There may be no solution that is fair both to animals and to human beings but all scientists can work towards putting into effect what Russell and Burch in their admirable text on the subject describe as the three Rs of humane experimental practice: *R*eplacement of animals by non-sentient systems wherever possible; *R*efinement of experimental procedures, and *R*eduction of numbers of animals used to the very minimum that will serve a useful purpose.

The Lancet, 1981

TOTAL PARENTERAL NUTRITION

effect on hepatic function, intestinal adaptation and tumor growth

An experimental study in rats

(Invloed van totaal parenterale voeding op leverfunctie, darmadaptatie en tumorgroei in de rat)

PROEFSCHRIFT

TER VERKRIJGING VAN DE DE GRAAD VAN DOCTOR IN DE GENEESKUNDE
AAN DE ERASMUS UNIVERSITEIT ROTTERDAM
OP GEZAG VAN DE RECTOR MAGNIFICUS
PROF. DR. M.W. VAN HOF
EN VOLGENS BESLUIT VAN HET COLLEGE VAN DEKANEN.
DE OPENBARE VERDEDIGING ZAL PLAATSVINDEN OP
WOENSDAG 11 JANUARI 1984 TE 14.00 UUR.

DOOR

ROELOF UBBO BOELHOUWER

geboren te Semarang (Indonesië)

PROMOTOREN: PROF. DR. D.L. WESTBROEK

PROF. DR. H. VAN HOUTEN

The work described in this thesis was performed under the supervision of Dr. R.A. Malt, Professor of Surgery, in the Surgical Services of the Shriners Burns Institute and the Massachusetts General Hospital, Department of Surgery, Harvard Medical School, Boston, Massachusetts, USA.

In memory of my parents

To Charlotte,

Bobby, Dirk-Jan, and Michiel

CONTENTS

CHAPTER I

INTRODUCTION

1.1 Motivation

Within the past two decades total parenteral nutrition (TPN) has become an acceptable mode of treatment for the patient who cannot be adequately nourished by the oral route. Energy fuels and essential nutrients can be delivered intravenously to major organs of the body for utilization. However, certain metabolic limitations exist. Hepatic dysfunction, for instance, is a common but poorly understood metabolic complication of TPN. Hepatic changes consisting of fatty metamorphosis and progressive intrahepatic cholestasis develop during TPN. Associated biochemical changes include increased serum alkaline phosphatase, bilirubin and SGOT levels.

Essential fatty acid deficiency, calorie or protein excess, amino acid deficiencies or excess, intralipid and toxic effects of certain amino acids have all been postulated as causative factors for hepatic dysfunction.

It has also been suggested that amino acid imbalance may be a causative factor.Harper defined amino acid imbalance as a "change in the proportions of amino acids in a diet which results in a depression of food intake or growth rate that can be completely prevented by a supplement of the indispensible amino acid present in least amount in the diet in relation to the amount required for optimal performance".

Experimental animals, fed a diet devoid of a single indispensible amino acid or diets with amino acid imbalance adapted themselves by decreasing their food intake. However, when they were force-fed with these diets histopathological changes in the liver ensued.

With conventional parenteral feeding, instead of nutrients entering the body via the portal venous system, the infused nutrients are first exposed to "peripheral tissues". Therefore, amino acid imbalance may occur due to prior uptake of certain amino acids by the metabolically-active peripheral tissues (e.g. muscles) before release of amino acids from the periphery for hepatic utilization. If TPN solution infused by portal vein reduces hepatic dysfunction, it would support the theory that amino acid imbalance due to uptake of essential amino acids by peripheral tissues is a cause of hepatic dysfunction. However, if TPN solution given by systemic vein, portal vein or enteral route shows similar degree of hepatic dysfunction, then it would suggest that force-feeding of TPN can result in hepatic dysfunction unrelated to the route of delivery. In that case, hepatic dysfunction can be due to the direct toxic effect of TPN solution or to the force-feeding of a

high calorie, low quality protein diet comparable to that consumed by patients with Kwashiorkor's disease.
Lipid in the form of fat emulsion has been increasingly used next to glucose for the energy supply in TPN. Several studies have demonstrated that weight gain and positive nitrogen balance can be obtained by either fat or glucose-based TPN, although fat-based TPN may be less efficient. It has been suggested that a fatty infiltration of liver-tissue associated with glucose-based TPN can be reversed by using fat as part of the energy source.
However, there are reports that show fat accumulation in the liver of animals and in the lung in the pre-term infant when fat is given as a major caloric source.
In summary, the efficacy of fat-based TPN compared to glucose-based TPN still has to be evaluated and their effects on liver function and structure have to be studied.
In humans massive intestinal resection in the neonatal period may result in poor physical and mental development during the subsequent periods of time. Neonates that undergo major abdominal surgery without intestinal resection fare better than those after intestinal resection. It appears that the interference with nutrition after resection may be responsible for the poor physical and intellectual development.
Intravenous nutrition diminishes the adaptive response of the gut to intestinal resection in the adult as well as in the young rat. The lack of nutrients passing through the gastrointestinal tract seems to be a major factor. Nutrients given orally can reinstate that response.
The elongation and villous hypertrophy seen after intestinal resection in the young growing rat could be permanently jeopardized by a delay in the starting of oral feeding. However, it is also possible that catch-up growth of the intestine occurs, even after a prolonged period of TPN.
The widely recognized syndrome of cancer cachexia is still poorly understood. Anaerobic glycolysis is high in tumor cells and becomes the main source of energy for the tumor. Increased lactic acid production has been observed in tumor bearing rats and patients with extensive malignant disease. An explanation for cancer cachexia could be the stimulation of hepatic gluconeogenesis by increased lactate production via the Cori cycle. This inefficient metabolic circuit provides a continuous supply of glucose to the tumor while the host is drained of important amino acids and other gluconeogenic substrates resulting in continuing lean tissue mass loss.
TPN has been reported to avert cancer cachexia and improve tolerance for chemotherapy and radiation. Several studies showed that TPN stimulated tumor growth in rats. However, other studies showed that fat-based TPN promoted host maintenance equivalant to carbohydrate-based TPN

without tumor stimulation. Conflicting reports have been published as to whether carbohydrate-based TPN also stimulates tumor growth. Some studies were unable to demonstrate a stimulation of tumor growth by carbohydrate-based TPN.They actually showed that hyperglycemia decreased the DNA specific activity of tumors. Furthermore other authors have observed that hyperglycemia does not induce tumor growth. The mechanism is not yet clearly understood.
An experiment using hepatoma-bearing rats was designed to answer the question: which form of total parenteral nutrition, carbohydrate-based or fat-based, best maintains host weight with minimal tumor stimulation in the syndrome of cancer cachexia?

1.2 Objectives

The objective of the experiments described in this thesis was to get an answer to the following questions:

1. Will nutrient substrates infused via the portal venous system prevent hepatic dysfunction as is associated with central venous infusion and optimize nitrogen balance and hepatic protein synthesis?
2. What is the efficacy of fat-based TPN compared to glucose-based TPN and what is their effect on liver function and liver structure in the rat?
3. What are the effects of TPN on the development and adaptive response of the intestine in the young growing rat after small bowel resection, and are these effects irreversible?
4. Which form of TPN, carbohydrate-based or fat-based, best maintains host weight with minimal stimulation of tumorgrowth in the syndrome of cancer cachexia?

Major relevant data from the literature, which have been mentioned without references so far, can be found in the articles listed at the end of this paragraph.

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CHAPTER II

SURVEY OF THE LITERATURE

2.1 Total Parenteral Nutrition

The advantages of administering nutrients parenterally as a means for correction or prevention of malnutrition have been recognized for centuries. Nonetheless, most attempts to do so either failed or were not practical. These attempts included infusions of a mixture of protein hydrolysates, glucose and olive oil [96] or infusions of wine or various oils [73]. The following three scientific achievements contributed particularly to the succesfull application of parenteral nutrition in clinical medicine today:

1. The preparation of a protein hydrolysate suitable for intravenous use by Elman in 1937 [59]. Wretlind [219] refined the production process and the modified solution was used for many years until crystalline amino acid solution became available.
2. The development by Wretlind [220] of an intravenous fat emulsion (Intralipid ®) with few side effects facilitated the administration of non protein energy.
3. The introduction of the subclavian venipuncture by a French surgeon Aubaniac [8] in the early 1950s as a means of rapidly administering blood to battle casualties, which Dudrick [55] used to overcome the technical problems associated with the administration of hypertonic glucose solutions. His investigations showed that enough protein and glucose could be infused to promote normal growth in puppies as well as in infants by the use of catheters placed in the superior vena cava. The work of Dudrick and colleagues [55] has very significantly contributed to the widespread use of TPN.

Since 1968 many patients with unique nutritional needs resulting from a variety of debilitating physical conditions have benefitted from total parenteral nutrition.

In some instances parenteral nutrition may be considered a primary therapeutic modality. In such cases where normal oral intake ceases, nutritive needs can be supplied via the parenteral route. The therapeutic principle involved is that of allowing tissues or organs such as the gastrointestinal tract to rest while restorative and healing processes are enhanced by adequate nutrition provided via an alternate route.

One distinct entity which has been treated successfully in this way is enterocutaneous fistula [180]. Another area in which parenteral nutrition has been employed as primary therapy is inflammatory bowel disease [64]. Crohn's disease, or regional enteritis, and granulomatous as well as

ulcerative colitis have been treated with periods of bowel rest and parenteral nutrition [156].
Parenteral nutrition may also be used to support patients during various types of complex illness. Under such circumstances, parenteral nutrition can be thought of as being a secondary rather than a primary modality. Surgical patients will have a need for such supportive therapy more often than medical patients, as surgical patients, for a wide variety of reasons, suffer impairment of gastrointestinal tract function more frequently. Patients with acute pancreatitis may be greatly helped by supportive parenteral nutrition [75]. Operative intervention in the form of drainage of the pancreatic bed often combined with triple osteotomy may be required. In these cases prolonged ileus is the rule, and further absolute gastrointestinal tract rest may be helpful in allowing the inflammatory processes to subside [203].
The burned patient also presents special nutritional problems. The loss of integrity of the skin and subsequent inability to retain heat leads to a substantial increase in caloric expenditure. This nutritive problem is compounded by the electrolyte and protein loss that also occurs. Many patients with major burns may continue oral intake, but this is rarely sufficient to meet the increased caloric needs imposed by the so-called hyper-catabolic state. The burn victim is an example of a patient who may require true hyperalimentation in the form of greater than normal quantities of calories [216].
Patients receiving chemotherapy and/or radiotherapy for cancer often suffer debilitating side effects from these treatments which can be severe enough to prevent continuation of the therapy. The side effects of cancer chemotherapy may be ameliorated by supportive parenteral nutrition, which provides a normal or relatively normal nutritonal status and allows the continuation of the therapy in the presence of anorexia and nausea [41]. In addition, patients receiving radiotherapy to the region of the abdomen may develop inflammatory processes within the gastrointestinal tract itself, which may interfere with oral nutrition. Patients in the latter category may also be treated with bowel rest and supportive parenteral nutrition.

2.2 Complications of Total Parenteral Nutrition

Succesful central venous parenteral nutrition is not easy to achieve, and is not obtained if it is approached simply as another form of routine fluid therapy. Most have found that success is more likely if the responsibility for administering this form of nutritional support is delegated to a parenteral nutrition support team. For safe parenteral nutrition in

patients biochemical and microbiological laboratory facilities are essential. There are two general types of complications associated with parenteral nutrition: those related to the indwelling catheter (catheter related complications) and those related to the infusate (metabolic complications) (Table 2.I).

