute Metabolic Illness" ed. Lea, H.A. p. 279, Academic Press, New York.

Lefebvre, P. (1965). Ann. Endocr. (Paris), 26, 602.

Lefebvre, P. (1966). Diabetologia, 2, 130.

Lepisto, P.V. (1976) Journal of Trauma, 16, 52.

Levenson, S.M., Green, R.W., Taylor, F.H.L., Robinson, P., Page, R.C., Johnson, R.T. and Lund, C.C. (1946). Ann. Surg., 124, 840.

Levine, R.J. (1974). In "Diseases of Metabolism" eds. Bondy, P.K. and Rosenberg, L.E. .p. 1651, Sanders, Philadelphia.

Liaurdo, J.G. (1955), Lancet, 1, 1295.

Lindsey, A., Santeusanio, F., Braaten, J., Faloona, G.R. and Unger, R.H. (1975). Am. Med. Asso. 18, 227.

Lipsett, M.B. (1965) Cancer Res., 25, 1068.

Lipsett, D., Madras, B.K., Wurtman, R.J. and Munro, H.N. (1973) Life Science, 12, 57.

Long, C.L., Jeevanandam, M., Kim, B.M., and Kinney, J.M. (1977) Am. J. Clin. Nutr., 30, 1340.

Long, C.L., Spencer, J.L., Kinney, J.M. and Geiger, J.W. (1971) J. Appl. Physiol., 31, 102.

Madison, L.L., Mebone, D., Unger, R.H. and Lochner, A. (1964) J. Clin. Invest., 43, 408.

Manchester, K.L. (1968) In "Biological Basis of Medicine", eds. Bittar, E.E. and Bittar, N., Vol. 2, p. 221, Academic Press, New York

Manchester, K.L. (1970). Biochem. J., 117, 457

Marcial, V.A., Tome, J.M., Ubinus, J., Boch, A., and Correa, J.N. (1966) Radiology, 67, 231.

Mariani, G., Strober, W., Keiser, H., and Waldmann, T.A. (1976) Cancer, 38, 854.

Marks, R.A. and Bishop, J.S. (1957) J. Clin. Invest., 36, 254.

Marliss, E.B., Aoki, T.T., Unger, R.H., Soeldner, J.S. and Cahill, G.F. Jr. (1970). J. Clin. Invest. 49, 2256.

Martin, C.H. and Robinson, R. (1922). Biochem. J. 16, 407.

Martinez, I. (1964). Cancer, 17, 1278

Mathews, R.W. and Schnabel, T.G. (1935) J.A.M. Ass., 105, 1591.

Mattingly, D. (1962) J. Clin. Path., 15, 374

Mayhew, D.A., Wright, P.H. and Ashmore, J., (1969) Pharmacol. Rev., 21, 183.

Meguid, M.M., Brennan, M.F., Aoki, T.T., Muller, W.A., Ball, M.R. and Moore, F.D. (1973). Surg. Frum. 24, 97.

Mider, G.B. (1951). Cancer Res., 11, 821.

Mider, G.B., Alling, E.L. and Morton, J.J. (1950) Cancer 3, 56.

Miller, L.L. (1962). In "Amino Acid Pools" ed. Holden, J.T. p. 708, Elsevier Publishing Inc. Amsterdam.

Moore, F.D. (1954) Ann. Surg., 139, 253.

Moore, F.D. (1959). "Metabolic Care of the Surgical Patients", ed. Moore, F.D., Saunders, P., Philadelphia.

Moore, F.D., Steenburg, R.W., Ball, M.R., Wilson, G.M. and Myrden, J.A. (1955). Ann. Surg., 141, 145.

Moran, W.H., Rosenberg, J.C. and Zimmerman, B. (1959). Surg. Forum, 9, 120.

Munro, H.N. (1966). In "Wound Healing" ed. Illingworth, Sir Charles, Churchill, London.

Munro, H.N. (1970). In "Mammalian Protein Metabolism" ed. Munro, H.N. vol. 4, p. 299, Academic Press, New York.

Munro, H.N. and Cuthbertson, D.P. (1943). Biochem. J. 37, 12.

McMenamy, R.H. and Oncley, J.L. (1958) J. Biol. Chem., 233, 1436.

McCance, R.A. (1936) Proc. Roy. Soc. Med., 119, 245.

Nadal, J.W., Pederson, S., Maddock, W.G. (1941). J. Clin. Invest., 20, 691.

Naish, J.M. and Read, A.E.A. (1974). "Basic Gastroenterology" ed. Naish, J.M. and Read, A.E.A., p. 24, J. Wright Inc. Bristol.

Nakayama, K. (1964). Clin. Radiol., 15, 232.