Table 2.I Metabolic and Catheter-related Complications of TPN

Complications	Usual cause
CATHETER-RELATED COMPLICATIONS	
Disorders related to catheter site	
Malposition	Failure to confirm site of catheter tip
Pneumothorax	
Hemothorax	
Disorders related to use of catheter	
Sepsis	Inadequate catheter and catheter exit site care
Thrombosis	Unknown: pump dysfunction
Catheter dislodgement	Unknown
Perforation and/or infusion leaks	More common with polyvinyl catheters
METABOLIC COMPLICATIONS	
Disorders related to metabolic capacity of patient	
Hyperglycemia	Excessive intake (either excessive concentration or increased infusion rate) Change in metabolic state (e.g., sepsis, surgical stress
Hypoglycemia	Sudden cessation of infusion
Azotemia	Excessive nitrogen intake
Electrolyte disorders	Excessive or inadequate intake
Mineral disorders	Excessive or inadequate intake
Vitamin disorders	Excessive or inadequate intake
Essential fatty acid deficiency	Inadequate intake
Disorders due to infusate components	
Metabolic acidosis	Use of hydrochloride salts of cationic amino acids
Hyperammonemia	Inadequate arginine intake
Abnormal plasma aminograms	Amino acid pattern of intake
Hepatic disorders	Unknown

Catheter related complications include thrombosis, including superior caval vein thrombosis, dislodgement of the catheter, perforation, infection, pneumothorax, and hemothorax. The clinical incidence of thrombosis from central venous catheterization is less than 5 percent, but as high as 25 percent in autopsy studies [164].
Burt et al.[24] used elective venograms to evaluate prospectively the appearance of fibrin sleeves, significant venous narrowing or complete thrombosis distal of the catheter. They found a 25 percent incidence of clinically undetected central venous thrombosis.
However the most common complication of intravenous alimentation has been infection. Infection rates of 40 to 50 percent have been reported [47].
The most common metabolic problems associated with TPN are due to glucose intolerance and ion deficiencies. These can be managed successfully by close monitoring of the patient with routine analysis of blood chemistry. Judicious regulation of fluid rates and addition of insulin and ions when necessary relieve most problems.
Other metabolic complications are related to the differences between enteral and parenteral requirements and the fact that the appropriate mixture of infusate components is not as yet known [95].

2.3 Nutrient Infusate

The nutrient infusate should include an amino acid source as well as an energy source, electrolytes, minerals, and vitamins.

2.3.1 *Amino Acids*

Hydrolysates of fibrin and casein as well as crystalline amino acid mixtures have been used successfully as the nitrogen source for parenteral nutrition. The crystalline amino acid mixtures are used more commonly, but there is no evidence showing that they are a more efficient nitrogen source. These mixtures permit a more rigidly controlled intake of protein precursors than do the hydrolysates. Although the magnitude of nitrogen retention seems to be related directly to the magnitude of nitrogen intake, amino acid intakes ranging from 0,5 to 1 g/kg/day results in nitrogen retention comparable to that observed in enteral-fed normal men [92]. Administration of amino acids to a dehydrated patient or to a patient with hepatic insufficiency may result in serum amino acid imbalance and prerenal azotemia. Hyperammonemia also occurs, especially in patients with hepatic disorders. Crystalline amino acids are usually better tolerated if hyperammonemia occurs with casein

hydrolysates or in patients with liver disease, since there is almost no free ammonia in crystalline amino acids [56]. To ensure an adequate calorie to nitrogen ratio avoiding the problems associated with excess glucose administration [7,130], ratios of at least 150 non-protein calories per gram of nitrogen have been recommended.

2.3.2 *Energy Requirement*

A satisfactory estimate of basal caloric requirement or basal energy expenditure can be calculated from the formulas that Harris and Benedict derived over 60 years ago from the results of indirect calorimetry [90] (Table 2-II).
Their results correlate well with the values obtained with contemporary techniques of continuous expired air analysis in healthy subjects [127]. The Harris-Benedict equations take into account sex, height, age, and weight. If the patient has recently lost more than 10 percent of his weight, his original weight is entered into the equation [135].

WOMEN

$$BEE = 655 + (9.6 \times W) + (1.8 \times H) - (4.7 \times A)$$

MEN

$$BEE = 66 + (13.7 \times W) + (5 \times H) - (6.8 \times A)$$

Table 2.II Harris-Benedict Equations for Calculation of Basal Energy Expenditure (BEE). W denotes actual or usual weight in kg, H height in centimeters, and A age in years.

2.3.3 *Energy Source*

Either carbohydrate alone or carbohydrate in combination with fat can be used as the caloric source. TPN solutions are usually administered with final carbohydrate concentrations of 20 percent to 50 percent, making them extremely hyperosmolar. Therefore they must be administered into a large-caliber vein, such as the superior vena cava to avoid intimal irritation. Because the caloric value of fat is twice that of carbohydrate, and because emulsified fat does not exert an osmotic pressure, it is possible to administer a large number of calories in small volumes of a fat emulsion through peripheral veins. Fat is not only a valuable, physiological non-protein energy source but also a necessary part of any diet to supply the essential fatty acids that are important to maintain the normal composition of the structural body lipids, such as phospholipids of the cell membranes. The essential fatty acids are also the precursors of the various prostaglandins that are of great physiological importance [220].

Following the infusion of fat emulsion, the fat particles are rapidly cleared from the bloodstream at the same rate as natural chylomicrons [87]. The uptake of fat particles by various organs when fat (Intralipid ®) is eliminated from the bloodstream has been studied by Rossner et al.[160]. Most of the fat is taken up by the muscles (47%), some by the subcutaneous adipose tissues (13%), splanchnic area (25%) and myocardium (14%), the liver does not show any uptake.

There have been and will certainly continue to be conflicting opinions about the optimal proportion of fat to carbohydrate in TPN. A number of comparisons of the nitrogen (N)-sparing effect of intravenous fat and glucose have been made.

One of the best defined studies is that of Jeejeebhoy et al.[106], which showed that glucose alone and lipid systems, in which 83 percent of the nonprotein energy is supplied by Intralipid ®, can both produce a sparing effect on N balance. Broviac [21] used a lower fat content, 35 percent of nonprotein energy, without noticing any difference compared with carbohydrate alone as a nonprotein source of energy. Other studies have shown that exogenous fat supplied without glucose and amino acids has only little N sparing effect.

On the other hand, fat as the only nonprotein energy source readily promotes protein synthesis when amino acids are supplied simultaneously [218].

Most of these comparisons have been made in patients with only slightly increased metabolism. However, there are also some investigations performed in hypercatabolic patients by Long [126] and Allison [3] showing a more favorable effect on the N balance when nonprotein energy is

administered in the form of glucose alone compared with a combination of glucose and fat.
It can be concluded from these and other studies that, provided N intake is adequate, the N balance is influenced by changes in the energy supply, when these changes are derived from glucose plus fat. In the short-term at least, glucose spares more N than fat does but the overall differences between a glucose system and a lipid system are probably small in most cases. However, different effects of the two TPN regimens on N balance may be observed in patients wo remain hypercatabolic. In these patients high intake of glucose, particularly with added insulin, may be very effective [3]. In TPN the attempt is made to meet all the nutritional requirements imposed by the existing metabolic status.
The metabolic pathways of the body have been developed in such a way that the ratio of fat to glucose supply can vary very much without important negative effects. On the other hand, in most patients, there seems to be no reason to deviate too much from the ratio of 50:50 between energy supply from fat to carbohydrate which exists in our normal food [61]. An excess of either fat or glucose should be avoided.

2.3.4 *Electrolytes, Minerals and Vitamins*

Correction of electrolyte imbalances should not be limited to the more commonly monitored electrolytes, such as potassium, sodium, chloride, phosphate, calcium, and magnesium but should include correction of imbalance and deficiencies in trace elements. Furthermore vitamin deficiencies should be corrected. Both are essential because they regulate metabolic processes in many different ways and act as co-enzymes or as essential constituents of enzyme complexes regulating the utilization of carbohydrates, proteins and fat.
In animal studies, 15 trace elements have been found to be essential for health. They are iron, zinc, copper, chromium, selenium, iodine, cobalt, mangenese, nickel, molybdenum, fluorine, tin, silicon, vanadium, and arsenic [45]. Shils [173] categorized many needed minerals in man in terms of their relative quantitive needs.

2.4 Hepatic Dysfunction During TPN

With the current increased interest in assessing the requirements for and benefits of total intravenous nutritional support in surgical patients, a multitude of questions have arisen which cannot be answered readily by studies in human beings.

Many metabolic disturbances due to parenteral nutrition have already been described above, but one of the least understood is the development of abnormalities in liver function during total parenteral nutrition.
Metabolically, the liver is a highly active organ and an impairment of its function constitutes a serious situation. Elevations in serum levels of Alkaline Phosphatase, SGOT, SGPT, and less frequently bilirubin are recognized as being associated with TPN therapy. Although the exact cause of these increases of liver enzymes and bilirubin is unknown, various factors have been implicated as being responsible, including fatty infiltration of the liver secondary to hypertonic glucose infusion [37,172], essential fatty acid deficiency [117,155], amino acid deficiencies [177] or excess [39], and toxic manifestations of certain amino acids [197]. Also suggested as causative agents are degradation products of tryptophan generated in the presence of sodium metabisulphite as an antioxidant [82]. Similar increases in hepatic enzyme levels have also been seen during artificial enteral nutrition [192].
It is very difficult to identify from the available literature what constituent of an intravenous feed might be responsible for any changes that do occur. One of the specific difficulties in interpretation is to separate in those patients in whom changes do occur, the effects of their disease process and those of the nutritional support.
Contradicting results have been reported concerning the ability of amino acid solutions to normalize the fatty infiltration of the liver after hypertonic glucose infusion in protein-depleted rats. Chang [37] infused hypertonic dextrose in rats under restrained conditions and found a gross fatty infiltration of the liver. An addition of essential amino acids did not prevent these changes. Pulito et al.[155] found gross fatty infiltration in dog-livers on fat-free TPN during positive nitrogen balance. On the other hand Steiger et al.[182] showed that fatty livers of protein-depleted rats were restored to normal by intravenous feeding of 30% glucose and 5% amino acid solution.
Several authors [106,134,157,223] have shown that the combination of carbohydrates and fat in the nutrient substrate can alleviate or prevent early fatty infiltration of liver cells and hepatic dysfunction. Kronevi [116], however, showed diffuse infiltration of droplets of fat in both parenchymal and reticuloendothelial cells of rat-liver after intravenous feeding regimens with fat, while Levene [122] reported pulmonary fat accumulation after Intralipid ® 20% infusion in the preterm infant.
Vileisis [197] investigated the development of transient cholestatic jaundice during parenteral nutrition. However, he could not demonstrate either essential fatty acid deficiency or lipid administration as the cause of parenteral nutrition-associated cholestatic jaundice. He suggested that a relation between the relative nutrient excess in TPN, containing both

protein and carbohydrate but not lipid, and cholestatic jaundice developed as a consequence of impaired bile flow.
Cohen [39] showed that incubation of guinea pig liver explants with protein hydrolysates and with certain amino acids such as glycine, leucine, threonine, and isoleucine leads to liver impairment.
Grant and associates [82] reported the histologic appearance of localized periportal fatty change in liver of man and experimental animals when they are fed parenterally. Rats receiving solutions containing tryptophan conversion products, either as a complete nutrition solution or as a single amino acid solution, demonstrated periportal fatty changes, while solutions containing no tryptophan conversion products did not cause histological abnormalities of the liver.
This phenomenon can be understood when one takes into account the time interval between manufacturing and administration of amino acid solutions, containing sodium bisulphite as an anti-oxident. The interval often exceeds several months, and provides ample time and conditions for production of tryptophan conversion products. If, however, as demonstrated by Kleinman et al.[112], protein solutions are prepared without sodium bisulphite and are protected from light, up to 96 percent of the initial tryptophan, and other amino acids as well, can be recovered, even if stored for six months or more at room temperature.
It has further been suggested that amino acid dysbalance may cause histopathological changes in the liver [175,176]. Sidransky demonstrated that pathological changes, consisting of periportal fatty infiltration of the liver and excess hepatic glycogen, could be attributed to a nutritional imbalance created by an essential amino acid deficiency, notwithstanding an adequate or high calorie diet.

2.5 Intestinal Adaptation During TPN

It has been demonstrated in many animal species and man that after resection of one part of the small bowel, the residual intestine develops both structural [11,67,144,145,147,199,212,213,214] and functional [26,52,143,199,209,210,213,214] changes. These include small bowel dilatation and elongation, villus size enlargement and increased crypt depth, epithelial hyperplasia, more rapid cell migration, precocious mucosal maturation and consequently an increased function per unit length in the residual bowel.
There are several mechanisms that possibly can affect intestinal adaptation. These include changes of enteral content [53,63,103,123,124,137,213,214,215], changes in the trophic effects of pancreatico-

biliary secretions [5,161], and in the influence of hormonal factors [11,128,213,214].

Sick or low-birthweight infants may be deprived of enteral feeding as part of their routine management. In many centers these infants are fed routinely by the intravenous route during the early weeks of postnatal life, employing TPN either as the sole source of nutritional support [54] or as a supplement to tolerated enteral nutrients [35]. This policy has been defended partly on the grounds that this is the route by which the infant would have been fed had it remained in utero.

Whereas mortality rate was as high as 70 to 90 percent prior to routine use of TPN, the improved survival data in these groups of infants (mortality less than 10 percent) seems to be partly due to the improved nutritional management with TPN.

However, TPN in neonates might be criticized on several grounds, for example, its potential for inducing serious metabolic complications, the hazards of central venous lines (infection, haemorrhage, thrombosis, emboli), its expense, and the need for intensive monitoring. It also lacks the protective effect against infections as compared with breastmilk.

A more important objection, however, would be that TPN may preclude possible beneficial effects of enteral feeding in terms of stimulating adaptation of the gut [131]. Some studies have demonstrated improvements in intestinal structure and function during periods of exclusive parenteral intake [83,168]. However, only after enteral feeding is resumed, does the gastrointestinal structure and function return to its normal state [83].

The absence of oral nutrition in animals with an intact intestinal tract and fed parenterally led to a marked atrophy of intestinal mucosa [63,91,109,123]. However, atrophy following a period of starvation does not worsen during parenteral feeding [91]. The increase in mucosal mass that occurs when previously starved animals are re-fed with rat-chow does not occur in animals that are re-fed parenterally [95]. Whether or not atrophy of intestinal mucosa occurs in low birthweight infants when only parenteral nutrients are given, is not known. The question emerges if postponement of enteral feeding for a longer period of time, for example following neonatal gut surgery, permanently suppresses intestinal adaptation and growth.

2.6 Host-tumor Interaction and Nutrient Supply

Cachexia is one of the commonest metabolic manifestations of a malignant tumor in man and it complicates gravely the treatment and cure of malignant disease. The derivation of the term cachexia is from the Greek "kakos" and "hexis" meaning "bad condition"; this adequately describes

both the physical and mental state of the patient suffering from cachexia. The clinical features are characterized by a wide range of syptoms including: the classic loss of weight; nutritional disturbances such as anorexia, nausea, depletion and redistribution of host components; diarrhea; muscle weakness; anemia; and frequently interference in the functioning and intermediary metabolism of organs and tissues of the host.
Cachexia is at least in part due to anatomical alterations evoked by the tumor that are present in a cancer patient. It has been repeatedly observed, however, that the degree of cachexia bears no simple correlation to caloric intake, tumor burden, tumor cell type, or anatomical site of the tumor [44].
In the attempt to clarify the etiology of cachexia, the attention of investigators has been focused on distant metabolic effects produced by cancers. Such effects are well documented in both cancer patients and experimental models and are known collectively as „systemic effects of tumors" or „paraneoplastic syndrome" [43,44,85,100,115,186,204,206,221].
The cachectic patient exhibits a much narrower therapeutic margin for the modern forms of cancer therapy (e.g., chemotherapy and radiotherapy), which often results in a further progression of cachexia [51,146]. Similarly, cachexia is thought to have an adverse effect on the immunologic ability of the host to respond to cancer [20,22].
The negative balance between caloric intake and caloric expenditure seems to be caused by a combination of many different factors. The possible contributors to this imbalance will be considered in turn.

2.6.1 *Food Intake*

The size and location of the tumor mass can cause dysphagia, and interference with eating. Following the diagnosis of a malignant disease, psychological and emotional disturbances may result in the lowering of a patient's appetite.
The regulation of food intake is under central nervous system control in the hypothalamic region of the brain, which can either stimulate orsuppress feeding behaviour [6].
The altered serotoninergic status in tumor-bearing humans and animals may cause tumor associated anorexia [115]. Other mechanisms rely on registration of substances in the blood giving rise to glucosensitive, liposensitive and amino acid sensitive control mechanisms [185]. Reduced levels of glucose or amino acids in the blood have been reported to result in a decreased appetite [121] and this mechanism may operate in the patient with cancer where amino acid patterns are known to vary [162]. Theologides

[186] has advanced the hypothesis that tumors produce peptides, and oligonucleotides which have an anorexigenic effect, either through a direct effect on hypothalamic sensory cells or through peripheral neuroreceptor cells.

2.6.2 *Intestinal Absorption*

Functional defects of the gastrointestinal tract in patients with malignant disease are well documented and may result in loss of weight. Involvement of the intestinal tract, especially in cases of lymphoma, can lead to malabsorption syndrome with flattening of the intestinal villi and steatorrhoea [58]. There are many instances where abnormalities of mucosal architecture and disorders of absorption occur in patients with neoplasms arising at sites distant to and not involving the gastrointestinal tract [10,46]. The reason for derangement in absorptive function of the gut can be understood when the primary tumor involves the gastrointestinal tract itself or when there is metastatic involvement. It is less clear why extra-gastrointestinal tumors give rise to this syndrome. These changes clearly represent distant systemic effects of tumors. The production of humoral agents has been proposed, but not substantiated [12]. More recently, it has been established that tumors of the endocrine system and those exhibiting paraendocrine behaviour can secrete pharmacologically active substances which may stimulate secretions of the gastrointestinal tract [185]. Protein-loss, due to the loss of plasma proteins into the intestinal tract, represent another gastrointestinal disorder which may contribute to weightloss, particularly in patients with a malignancy which involves the gut [200]. Dudrick and Shils have proved that the techniques of enteral nutrition by tube feeding and total parenteral nutrition are highly beneficial to the patient who is unable to eat normally [57,174].
Although there is much to be gained by nutritional repletion, for many cancer patients adequate dietary intake is not sufficient to halt the process of progressive weight loss. Additional factors to explain the phenomena, therefore, must by sought to explain the incidence of cancer-induced cachexia in patients whose nutritional intake appears unaffected.

2.6.3 *Host Metabolism and Energy Expenditure*

A wide range of factors are known to influence metabolic rate and energy expenditure. These include age, nutritional status, temperature, the level of various hormones and pathological conditions such as trauma and infection [222]. In contrast to starvation and malnutrition where there is a

fall in the resting metabolic rate and energy expenditure [72], in cancer patients [185], an increase in this rate may take a significant contribution to the accruing weight-loss.
Many studies have documented abnormalities of energy expenditure and metabolism in a variety of host-tumor systems. Abnormalities of carbohydrate metabolism including glucose intolerance [132], insulin resistance [129], increased rates of anaerobic glycolysis [74], and impaired adaptation to a glucose load with continued non-oxidative metabolism [205] have been reported. Similarly, lipid disorders including decreased carcass fat, abnormalities in clearance of fat from serum, and elevated plasma free fatty acids are seen in a variety of experimental tumor systems [30]. Brennan [18] suggested that the normal adaptive mechanisms of the non-tumor-bearing host to starvation that results in body protein conservation are not functioning in the tumor bearing host.

2.6.4 *Metabolism of Tumor Cells*

The increase in nitrogen content of the growing tumor may exceed the dietary intake of nitrogen [34]. The tumor, therefore, acts as a „trap" for available amino acids, and, in consequence, protein synthesis in the peripheral tissues of the host is inhibited. The net result is a depletion of host tissue protein [185]. Another mechanism whereby tumors induce weight loss in the host has been advanced by Gold[74]. In general, because tumor cells display high rates of anaerobic glycolysis, they produce large quantities of lactic acid; the end product of the anaerobic glycolytic pathway. This lactic acid is recycled to glucose via the gluconeogenic pathway in the liver and kidneys. The specific recycling process is referred to as the Cori cycle after its discoverer [42]. Anaerobic catabolism of glucose is a relatively inefficient process, yielding a net 2 moles ATP per mole of glucose. This energy is utilized by the tumor. This compares with a net yield of 36 moles ATP from the aerobic respiration of 1 mole of glucose via the tricarboxylic cycle. The resynthesis of 1 mole of glucose from lactic acid, however, requires 6 moles ATP supplied by the liver or kidney of the host. Thus, the tumor derives relatively little energy from the catabolism of glucose, while the host expends considerably more in the resynthesis. In the presence of a large tumor mass, this metabolic circuit constitutes a severe drain on the energy reserves of the host, ultimately leading to weight loss.

2.6.5 *Nutritional Consequences of Host-Tumor Interaction*

Numerous studies have shown that extended periods of force feeding in tumor-bearing animals may temporarily improve nutritional status. However, controlled studies in patients demonstrated that, in spite of better weight gain in a parenterally alimented group, there were no differences in long term follow-up, particularly with respect to mortality [19]. Limited periods of adjuvant nutritional repletion and/or maintainance, either by enteral or parenteral route, may be of substantial benefit in patients so as to increase their tolerance and possible response to primary antineoplastic radiotherapy, chemotherapy and surgery [30].

As accelerated tumor growth secondary to nutritional support can not be documented in humans, Cameron et al.[32,33] have studied tumor growth in animals by providing carbohydrate-based TPN. They did find increased tumor growth. On the other hand, Goodgame et al.[79] were unable to substantiate these findings. Buzby [30] showed in rats bearing mammary tumors that fat-based TPN promoted host maintainance equivalant to carbohydrate-based TPN without stimulation of tumor growth.

The different utilization of energy substrates by host and tumor suggests the possibility of manipulating energy substrates in intravenous nutrient regimens in order to feed the patient and starve the tumor.

CHAPTER III

THE GENERAL EXPERIMENTAL DESIGN AND METHODS

3.1 Introduction

The aim of part of the study presented in this thesis is to evaluate if the effect of total parenteral nutrition (TPN), given via the portal venous system, will minimize hepatic dysfunction associated with systemic vein infusion of TPN and optimize nitrogen balance and hepatic protein synthesis.
Futhermore, the role of fat-based (Intralipid ® 20%) TPN in preventing or ameliorating hepatic dysfunction is investigated.
It further attempts to answer the question if the inhibition of postresectional intestinal adaptation by TPN in young growing animals is reversible, and, if TPN with and without fat (Intralipid ® 20%) maintains host nutritional adequacy without stimulating tumor growth in tumor-bearing rats.

3.2 Experimental Animals

In the first experiment, described in chapter IV, male C.D.-1 rats, weighing 214-260 g, supplied by Charles River Breeding Laboratories, Wilmington, MA, were used. 7-Week old male Wistar rats (80-90 g.) (Charles River Breeding Lab.) were used in experiment 3 (chapter VI). Experiments 2 and 4 (chapters V and VII) were carried out in adult male A.C.I.-N rats weighing 180-210 g. (Harlan Industries Inc., Indianapolis, IN).
All rats were allowed a minimum of 3 days acclimatisation before surgery and were kept in suspended cages with open wire-mesh bottom under alternate 12 hour lighting cycles.
Water and Purina rat-chow pellets, consisting a minimum of 4.5% fat and 22.5% protein (Ralston Purina Company, U.S.A.; no. 5012), were allowed ad libitum before surgery. After surgery all experimental animals werd housed individually in metabolic cages in a temperature-controlled environment with 12-hour cycles of light and darkness.

3.3 Surgical Procedures

Before intestinal resection (experiment 3) rats were fasted for 12 hours. In the other experiments (1, 2, 4) the animals were not starved. All

operations, utilizing a simple operation microscope, were performed under ether anesthesia. The operative techniques employed in the different experiments will be described under the relevant chapter headings, but the general operative procedure is described here.

3.3.1 *Laparotomy*

Laparotomies were carried out through a midline incision. The abdomen was closed with a continuous 4.0 silk suture taking all layers of the abdominal wall. Intestinal anastomoses were made with 6.0 or 7.0 interrupted silk sutures on an atraumatic needle taking all layers of the bowel wall.