Natelson, S. (1963). "Microtechniques of Clinical Chemistry" Charls Thomas, Springfield, Illinois.

Nature, (1973) "News and Views", Nature, 246, 186.

Neeley, W.E., Pollack, M. and Cupas, C.A. (1975). Clin. Biochem., 8, 273.

Neethling, A.L. and Shanley, B.C. (1976). Lancet, ii, 578.

O'Keefe, J.D., Sender, P.M. and James, W.P.T., (1974). Lancet, 2, 1035.

Ochsner, A. and De Bakey, M. (1941). J. Thorac. Surg., 10, 401.

Oettle, A.G. (1963). S. Afr. Med. J., 37, 957.

Owen, J.A. (1967). In "Advances in Clinical Chemistry" ed. Sobotka, H. and Stewart, C.P., p. 9, Academic Press, New York.

Parker, E.F. and Gregorie, H.B. Jr. (1967). "Current Problems in Surgery", p. 28, Year Book Medical Publishers, Inc., Chicago.

Paul, M.J., Cramer, H. and Goodwin, F.K. (1971). Arch. Gen. Psychiat., 24, 327.

Peaston, M.J.T. (1974). In "Parenteral Nutrition in Acute Metabolic Illness" ed. Lee, H.A., p. 139, Academic Press Inc., New York

Pekkarinen, A. (1960) Am. J. Cardiol. 5, 604.

Peters, J.P. (1948). Am. J. Med., 5, 100.

Pitts, R.F. (1963). "Physiology of the kidney and body fluids", 2nd ed. Year Book Medical Publishers, Chicago.

Porte, D., Graber, A.L., Kuzuya, T. and Williams, R.H., (1966), J. Clin. Invest., 45, 228.

Porter, D., Jr. (1969). Arch. Intern. Med., 123, 252.

Prendergast, J.J., Fenichel, R.L. and Daly, B.M. (1952). Arch. Surg., 64, 733.

Randall, H.T. (1975). In "Manual of Surgical Nutrition" eds. Ballinger, W.F., Collins, J.A., Drucker, W.R., Dudrick, S.J. and Zeppa, R., Saunders, Philadelphia.

Randall, H.T. (1976). In "Modern Nutrition in Health and Disease" eds. Goodhart, R.S. and Shils, M.E. 5th ed. p. 950, Lea and Febiyer, Philadelphia.

Randle, P.J., Garland, P.B., Hales, C.N. and Newsholme, E.A., (1963). Lancet 1, 785.

Ravdin, I.S., McNamee, H.G., Kamholz, J.H. and Rhoads, J.E. (1944). Arch. Surg., 48, 491.

Rhoads, J.E. and Alexander, C.E. (1955). Ann. NY Acad. Sci., 63, 268.

Robbins, S.L. (1974). "Pathologic Basis of Disease" ed. Robbins, S.L. Saunders Company, Philadelphia.

Roberts, K.E., Randal, H.T., Philbin, P. and Lipton, R. (1954) Surgery, 36, 599.

Robison, G.A., Butcher, R.W. and Sutherland, E.W. (1971). "Cyclic AMP. Academic Press, New York.

Rose, D.P. (1967). Lancet, 4, 239.

Ross, H., Johnston, I.D.A., Welborn, T.A. and Wright, A.D. (1966) Lancet, 2, 563.

Roth, J.S. (1968). In "Methods in Cancer Research" ed. Bush, H., p. 153, Academic Press, New York.

Rouser, G., Kelly, K., Samuels, A.J., Jelinek, B. and Heller, D. (1962) In "Amino Acid Pool", ed. Holden, J.T., p. 373, Elsevier, Amsterdam.

Russell, R.C.G., Walker, C.J. and Bloom, S.R. (1975). Brit. Med. J. 1, 10.

Salter, J.M., Tzrin, C., Laidlaw, J.C. and Gornall, A.G. (1960) Metabolism, 9, 753.

Samols, E. and Marks, V. (1965). Lancet, 1, 462.

Sandberg, A.A., Eik-Nes, K., Samuel, S.T., and Tyler, F.A. (1954) J. Clin. Invest., 33, 1509.

Sass-Kortsak, A. (1965). Adv. Clin. Chem. 8, 36.

Sawyer, R.B., Drew, M.A., Gesink, M.H., Sawyer, K.C. Jr. and Sawyer, K.C. (1970). Arch. Surg. 100, 343.

Schoemaker, W.C. and Van Ittallie, T.B. (1960) Endocrinology, 66, 260.

Schonheyder, F., Bone, J. and Skjoldborg, H. (1974) Acta. Chir. Scand., 140, 271.

Schreier, K. and Karch, H.L. (1954 - 1955). Arch. Kin. Chir. 280, 518.