3.3.2 *Cannula Placement*

When chronic intravenous feeding was given, the neck and midscapular area of the rat were shaved and desinfected. The animal was restrained in the supine position. A small transverse incision was made in the neck lateral to the midline and 0.25 cm above the clavicle. The jugular vein was isolated and ligated 0.5 cm above the clavicle with a 5.0 silk suture, while a second suture was placed around the vein immediately above the clavicle and left untied until the catheter had been inserted. The beveled tip of a sterile silastic catheter (0.020-0.030 in inside diameter, 0.037-0.047 in outside diameter, Dow Corning Corp., Midland, MI) was inserted through a phlebotomy in the jugular vein between the sutures, and advanced into the superior vena cava or right atrium. The catheter was then secured with each of the two sutures. The free end of the catheter was fitted into a clamp, which was passed subcutaneously through the skin of the midscapular area to the incision (fig. 3.1). Intermitted irrigations with a saline-filled syringe maintained patency of the catheter until final connection was made to an infusion apparatus. The catheter was passed through an opening in a metal harness assembly, and a tightly wound stainless steel protective coil, which was attached distally to the swivel (Becton, Dickinson and Company, Rutherford, NJ). The upper portion of the swivel was fixed to a support assembly over the metabolic cages and connected with a no. 24 clear Transflex tube to a Harvard infusion pump. The harness was attached and secured to the animal with 3.0 metal stitches [181].
When nutrient substrates were infused into the portal vein, a laparotomy was carried out and a catheter was inserted and secured in one of the last branches of the portal vein and passed subcutaneously through the abdominal wall to the midscapular area as described above.

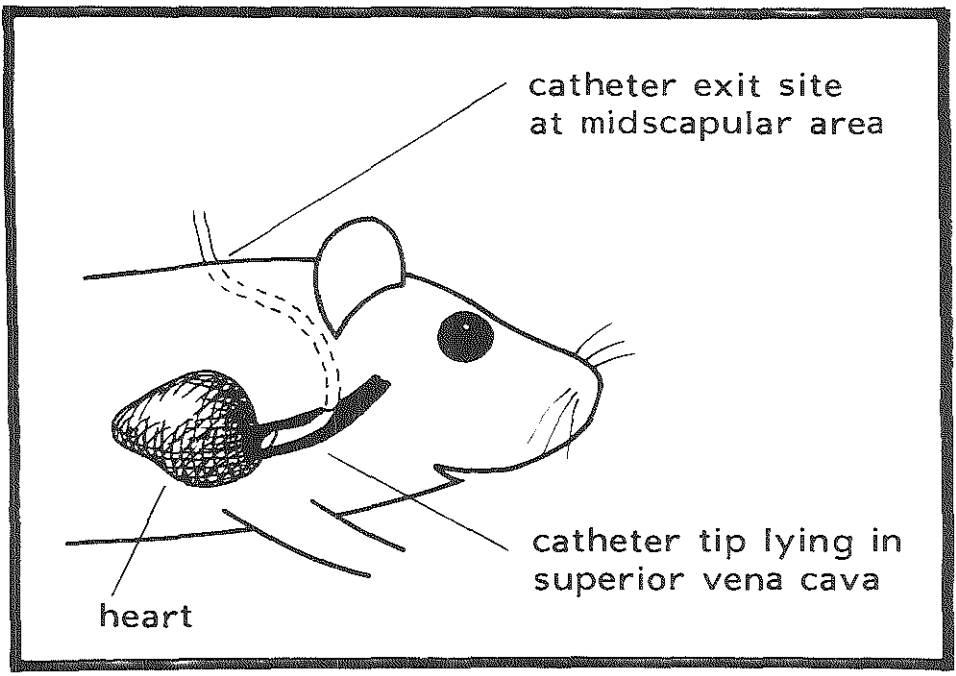


Fig 3.1 *Sterile silastic catheter passed down jugular vein into superior vena cava or right atrium. The right atrium in the rat is at the level of the axilla.*

3.4 Specimens for Histological and Biochemistry

At the time of sacrifice the abdominal cavity was quickly opened by transverse incision. After exposing the inferior caval vein it was punctured to withdraw blood for hepatic function tests when necessary. The liver and both tibialis anterior muscles were removed and weighed. Weighed samples of the liver and one tibialis anterior muscle were quickly frozen in dry ice and stored at -20°C till further processing.
After marking the position of Treitz ligament, the stomach, and the small and large bowel were rapidly removed, and carefully dissected free of fat and mesentery in ice-cold saline. The location of histological and biochemical specimens was identified as described in chapter VI.
Samples for histometry were cut open, rinsed in ice-cold saline and preserved in a coded vial with 10 percent buffered formalin solution.

3.5 Biochemical Assays

3.5.1 *Nucleic Acids*

Ribonucleic acids were determined by the methods of Scott et al.[170], as modified by Hinrichs et al.[99]. The method is based upon the ultraviolet absorption of the purine and pyrimidine constituents of nucleic acids. Specimens were thawed, homogenized in citric acid sucrose and treated with cold perchloric acid to precipitate the macromolecular fraction, including RNA and DNA. After treatment with 80 percent ethanol and cold alcohol-ether to extract the lipids, the precipitate underwent alkaline digestion for one hour in order to hydrolize the RNA and render it acid soluble. Subsequently the optical density (OD) of the RNA was measured at two different wavelengths (260 nm and 280 nm) in order to minimize errors due to contaminants, which might contribute to the ultraviolet absorption [189]. The residual pellets were used for DNA determination according to the method of Burton [25]. They were precipitated by 0.5 N perchloric acid and separated by incubation at 70°C for 15 minutes to hydrolyze the DNA fraction. The DNA in the supernatant was calculated from its OD at 260 nm.

3.5.2 *Protein Content*

Liver and muscle protein contents were determined by the Bio-Rad Protein Assay [17], based on the binding of a protein reagent to proteins.

Up to 0.1 ml of the homogenized specimen was pipetted into a test tube. After adding 5 ml of protein reagent to the test tube, the contents were mixed by inversion. After 5 to 20 minutes the protein concentration was read from its OD at 595 nm and thereafter protein weight could be read from a standard curve.

3.5.3 *Nitrogen Content and Nitrogen Balance*

The cumulative urinary nitrogen excretion was determined by the micro Kjeldahl method [136].
Nitrogen balance was estimated by subtracting total urinary nitrogen excreted from total nitrogen intake, which was calculated from the volume of TPN solution administered or the amount of chow consumed. Fecal nitrogen measurements were not carried out, since significant amounts of fecal material were not excreted until the animal resumed oral alimentation [30].

3.5.4 *Serum Biochemistry*

Determinations of levels of serum glucose, cholesterol, albumin, alkaline phosphatase activity, glutamate-oxalo-acetate transaminase (SGOT) and glutamate-pyruvate transaminase activity (SGPT) were carried out on a multichannel auto-analyser by standard methods.

3.6 Histology

All specimens were embedded in paraffine, sectioned to 4 u-thick slices and stained with hematoxylin-eosin and periodic Acid Schiff (PAS). In addition liver-tissue was snap frozen in liquid nitrogen. Cryostat sections were stained with oil-red-0 to examine fatty changes in liver cells.
Specimens were coded to eliminate observer bias.

3.7 Classification of Hepatic Steatosis

Fatty liver has been classified to its etiology, distribution in the hepatic lobule and severity.
The liver may show considerable lipid on chemical analysis and exhibit little fat on histological examination; however, in most instances there is a

good correlation between chemical and morphological findings.
In our experiments grading of liver biopsy specimens was based on the amount of the liver section occupied by fat globules [120]. When fat globules occupy less that 10% of the histological section, it was designated 1 plus and considered abnormal but not clinically significant. A designation of 2 plus or mild fatty liver has been used when fat globules occupied 10 to 30% of the biopsy specimen; 3 plus or moderate fatty liver was present when 30 to 70% of the histological section consisted of fat globules, and 4 plus or severe fatty liver was characterized by fat globules occupying over 70% of the section.

3.8 Statistics

Student's t-test for unpaired data was used throughout. When the term significant is used this means statistical significant ($p < 0.05$ for the two sided test). Variance of the mean is expressed as the standard error of the mean (SEM).

CHAPTER IV, Experiment 1

NUTRITIONAL EFFICACY AND LIVER CHANGES: EFFECT OF PREHEPATIC, CENTRAL VENOUS AND INTRAGASTRIC FEEDING

4.1 Introduction

Although total parenteral nutrition (TPN) is capable of delivering complete nutrients intravenously to major organs of the body for utilization, certain metabolic limitations exist. Fatty infiltration of liver [125,172], inefficient nitrogen utilization [76], and suboptimal hepatic protein synthesis [149] during TPN are thought partly due to the absence of nutrients passing through and processed by the gastrointestinal tract and the liver. It has been suggested, that prehepatic (portal vein) infusion of nutrient substrates permits primary hepatic modulation of the infused nutrients and may reduce the metabolic limitations of TPN [152]. We studied the nutritional efficacy of intragastric, central venous and prehepatic administration of a TPN solution in rats over a 4-day period, with special emphasis on changes in liver function and structure.

4.2 Material and Methods

Male CD-1 rats weighing 214-260 g. were assigned to one of 4 groups of nutritional regimens: 1.rats in the *control-group* (n = 10) had a sham laparotomy and were given chow and water ad libitum. 2.*Intragastric-TPN* rats (n = 11) had a laparotomy with insertion of a gastric tube (Dow Corning Silastic catheter 0.030 in id.) for intragastric administration of TPN solution. 3.*Central-venous TPN* rats (n = 13) had a sham laparotomy and insertion of a catheter via the internal jugular vein into the superior vena cava for intravenous infusion of TPN solution. 4.*Prehepatic-TPN* rats (n = 15) had a laparotomy with insertion of a silastic catheter into a branch of the portal vein for prehepatic (intraportal) infusion of TPN solution.
Rats given TPN solution by various routes (groups 2, 3, 4) received a continuous infusion of equal amounts of isocaloric and isonitrogenous TPN solution. (Table 4.I).

Table 4.I Composition of TPN Solution

8.5% Freamine ® III*	500 ml
50.0% Dextrose	500 ml

Additives/liter

K acetate	34.6	meq
KCl	26.8	meq
Ca Gluconate	9.3	meq
MgSO4	1.7	meq
NaCl	20.6	meq
MVI ®**	0.4	ml
Trace elements***	1.0	ml
Penicillin	1 x 10^6	IU
Gentamicin	16.0	mg

** Multi-vitamin infusion, USV Laboratories, Tuckahoe, NY 10707
*** Each 1 ml contains zinc 1.0 mg, copper 0.5 mg, manganese 0.2 mg, chromium 0.005 mg, iodide 0.028 mg
* Each 100 ml FreAmine III 8.5% contains:

Essential Amino Acids:
Nonessential Amino Acids:

L-Isoleucine	590 mg	L-Alanine	600 mg
L-Leucine	770 mg	L-Arginine	810 mg
L-Lysine Acetate	870 mg	L-Histidine	240 mg
(free base	620 mg)	L-Proline	950 mg
L-Methionine	450 mg	L-Serine	500 mg
L-Phenylalanine	480 mg	Ami Aminoacetic Acid	1.19g
L-Threonine	340 mg	L-CysteineHCl H2O	20 mg
L-Tryptophan	130 mg		
L-Valine	560 mg		

Phosphoric Acid NF	0,115 g
Sodium Bisulfite USP (as antioxidant)	0.1 g
Water for Injection USP	qs

pH adjusted with Glacial Acetic Acid (pH: Approx.6.5)

To avoid portal vein thrombosis, prehepatic-TPN rats (group 4) had heparin added to the TPN solution (2000 U/liter). Body weight, caloric intake and urine volume were recorded daily. After 4 days of nutritional regimen, all surviving rats were sacrificed under anaesthesia. Blood samples, liver and both tibialis muscles were collected for biochemical analysis.

The cummulative urinary nitrogen output over 4 days of nutritional regimen and the nitrogen balance during the 4 day period were calculated as described in section 3.5.3. DNA, RNA, protein and serum-biochemistry determinations were performed as described in section 3.5. Total liver lipid content was determined by the chloroform-methanol extraction method [68]. Histological measurements were done as described in section 3.6. Appropriate statistical analysis was carried out as described in section 3.8.

4.3 Results

6 of 21 prehepatic TPN rats with clinical or histological evidence of portal vein thrombosis were excluded.

4.3.1 *Body Weight and Nitrogen Balance*

Chow-fed rats ate approximately 270 kcal/kg/day and 14.5 g/kg/day of protein, which was significantly more than the estimated 205 kcal/kg/day and 8.3 g/day of protein received by rats given TPN solution by different routes ($p < 0.001$). As a result, chow-fed rats were in better positive nitrogen balance (Table 4.II).

Table 4.II Body weight, nitrogen balance, and liver structure during prehepatic, central-venous and intragastric feeding.

Group	chow	Intragastric TPN	Central-venous TPN	Prehepatic TPN
n	10	11	13	15
Wt. change (g)	0.2 ± 3.9	0.5 ± 2.8	-8.6 ± 2.1a,e	-6.6 ± 2.5g
Nitrogen balance (mg/kg//d)	+1578 ± 164	+472 ± 62b	+353 ± 67b	+345 ± 35b
Liver Wt. (g)	8.56 ± 0.22	8.63 ± 0.31	10.72 ± 0.43c,f	10.14 ± 0.42c,g
Liver DNA (mg/g)	2.63 ± 0.14	2.53 ± 0.10	2.81 ± 0.11	2.86 ± 0.11
Liver RNA (mg/g)	15.4 ± 0.8	14.3 ± 1.0	14.1 ± 0.8	13.1 ± 0.4c
Liver protein (mg/g)	235 ± 5	212 ± 9	215 ± 8	208 ± 7c
RNA:DNA	5.96 ± 0.38	5.70 ± 0.47	5.04 ± 0.74	4.66 ± 0.15c
Liver lipid (mg/100g body wt.)	126 ± 5	137 ± 9	202 ± 34a	172 ± 18

Relative to chow: a = p value < 0.05,
b = p value < 0.001,
c = p value < 0.01,
Intragastric-TPN *vs.* intravenous-TPN: e = p value < 0.02,
f = p value < 0.001
Intragastric-TPN *vs.* prehepatic-TPN: g = p value < 0.02

There was no statistical difference in nitrogen balance between rats given TPN solution by the intragastric, central venous or prehepatic route. The mean initial body weights of the 4 groups were similar: Chow-fed rats weighed 238.8 ± 4.0 g., intragastric-fed TPN rats: 235.6 ± 4.3 g., central venous TPN rats: 238 ± 3.9 g., and prehepatic-TPN rats: 231.1 ± 3.0 g. Body weight was maintained in rats fed intragastrically, while parenterally fed rats lost an average of 8.6 g. (central-venous) and 6.6 g. (prehepatic) as compared with chow-fed rats. The differences in weight gain in the 4 groups were not accurately reflected in the cumulative nitrogen balance, which remained positive in all 4 groups.

4.3.2 *Liver Changes*

Although rats given TPN solution by the intragastric, central venous or prehepatic route received fewer calories as compared with rats fed chow, major differences in hepatic changes were observed. Both groups of rats given TPN solution parenterally (by either the central venous or prehepatic route) had approximately 17 percent increase in liver weight as compared with rats fed chow or given TPN solution intragastrically (Table 4.II). The presence of hepatomegaly in parenterally fed-rats was associated with an increase in liver lipid content of 60 percent in rats given central venous TPN and 37 percent in rats given prehepatic TPN. Histological examination showed that 7 of 13 rats given TPN central venously and 9 of 15 rats given TPN prehepatically developed minimal to mild fatty changes of the liver (Table 4.III).

Table 4.III Fatty change of liver during intragastric, central venous and prehepatic feeding

Group	No.	No. of rats with fatty change of liver			Total
		Minimal	Mild	Moderate	
Chow	10	0	0	0	0
Intragastric-TPN	11	2	1	0	3
Central-venous-TPN	13	3	3	1	7
Prehepatic-TPN	15	7	2	0	9

Fatty changes of liver were not observed in chow-fed rats, and only 3 of 11 rats given intragastric TPN developed mild fatty changes of the liver, with fat globules occupying 5 to 10 percent of the liver section.
Abnormal hepatic function tests were only observed in rats given prehepatic TPN. Both SGOT and SGPT seemed elevated in rats given

prehepatic TPN as compared to rats given chow, intragastric or central venous TPN (Table 4.IV); however, the differences were not statistically significant. The serum albumin concentration was significantly decreased in rats given TPN solution by either oral or parenteral route. Hepatic protein content was reduced in rats given prehepatic TPN relative to chow-fed rats.

Table 4.IV Hepatic function tests during intragastric, central venous and prehepatic feeding

Group	chow	Intragastric TPN	Central-venous TPN	Prehepatic TPN
n	10	11	13	15
Albumin (g/l)	31.22 ± 0.69	28.51 ± 0.70a	26.13 ± 0.70a	27.94 ± 0.53a
Alkaline Phosph. (IU/l)	270.92 ± 23.93	169.40 ± 17.43	239.38 ± 23.84	297.89 ± 26.24
SGOT (IU/l)	73.08 ± 21.78	51.87 ± 6.19	69.92 ± 8.89	185.11 ± 47.93
SGPT (IU/l)	11.54 ± 2.36	6.60 ± 0.83	8.31 ± 1.82	42.21 ± 12.46

Relative to chow = a: p value 0.05

Hepatic DNA content was similar in all 4 groups of rats. There was a 15 percent reduction in hepatic RNA, a 22 percent reduction in hepatic RNA to DNA ratio and a 12 percent reduction in hepatic protein in rats given prehepatic TPN as compared with chow-fed rats; however, no significant difference was observed in these values between rats given TPN by the central venous or the prehepatic route (Table 4.II).

4.3.3 *Muscle Changes*

Individual muscle weight of the tibialis anterior muscle and muscle protein content were not statistically different in the 4 groups of rats. suggesting that these values were not sensitive indices of minor differences in nutritional adequacy (Table 4.V).

Table 4.V Muscle weight and protein content during prehepatic, central venous and intragastric feeding

Group	chow	Intragastric TPN	Central-venous TPN	Prehepatic TPN
n	10	11	13	15
Muscle wt (g)	0.40 ± 0.01	0.41 ± 0.01	0.43 ± 0.01	0.40 ± 0.01
Muscle protein (mg/g)	262 ± 4	247 ± 11	253 ± 13	248 ± 15
Muscle protein per cell (mg/mg DNA)	605 ± 76	586 ± 44	537 ± 69	451 ± 48

4.4 Discussion

The results of this experiment suggest, that there are no metabolic advantages in administering total parenteral nutrition-solutions into the portal vein instead of into a systemic vein.
Despite the use of a low-calorie regimen designed to minimize fatty changes of liver during TPN(chapter II) both groups of rats parenterally fed by either the standard central venous or by the prehepatic route via the portal vein, demonstrated hepatomegaly with an increased total hepatic lipid content. It appears that the provision of nutrient substrates in the TPN solution directly to the liver for primary hepatic processing does not prevent the development of fatty infiltration of the liver.
The presence of only minimal fatty infiltration of liver in rat given the TPN solution intragastrically suggest that both the modulation of nutrient substrates by the gastrointestinal tract and the associated postabsorptive hormonal interaction are important factors in minimizing the development of fatty infiltration of liver. Nevertheless, the previous observation of the development of fatty liver in rats force-fed a high carbohydrate and amino-acid deficient diet (devoid of threonine or valine) [175] would suggest that limits exist to which the gastrointestinal tract can protect the liver from injury caused by a nutritionally deficient diet.
In evaluating nutritional efficacy, there appears to be no significant difference between rats given TPN by the central venous or prehepatic

route. Maintenance of body weight, nitrogen balance, liver protein, individual muscle weight and muscle protein were similar in the two groups of rats. Similar results are observed by others in Lewis-Wistar rats [16] and in chair-adapted primates [62]. In contrast, rats given TPN solution via the gastrointestinal tract maintained better body weight.

Our reslts are in agreement with previous reports that amino acid solution is more effectively utilized when administered through the gastrointestinal tract instead of intravenously [27,118].

Mechanical complications occurred with the use of prehepatic TPN.

Despite the use of heparin in the TPN solution, 6 out of 21 rats that received TPN solution intraportally showed evidence of partial or complete portal vein thrombosis. Although this complication of prehepatic TPN may be more prevalent in rats with small portal veins, the possibility of portal vein thrombosis resulting from the infusion of a hyperosmolar solution is real. It is possible that the elevation of SGOT and SGPT levels observed in rats given prehepatic TPN partly resulted from liver injury caused by microscopic thrombosis or emboli in the portal circulation.

It would appear that in the absence of substantial benefit in improving the nutritional efficacy of TPN, the clinical usefullness of prehepatic TPN with its added risk of portal vein thrombosis is none.

CHAPTER V, Experiment 2

FAT-BASED VERSUS CARBOHYDRATE-BASED TOTAL PARENTERAL NUTRITION: EFFECTS ON HEPATIC STRUCTURE AND FUNCTION IN RATS

5.1 Introduction

Hepatic dysfunction characterized by cholestasis and fatty infiltration of liver occurs in adults [125,172] and infants [158,187] during fat-free total parenteral nutrition (TPN). Infusion of hypertonic dextrose alone produces fatty infiltration of the liver in Sprague-Dawley rats [37].
Because hepatic dysfunction during TPN may be alleviated or prevented by using a fat-based TPN with Intralipid ® 20% emulsion as a major caloric source [106,134,157,223], we studied the nutritional adequacy of carbohydrate-based versus fat-based TPN and the effect of these regimens on hepatic structure and function.

5.2 Material and Methods

10 Weeks old rats, weighing 180-210 g. were randomly divided into 4 groups: The first group *(chow-group)* was given chow and water ad libitum. The second group *(fasting-group)* received 0.9% saline solution intravenously.
The third group *(carbohydrate-based TPN-group)* received a TPN-solution consisting of dextrose 25%, and amino acids (FreAmine ® III), 4,25% intravenously.
The fourth group *(fat-based TPN-group)* received a TPN-solution consisting of fat emulsion 3,9% (Intralipid ® 20%), dextrose 12,5%, and amino acids 4,25% intravenously.
All rats were harnessed in metabolic cages. Rats in groups II, III and IV had a silastic catheter placed into the vena cava superior via the internal jugular vein. Intravenous solutions were administered as described in section 3.3. The chow group had a sham operation and was harnessed without catheter placement. Groups III and IV received a continuous infusion of isocaloric and isonitrogenous TPN solutions. The composition of the TPN solutions and additives are shown in
Table 5.I.

Table 5.I Composition of TPN Solution

	Carbohydrate-TPN ml	Fat-TPN ml
8.5% Freamine ® III*	500	500
50% Dextrose	500	250
20% Intralipid ®	---	193
Sterile water	---	57

Additives (per liter)

K Acetate	34.6	meq
K Cl	26.8	meq
Ca Gluconate	9.3	meq
MgSO4	1.7	meq
NaCl	20.6	meq
Na Phosphate	23.0	meq
Multivitamins**	0.4	ml
Trace elements***	1.0	ml
Penicillin	1 x 10^6	I.U.
Gentamicin	16.0	mg

* Solution content see Table 4.I
** MVI (USV Laboratories, Tuckahoe, NY)
*** Each ml contains zinc 1 mg, copper 0.5 mg, manganese 0.2 mg, chromium 0.005 mg, iodide 0.028 mg

The body weight, caloric intake, and urine volume were recorded daily. After 7 days of the nutritional regimen, all surviving rats were sacrificed under ether anesthesia. Blood, liver and both tibialis anterior muscles were collected for analysis. Nitrogen balance was calculated and biochemical assays as described in section 3.5 and histological studies as described in section 3.6 were performed.

5.3 Results

Rats that died before the 7-day period (catheter-thrombosis, septicemia, pneumonia) were excluded.

5.3.1 *Nutrient Intake*

Caloric intake was similar in the chow and the two TPN groups (Table 5.II). Protein intake was 24% higher in the chow group as compared with the TPN groups. Calorie and protein intake were similar in the TPN groups.

Table 5.II Nutrient intake and nitrogen balance during 7-day nutritional regimen

Group	No.	Calorie (Kcal/kg/d)	Protein (g/kg/d)	Non-Protein Calories Fat (%)	Non-Protein Calories Carbohydrate (%)	Cumulative N-Balance (mg/kg/day)
Chow	12	258 ± 14	13.3 ± 0.7	12	88	1122 ± 126
Fasting	14	0	0	0	0	-901 ± 29
Carbohydrate-TPN	13	263 ± 8	10.7 ± 0.3a	0	100	471 ± 92b
Fat-TPN	11	249 ± 5	10.3 ± 0.3a	50	50	291 ± 88b

Relative to chow = a: p value < 0.01
b: p value < 0.001

5.3.2 *Body Weight*

Initial body weights were similar in the 4 groups: the chow-group 202.5 ± 3.95 g.), the fasting-group (201.21 ± 2.20 g.), the carbohydrate-TPN-group (196.85 ± 3.81 g.), and the fat-TPN-group (201.09 ± 4.19 g.). Final body weights after 7 days of nutritional regimen were similar in the isocaloric groups: the chow-group (197.17 ± 2.45 g.), the carbohydrate-TPN-group (201.27 ± 4.35 g.), and the fat-TPN-group (201.27 ± 4.35 g.). There was a 33 percent reduction in final body weight of fasting rats (131.57 ± 3.76 g.).