Schultis, K. and Geser, G.A. (1970) In "Parenteral Nutrition" ed. Meng. H.C. and Law, D.H., p. 136, Charles Thomas, Springfield, Illinois.

Schwartz, A.E. and Roberts, K.E. (1957). Surgery, 42, 814.

Seelig, M.S. (1964). Am. J. Clin. Nutr., 14, 342.

Shils, M.E. (1971). Surgery Gynec. Obstet., 132, 709.

Shils, M.E. (1976). In "Modern Nutrition in Health and Disease" eds. Goodhart, R.S. and Shils, M.E. p. 981, Lea and Febiger, Philadelphia.

Shils, M.E. and Gilat, T. (1966). Gastroenterology, 50, 347.

Sigulem, D.M., Barsal, J.A., Velasco, G.G., Rosso, P. and Winick, M. (1973). Am. J. Clin. Nutr. 26, 793.

Simpson, H. and O'Duffy, J. (1967). Br. Med. J., 2, 538.

Singleton, W.S., Benerito, K.F., Brown, M.C., Lee Dit Vapani, L. and White, J.L. (1960). Metabolism, 9, 959.

Sjoerdsma, A., Weissbach, H., and Udenfriend, S. (1956) Am. J. Med. 20, 526.

Slack, N.H. (1970). Cancer, 25, 987.

Sprinson, D.B., and Rittenberg, (1949). J. Biol. Chem., 180, 715.

Sorroff, H.S., Pearson, E. and Artz, C.F. (1961). Surgery Gynec. Obstet., 112, 159.

Steinberg, D. (1966). Pharmacol. Rev., 18, 217.

Steiner, A.L., Pagliara, A.W., Chase, L.R. and Kipnis, D.M. (1972). J. Biol. Chem., 247, 1114.

Steinfeld, J.L. (1960). Cancer, 13, 974.

Stocus, P. (1966). Br. J. Cancer, 20, 595.

Sturdy, D.E. (1965). Br. J. Surg., 52, 245.

Sutherland, E.W. and Rall, T.W. (1958). J. Biol. Chem., 232, 1077.

Sutherland, E.W. and Rall, T.W. (1960). Pharmacol. Rev. 12, 265.

Sutherland, E.W., and Robison, G.A. (1966). Pharmacol. Rev., 18, 145.

Tagliamonte, A., Biggio, G., Vargiu, L. and Gessa, G.L. (1973) Life Sciences, 12, 277.

de Takats, G. (1931). Am. J. Surg., 11, 39.

Theologides, A. (1972). Cancer, 29, 484.

Theologides, A. (1977). In "Nutrition and Cancer" ed. Winick, M. p. 75, John Wiley, England.

Thomsen, V. (1938). Acta. Med. Scand. Suppl. 91.

Thompson, W.D., Ravdin, I.S. and Frank, I.L. (1938) Arch. Surg. 36, 500.

Tilstone, W.J. (1974). In "Parenteral Nutrition in Acute Metabolic Illness", ed. Lea, H.A. p. 113, Academic Press Inc., New York.

Torek, F. (1913). Surg. Gynec. Obstet., 16, 614.

Tweedle, D.E.F. and Johnston, I.D.A. (1971). Br. J. Surg., 58, 771.

Unger, R.H. (1971) Adv. Intern. Med., 17, 265.

Unger, R.H. and Orci, L. (1975). Lancet, i, 14.

Vallee, B.L., Wacker, W.E. and Ulmer, D.D. (1960). New Engl. J. Med., 262, 155.

Van Duren, B.L. (1958). J. Nat. Cancer Inst. 21, 1.

Van Goidsenhoven, G.M. T., Gray, O.V., Price, A.V. and Saunders, P.H. (1954). Clin. Sci. 13, 383.

Van Slyke, D.D., Philips, R.A., Hamilton, P.B., Archibald, R.M., Dole, V.P. and Emerson, K. Jr. (1944). Trans. Assoc. Am. Physicians, 58, 119.

Vinnars, E., Bergstrom, J. and Furst, P. (1965). Annals of Surgery, 182, 665.

Vinnars, E., Furst, P., Bergstrom, J. and von Francken, I. (1976) In "Metabolism and the Response to Injury" ed. Wilkinson A.W. and Cuthbertson, D.P., p. 336. Pitman Medical, London.

Vitek, V., Gill, W., Lang, D.J., Conn, A.K. and Cowley, R.A. (1976) Surgery, Gynerology and Obstetrics, 143, 901.

Wacker and Parisi (1968). New Eng. J. Med., 278, 658.

Wadge, J.H., De Campos, R., Kerr, A., Smith, R., Farrell, R., Ilie, V. and Williamson, D.H. (1976). Clin. Sci. and Mel. Med. 50, 393.