5.3.3 *Nitrogen Balance*

Rats parenterally fed were in positive nitrogen balance over the 7-day period. There was no significant difference in nitrogen balance between the carbohydrate-TPN group and the fat-TPN group receiving an isocaloric, isonitrogenous regimen (Table 5.II). Rats fed chow-pellets had a significant greater positive nitrogen balance ($p < 0.001$), reflecting the higher protein intake and retention compared with rats given TPN.

5.3.4 *Liver Changes*

There was a 65 percent increase in liver weight in rats given fat-TPN compared with rats fed chow (Table 5.III). The water content of liver in rats fed chow or given TPN was equal. Histological examinations showed fatty infiltration in both TPN groups. However, only 4 out of 13 rats given carbohydrate-based TPN had fat globules occupying more than 10 percent of the histological section, whereas, all 11 rats given fat-based TPN had over 10 percent of the histological section occupied by fat globules. In addition, periportal inflammation was observed in most rats given fat-based TPN.

Table 5.III Effect of nutritional regimen on liver

Group	Liver wt.	Content	Water	Liver DNA	RNA:DNA	Liver Protein	Liver Protein per cell	Liver
	(g)	(%)	(mg/g)			(mg/g)	(mg/mgDNA)	
Chow	6.58	± 0.20	71.83 ± 0.89	1.49	± 0.09	9.34 ± 0.56	260 ± 11	180 ± 11
Fasting	3.0	± 0.21a	73.77 ± 0.39	3.02	± 0.12	4.81 ± 0.29	239 ± 16	74 ± 3a
Carbohydrate TPN	6.09	± 0.32d	71.00 ± 0.97	1.73	± 0.16	10.41 ± 1.46	252 ± 10	157 ± 14
Fat-TPN	10.88	± 0.06d	71.55 ± 0.86e	1.80	± 0.09c	7.99 ± 0.40	245 ± 12	138 ± 8b

Relative to chow = a: p value ◁ 0.001
b: p value ◁ 0.01
c: p value ◁ 0.05
Diet III *vs.* IV = d: p value ◁ 0.001
Relative to fasting = e: p value ◁ 0.05

There was 20.8 percent increase in liver DNA content in rats given fat-TPN as compared with chow-fed rats. There was a near 2-fold increase in hepatic DNA content in fasting rats.
Histological examination showed smaller liver cells in fasting rats.
The hepatic protein contents in rats fed chow or given both types of TPN were similar. However, the fat-TPN group had a 27 percent decrease in protein content per cell (mg/mg DNA) compared with the chow group. No difference was observed between the two TPN groups.

5.3.5 *Serum Biochemistry*

After 7 days of nutritional regimen, hyperglycemia was not observed in rats given carbohydrate-based TPN (Table 5.IV). The serum cholesterol was elevated in all 11 rats given fat-based TPN and was approximately twice that of rats fed chow or given carbohydrate-based TPN (range: 1.70 - 6.6 mmoles/L). The serum albumin concentrations were similar in chow-fed and parenterally-fed groups. Serum alkaline phosphatase and glutamate-pyruvate transaminase (SGPT) activities were elevated in rats given fat-based TPN.

Table 5.IV Serum glucose and liver function tests following 7-day nutritional regimen

	Chow	Fasting	Carbohydrate-TPN	Fat-TPN
Glucose (mmoles/l)	10.0 ± 1.1	7.54 ± 0.58	6.77 ± 0.68c	7.15 ± 1.20
Cholesterol (mmoles/l)	1.45 ± 0.07	0.99 ± 0.11a	1.43 ± 0.13	3.31 ± 0.51b
Albumin (g/l)	39.7 ± 1.2	35.4 ± 1.7c	36.0 ± 1.57	34.0 ± 3.8
Alk.Phos. (IU/l)	164 ± 17.3	135 ± 22.8	95.8 ± 8.5a	305 ± 44.3b
SGPT (IU/l)	38.5 ± 9.2	36.0 ± 7	25.7 ± 1.8	197.3 ± 101.5

Relative to chow = a: p value < 0.001
b: p value < 0.01
c: p value < 0.05

5.3.6 *Muscle Changes*

The average weight of the paired tibialis anterior muscles in rats given fat-based TPN was 9.5 percent less than in rats fed chow, but was the same as those in rats given carbohydrate-based TPN (Table 5.V). Muscle DNA, protein content, and protein content per cell (mg/mg DNA) were similar in rats fed chow or parenterally fed. In contrast, fasting rats had 36 percent reduction in muscle weight (tibialis anterior muscle), 26 percent reduction in muscle protein and 47 percent reduction in muscle protein per cell (estimated from the DNA content).

Table 5.V Effect of 7-day nutritional regimen on muscle

Group	Muscle Weight (g)	Muscle DNA (mg/g)	Muscle Protein (mg/g)	Muscle Protein per cell (mg/mgDNA)
Chow	0.42 ± 0.01	0.34 ± 0.04	344.5 ± 20.4	1210 ± 164
Fasting	0.26 ± 0.01a	0.40 ± 0.04	255 ± 19b	642 ± 45b
Carbo hydrate TPN	0.42 ± 0.01	0.35 ± 0.02	388 ± 12	992 ± 73
Fat-TPN	0.38 ± 0.01c	0.40 ± 0.03	342 ± 22.0	884 ± 78

Relative to chow = a : $p < 0.001$
b : $p < 0.01$
c : $p < 0.02$

5.4 Discussion

After 7 days of TPN in these rats, hepatic dysfunction was not prevented by incorporating fat emulsion to provide 50 percent of the caloric source. In fact, the liver weight of rats given fat-based TPN was 78.6 percent greater than rats given carbohydrate-based TPN, presumably as a result of excess lipid accumulation in the reticulo-endothelial cells of the liver. The amount of fat emulsion infused (9-10 mg/g/day) may have exceeded the lipid tolerance of these ACI-N rats, resulting in fat overloading and hepatomegaly. On the other hand, the results of this study would suggest that infusion of excessive amounts of fat emulsion may have contributed to hepatomegaly and hepatic dysfunction.

In a previous experiment Kronevi and Roos [116] observed hepatomegaly and hepatic dysfunction in rats given 10 days of fat-based TPN (20% Intralipid ®), 9 g/kg/d). In puppies given 4-8 weeks of fat-based TPN (10% Intralipid ®), 2-4 g/kg/d), the liver-lipid content doubled [113]. However Buzby et al.[31] showed that fatty infiltration of the liver and hepatomegaly were prevented in rats given fat-based TPN when a smaller proportion of calories (25 percent) was from fat.

Fatty infiltration of liver in rats probably can be prevented by using a balanced TPN regimen with fat contributing less than fifty percent of the caloric requirements.

The nutritional adequacy of both carbohydrate-based and fat-based TPN was confirmed by having body weight, muscle weight, serum albumin concentration, liver and muscle protein of rats parenterally-fed similar to rats fed chow. Rats given either type of TPN were in similar positive nitrogen balance. A lower protein intake in the TPN groups (10 g/kg/day) compared with the chow groups (13 g/kg/day) may explain the smaller

positive nitrogen balance observed in rats given TPN. Nitrogen balance was not corrected for fecal nitrogen excretion, which was negligible in rats given TPN.

During TPN with 60 percent of the calories from 10% Intralipid ® (2 g/kg/d) elevated serum alkaline phosphatase activity and increased levels of bilirubin and cholesterol were observed in adult patients [165]. Histological examination of biopsies of the liver did not show fatty infiltration, but periportal inflammation and bile duct proliferation in the portal triads with canalicular bile plugs [165].

Portal triad inflammation as well as fatty infiltration of liver were observed in our rats, that have been given fat-based TPN. Serum cholesterol levels and alkaline phosphatase activity were elevated two-fold in our rats when fat-based TPN was given.

A dysfunction of the liver was not found in adult patients receiving fat emulsion at a dosage of 2.5 g/kg/d [88].

Similarly, hepatic dysfunction was not observed in home-TPN patients relying on 10% Intralipid ® to supply 40 percent of their caloric requirements over periods of months to years [107]. Recently, however, hypercholesterolemia and elevated levels of alkaline phosphatase are observed in patients on fat-based TPN (10% Intralipid ®, 2-3 g/kg/d) and are considered to be early signs of hepatic cholestasis [2].

In conclusion, this study shows that TPN regimens with or without fat are equally effective in maintaining body weight, positive nitrogen balance, muscle and hepatic protein content. However, hepatic dysfunction in rats during TPN is not prevented by using a fat emulsion to provide 50 percent of the caloric requirement.

CHAPTER VI, Experiment 3

POSTRESECTIONAL INTESTINAL ADAPTATION: EFFECTS OF TOTAL PARENTERAL NUTRITION AND ORAL FOOD INTAKE IN YOUNG RATS

6.1 Introduction

With the availability of total parenteral nutrition (TPN), neonates undergoing massive intestinal resection can be nutritionally supported for long periods of time [95]. Although TPN can be life saving, it could also inhibit intestinal adaptation and prolong the need for parenteral nutrition support because TPN inhibits post-resectional adaptive hyperplasia as well as normal growth in young animals [103,114,137,138].
Food is found to be essential to optimal postresectional intestinal adaptation in both mature and young growing animals [53,63,103,137,213,214]. When either young, growing rats or adult dogs are given TPN as their sole nutrient support after intestinal resection, compensatory mucosal growth in the residual intestine is inhibited [63,137].
Because Morin and his colleagues [137] speculated that early luminal nutrition is essential for the induction of a maximal adaptive response to intestinal resection in young, growing rats, we studied the inhibition of postresectional intestinal adaptation during TPN and after, to see if it is reversible.

6.2 Material and Methods

7-Week old male Wistar rats (n = 130) weighing 90-95 grams, were subjected to resection of 70 percent of the small bowel. Before surgery the rats were housed for ten days in wire-bottomed cages in an environment at constant temperature with 12 hours of alternating light and darkness. Control rats (n = 33) without an operation were housed similarly and followed in parallel.
Before resection under ether anesthesia, rats were fasted for 12 hours. The small intestine was measured along its antimesenteric border by gently stretching it along a measured silk thread. Seventy percent of the small bowel between the ligament of Treitz and the ileocecal valve was removed, leaving proximal jejunum and distal ileum each equivalant to 15 percent of the original intestinal length. An end-to-end anastomosis was carried out with interrupted 6-0 silk sutures.
Rats with small bowel resection were divided into 3 groups. The *first*, to be

given TPN (n = 50), had cannulation of the internal jugular vein with a silastic catheter as described in section 3.3.
TPN consisted of amino acids 4.25% (FreAmine(O)III) and dextrose 20%, supplemented with appropriate electrolytes, multi-vitamins, and gentamicin (3 mg/kg/day) [181]. The volume of TPN solution infused daily was adjusted to provide each rat with 200 ml/kg/day for the first 24 hours and 400 ml/kg/day for the subsequent 9 days.
Rats were allowed free access to water. The *second* group (n = 40) was allowed TPN-solution and water by mouth freely („elemental-diet" group), as the sole oral intake for 10 days. The *third* group (n = 40) had free access to Purina Rat chow pellets and water. *Control* rats (n = 33) had access to chow, but had no operation.
Zero-day points were established by sacrifice of 12 control rats. Approximately half the rats with intestinal resection plus 10 control rats were sacrificed after 10 days of dietary regimen by exsanguination under ether anesthesia.
As soon as the rats were anesthetized, the entire small bowel was removed from the ligament of Treitz to the ileo-cecal valve and rapidly cooled in ice-cold saline solution. The mesentery was rapidly stripped from the intestine and the small bowel was measured hanging vertically, stretched by a fixed mass of 5 g. attached to one end to provide uniform tension. Segments of intestine 5-cm long and 1-cm long were removed serially, starting 10 cm proximal to the anastomosis and 10 cm distal to it (Fig.6.1).

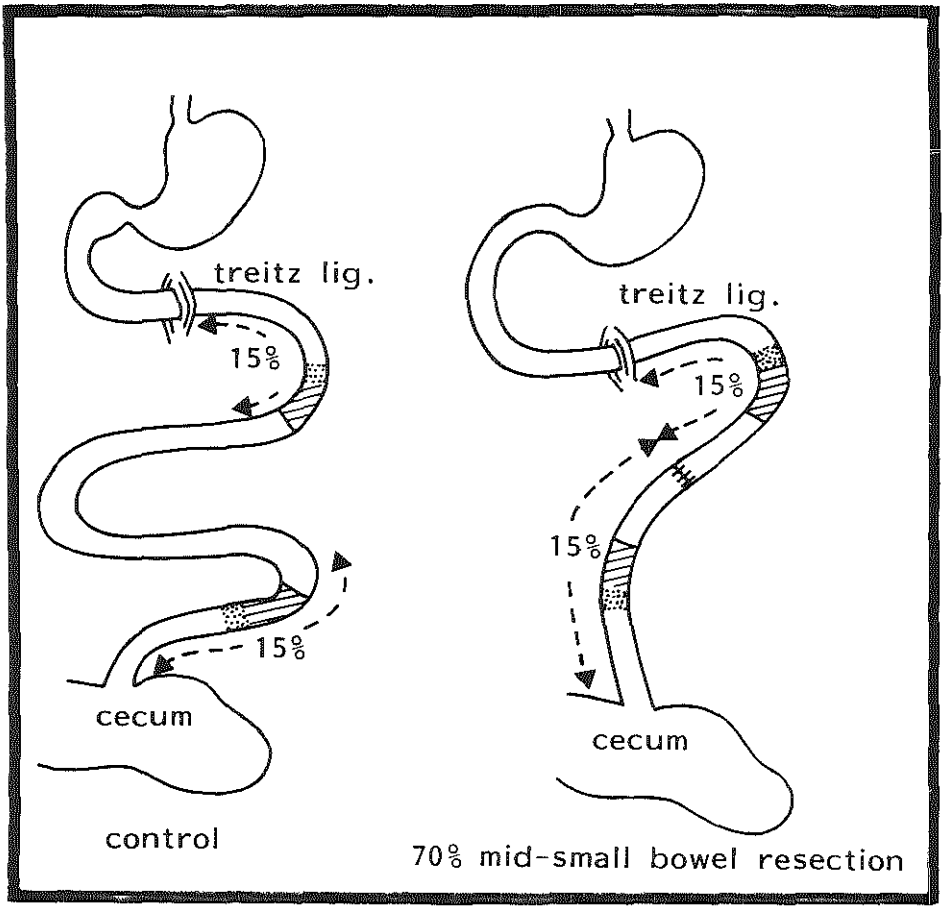


Fig. 6.1 *Dotted areas represent location of specimens for histology, hatched areas represent the sites used for biochemical analysis.*

The length of proximal bowel ranged from 12.0 ± 0.5 in rats fed intravenously to 13.9 ± 1.0 cm in those fed with chow-pellets and the length of distal bowel ranged from 13.1 ± 0.5 in those fed intravenously to 15.6 ± 1.4 cm in those fed with chow-pellets. Corresponding segments were collected from control rats. The 5-cm intestinal segments were opened longitudinally, rinsed in ice-cold saline solution, and their mucosa removed by scraping with a glass slide. Scrapings were frozen at -25 °C. The 1 cm-long segments were opened longitudinally, rinsed in saline solution, coded and stored in 10% Formalin.
The remaining rats of all 3 groups with intestinal resection and the control rats were allowed free access to chow and water for 4 additional weeks before sacrifice.
Fixed and stained intestinal segments were sectioned longitudinally. The tallest 5 villi and their complete crypts were measured using an eyepiece micrometer. The mean value of 5 measurements was used for computation.
The frozen mucosal scrapings were homogenized in 10 mM sodium pyrophosphate containing 0.002% Triton-100. DNA and RNA content were assayed according to the method described in section 3.5.
Sucrase activity was assayed using Glucostat reagent (Statzyme, Worthington Diagnostics, Freehold, NJ) [190].

6.3 Results

Of the fifty rats given TPN after 70 percent mid-small bowel resection, thirty three survived and gained weight. The remaining animals either failed to gain weight because of catheter disruptions or leakage, or they died of infective catheter complications. Of the eighty rats given elemental diet or chow after 70 percent mid-small bowel resection, sixty one survived and gained weight. All control rats survived until sacrifice.

6.3.1 *Somatic Growth*

Intestinal resection inhibited the increment of weight with time as compared with increments in control rats (Fig. 6.2 and 6.3).

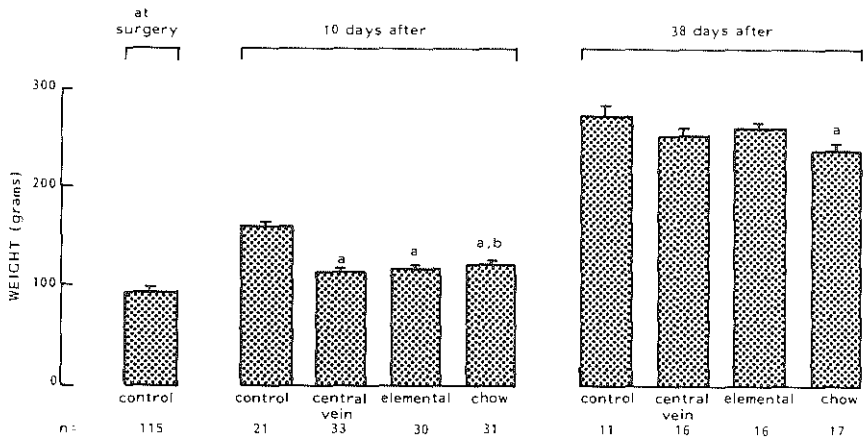


Fig. 6.2 *Weights at the two times of sacrifice*
a : $p < 0.001$ vs control
b : $p < 0.001$ vs central vein

In addition, after 10 days of dietary regimen, the resection-TPN group weighed 7 percent less than the resection-chow group. When all groups were maintained on chow for 4 weeks following the 10 days of dietary regimen, all weighed the same whether they had earlier been given TPN, elemental diet or chow. The body weight of the resection-chow group, however, was 13 percent less than that of the control rats.

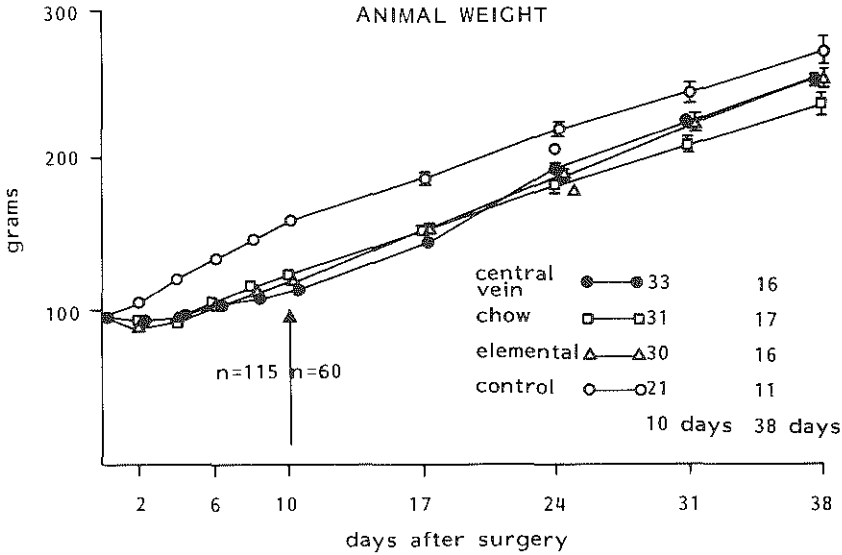


Fig. 6.3 *Animal weight during feeding regimen*

6.3.2 *Intestinal Elongation*

Ten days after resection, the length of the residual jejunum in rats on TPN was 13 percent less than in rats fed chow (Fig. 6.4). In rats fed an elemental diet it was 14 percent less. Ileal length was 16 percent less in rats given TPN and 26 percent less after an elemental diet as compared with chow-fed rats.

After returning to chow feeding for 4 weeks, however, the small bowel of rats previously given TPN was 17.5 percent longer than that of rats fed chow from the start, and the small-bowel of rats previously given an elemental diet was 13.3 percent longer than that of the chow group.

6.3.3 *Mucosal Growth*

Mucosal growth 10 days after intestinal resection was inhibited in rats given TPN, but was marked in rats fed an elemental diet or chow. As compared with the control (unoperated) rats, rats given TPN after intestinal resection had 31 percent shallower ileal mucosal crypts, a 38 percent decrease in jejunal DNA-content and a 53 percent decrease in ileal DNA-content, a 53 percent decrease in jejunal RNA-content, and a 51 percent decrease in ileal RNA-content (Fig. 6.5, 6.6 and 6.7).

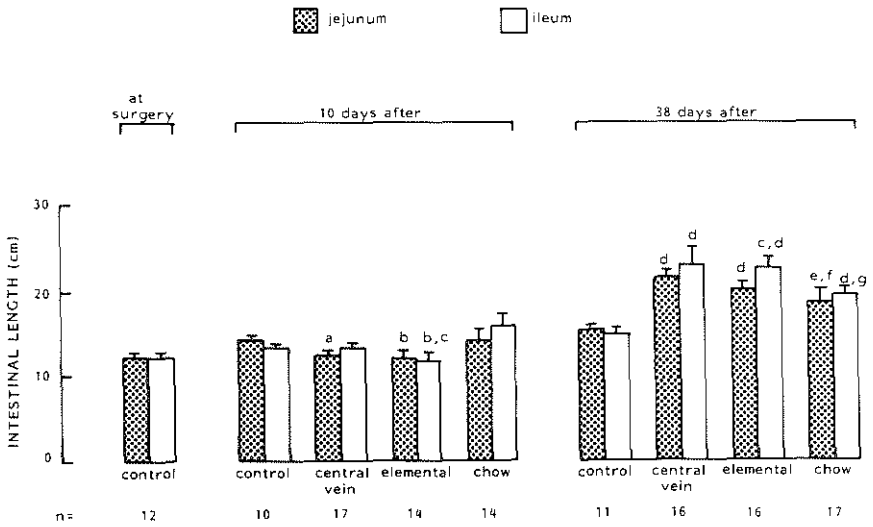


Fig. 6.4 *Lengths of jejunum and ileum*
a: $p < 0.02$ vs control; b: $p < 0.05$ vs control;
c: $p < 0.02$ vs chow; d: $p < 0.001$ vs control;
e: $p < 0.01$ vs control; f: $p < 0.02$ vs central vein.

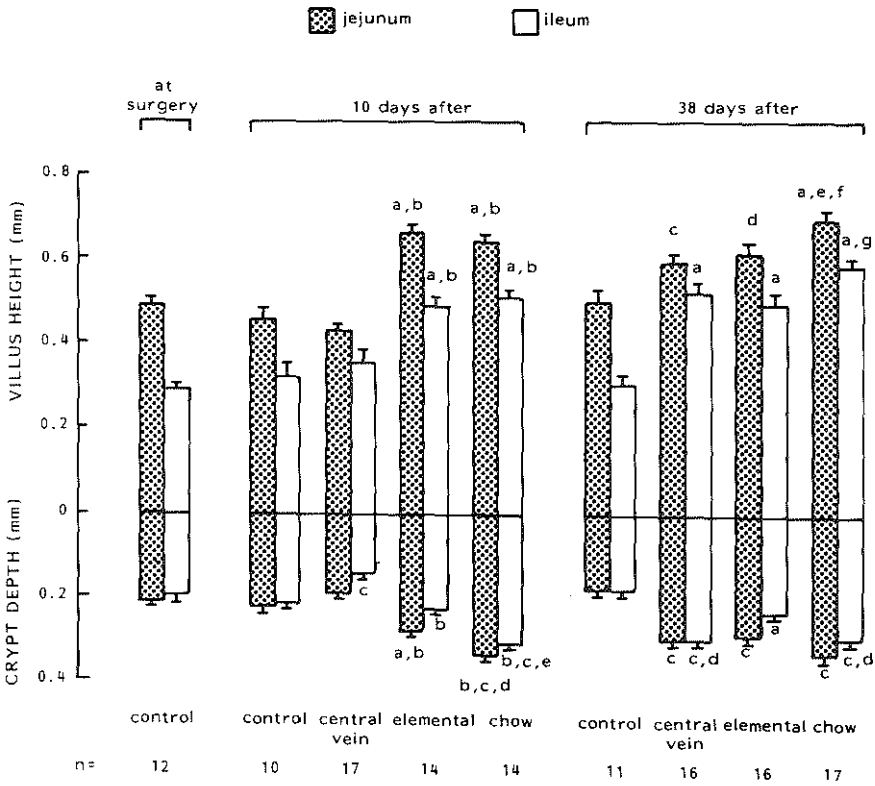


Fig. 6.5 *Villus height and crypt depth*
For villus heights: a: $p < 0.001$ vs control; b: $p < 0.001$ vs central vein; c: $p < 0.05$ vs control; d: $p < 0.01$ vs control; e: $p < 0.02$ vs central vein; f: $p < 0.05$ vs elemental; g: $p < 0.002$ vs elemental. For crypt depths: a: $p < 0.01$ vs control; b: $p < 0.001$ vs central vein; c: $p < 0.001$ vs control; d: $p < 0.01$ vs elemental; e: $p < 0.001$ vs elemental.