Wadstrom, L.B. and Wiklund, P.E. (1964). Acta. Chir. Scand., 325, 50.

Waldmann, T., Trier, J. and Fallon, J. (1963). J. Clin. Invest., 42, 171.

Waldmann, T., Broder, S. and Strober, W. (1977). In "Nutrition and Cancer", ed. Winick, M., p. 105, John Wiley, England.

Walker, W.F. (1965) Proc. R. Soc. Med., 58, 1015.

Walker, W.F., Johnston, I.D.A. (1971). The Metabolic Basis of Surgery" eds. Walker, W.F., Johnston, I.D.A., p. 13, Heinemann, England.

Walker, W.F., Watt, A., Morgan, H.G. and McCowan, M.A.A. (1964) Br. J. Surg., 51, 783.

Warren, R.L., Jelliffe, A.M., Watson, J.V. and Hobbs, C.B. (1969). Clin. Radiol., 20, 247.

Waterhouse, C. and Kemperman, J.H. (1971). Cancer Res., 31, 1273.

Waterlow, J.C. and Sender, P.M. (1976). In "Metabolism and the Response to Injury" eds. Wilkinson, A.W. and Cuthbertson, D.P. p. 215, Pitman Medical Inc. Tunbridge Wells, Kent.

Waterlow, J.C. and Stephen J.M.L. (1967). Clin. Science, 33, 489.

Werner, M. and Cohnen, G. (1969). Clin. Sci., 36, 173.

Werner, S.C., Habif, D.V., Randall, H.J. and Lokwood, J.S. (1951) Surg. Forum.

Werner, W.H.G. and Wirlinger, H. (1970). Z. Analyt. Chem. 252, 224.

White, F.R. (1945). J. Natl. Cancer Inst. 5, 265.

Widdowson, E.M. and Dickerson, J.W.T. (1964). "Chemical Composition of the Body" eds. Widdowson, E.M. and Dickerson, J.W.T. p. 39, Academic Press, New York.

Wicland, O. (1968). Adv. Metab. Dis. 3, 1.

Wilkinson, A.W. (1969). "Body Fluids in Surgery" ed. Wilkinson, A.W., p. 61, 89.

Wilmore, D.W., Lindsey, C.A., Moylan, J.A., Faloona, G.R., Pruitt, B.A. and Unger, R.H. (1974b). Lancet, 1, 73.

Wilmore, D.W., Long, J.M., Mason, A.D. and Pruitt, B.A. (1976) In "Metabolism and the Response to Injury" eds. Wilkinson, A.W. and Cuthbertson, D.P. p. 274, Pitman Medical Inc. Tunbridge Wells, Kent.

Wilmore, D.W., Long, J.M., Mason, A.D., Skreen, R.W. and Pruitt, B.A. (1974a). Annals. of Surgery, 180, 653.

Wilmore D.W., Moylan, J.A., Helmkamp, G.M. and Pruitt, B.A. (1973) Annals. of Surgery, 178, 503.

Wilmore, D.W., Mason, A.D. Jr. and Pruitt, B.A. Jr. (1976). Annals of Surgery, 183, 314.

Wilson, H., Lovelace, J.R. and Hardy, J.D. (1955). Annals of Surgery, 141, 175.

Winkler, A.W., Danowski, T.S., Elkinton, J.R. and Peters, J.P. (1944). J. Clin. Invest., 23, 807.

Wiseman, G. and Chadially, F.N. (1958). Brit. Med. J. 11, 18.

Woeber, K.A. and Harrison, W.A. (1971). J. Clin. Invest., 50, 378.

Wolff, H. (1956). Klin. Wochschr. 34, 409.

Woolf, L.I., Groves, A.C., Moore, J.P., Duff, J.H., Finley, R.J. and Loomer, R.L. (1976). Surgery, 79, 283.

Wootton, J.D.P. (1974). "Microanalysis in Medical Biochemistry" Churchill, London.

Wright, J.T. and Richardson, P.C. (1967). Br. Med. J., 4, 540.

Wu, C., and Bauer, J.M. (1960). Cancer Res., 20, 848.

Yoshida, O., Brown, R.R. and Bryan, G.T. (1970). Cancer, 25, 773.

Zachman, M., Cleveland, W.W., Sandberg, D.A. and Nyhan, W.L. (1966). Am. J. Diseases Children, 112, 283.

Zimmerman, B. (1972). In "Textbook of Surgery" Vol. 1., ed. Sabiston, D.C., p. 94, Saunders Company, Philadelphia.

Zimmerman, B., Casey, J.H. and Bloch, H.S. (1956). Surgery, 39, 161.