In contrast, rats fed chow after resection had a 39 percent increase in jejunal villous height, 51 percent greater jejunal crypt depth, 36 percent more DNA, and 184 percent more RNA. Ileal villus height increased 56 percent, DNA 11 percent, and RNA 104 percent. In rats fed an elemental diet as compared with control rats, jejunal villus height increased 44 percent and crypt depth 28 percent, ileal villus height 54 percent and crypt depth 6 percent. Jejunal DNA-content increased 23 percent while RNA-content increased 81 percent, while ileal RNA-content increased 23 percent.

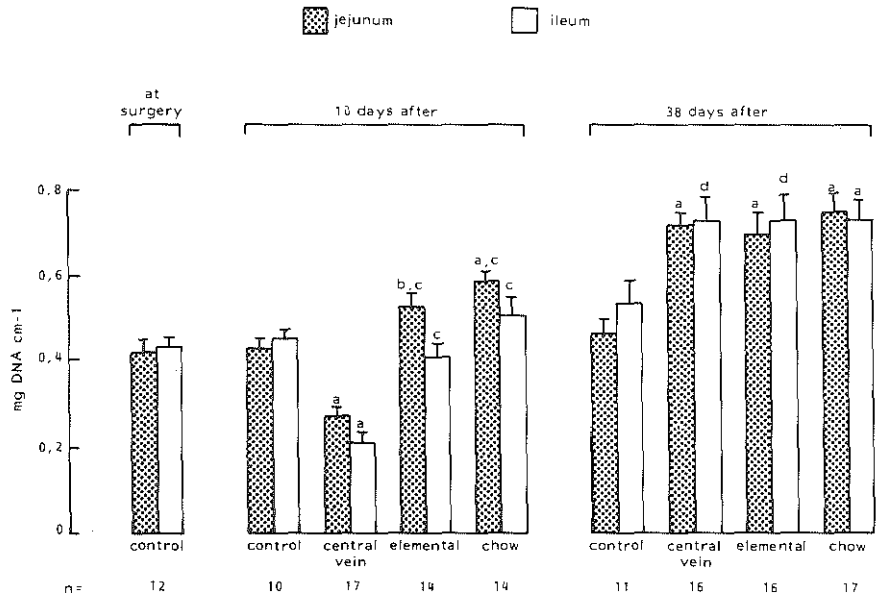


Fig. 6.6 *Mucosal DNA-Content*
a: $p < 0.001$ vs control; b: $p < 0.02$ vs control;
c: $p < 0.001$ vs central vein; d: $p < 0.01$ vs control.

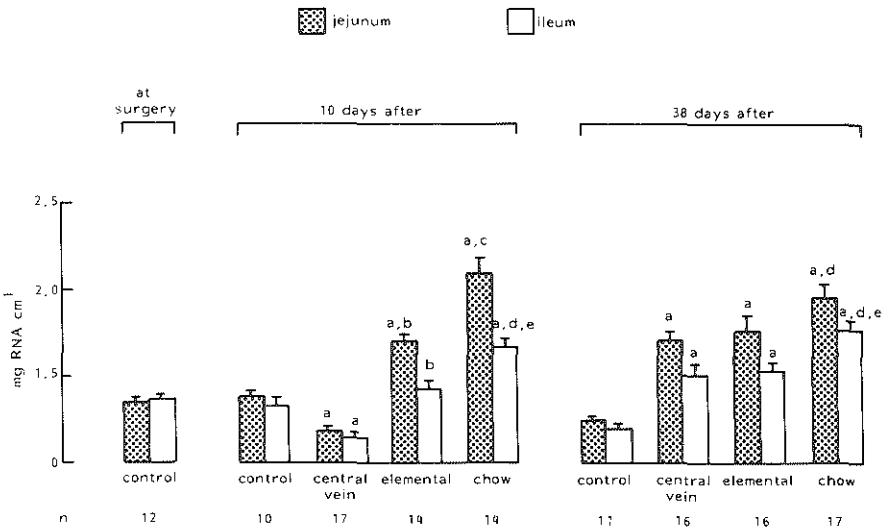


Fig. 6.7 *Mucosal RNA-Content*
a: $p < 0.001$ vs control; b: $p < 0.001$ vs central vein;
c: $p < 0.001$ vs elemental; d: $p < 0.01$ vs central vein;
e: $p < 0.01$ vs elemental.

After chow-feeding for 4 additional weeks, compensatory mucosal growth was apparent in all 3 groups of operated rats regardless of the initial regimens. Nevertheless, some differences in the mucosal growth remained. For example, in rats fed chow throughout, jejunal villus height was 18 percent higher than in rats initially given TPN and 12 percent higher than in rats initially given an elemental diet.
Ileal crypts were 25 percent deeper than rats given an elemental diet initially.
Although there were no significant differences in the longterm DNA-content of the jejunum or ileum among all groups with resection after 4 weeks of chow feeding, the jejunal and ileal RNA-contents of rats fed chow from the start were 33 percent and 53 percent higher, respectively, than in rats initially given TPN.

6.3.4 *Mucosal Cell Size*

Particularly in early cell growth, changes in mucosal cell size are reflected by the RNA to DNA-ratio [210,217].
In rats given TPN, the jejunal mucosal RNA:DNA decreased 24 percent as compared with the value in control rats. To the contrary, in rats fed an elemental diet, the jejunal RNA:DNA was 92 percent greater than in rats given TPN and 173 percent greater in rats fed a chow diet from the start. All rats further increased RNA to DNA-ratio after 4 weeks of chow-feeding; however, the compensatory increase in mucosal RNA:DNA in rats initially given TPN remained 21 percent less in the jejunum and 32 percent less in the ileum compared with values in rats fed chow throughout (Fig. 6.8).

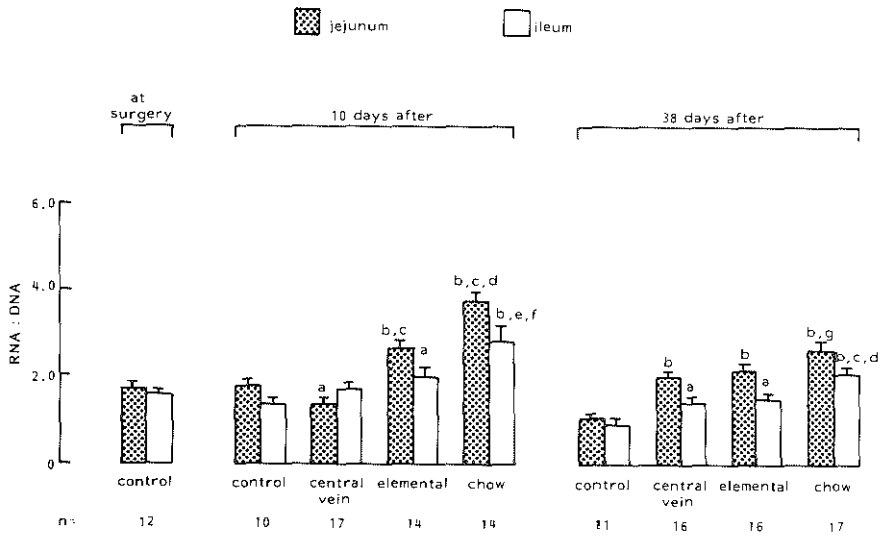


Fig. 6.8 *Ratio of RNA content to DNA content (RNA per cell).*
a: $p < 0.05$ vs control; b: $p < 0.001$ vs control;
c: $p < 0.001$ vs central vein; d: $p < 0.001$ vs elemental;
e: $p < 0.01$ vs central vein; f: $p < 0.05$ vs elemental:
g: $p < 0.05$ vs central vein.

6.3.5 *Mucosal Sucrase Activity*

Jejunal sucrase activity per cm of intestine 10 days after resection rose approximately 250 percent ($p < 0.001$) in rats fed an elemental diet compared with activity in rats given TPN. The rise in ileal sucrase activity was smaller, with 89 percent in rats fed an elemental diet ($p < 0.05$) and 53 percent in those fed chow (not significant).
After 4 weeks of chow-feeding following the ten day period of regimen feeding, all rats with intestinal resection had similar jejunal sucrase activities, which were 100-150 percent higher than in the control rats ($p < 0.001$). Furthermore, the normal jejunal-ileal sucrase gradient [193] was maintained despite having the distal intestinal mucosa in closer contact with higher concentrations of ingested sucrose.
To gain an indirect measurement of the sucrase activity per cell [103,196,210], sucrase activity was also expressed in micromoles per mg DNA.The sucrase activity per cell after intestinal resection demonstrated a response similar to that of the sucrase activity per centimeter (Fig. 6.9).

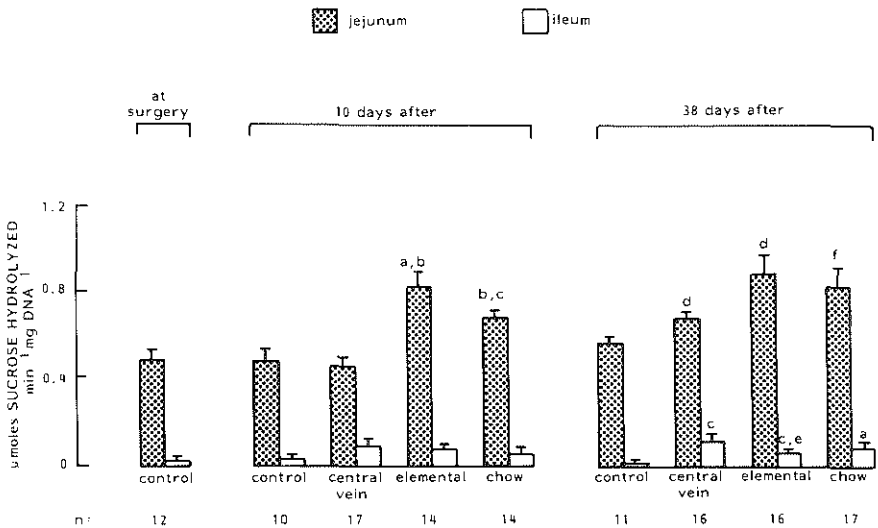


Fig. 6.9 *Sucrase activity per cell.*
a: $p < 0.001$ vs control; b: $p < 0.001$ vs central vein;
c: $p < 0.01$ vs control: d: $p < 0.02$ vs control;
e: $p < 0.05$ vs central vein; f: $p < 0.05$ vs control.

6.4 Discussion

This study demonstrates that ten days of total parenteral nutrition do not permanently inhibit adaptation following 70 percent mid-small bowel resection in young growing rats. The decrease in intestinal length, villus height, crypt depth, and mucosal DNA and RNA contents are reversed by oral feeding for 4 weeks. The initial inhibition of intestinal adaptation is apparently a consequence of early absence of food from the intestinal lumen. Oral food intake allows compensatory growth to occur in the residual intestine.

Luminal nutrient intake is required for normal growth, ontogenic development, and maintenance of mucosal mass in the intestinal tract [4,191,198,199,211,212,213,214]. When neonatal animals suckle, ingested nutrients allow rapid intestinal growth and the intestinal mucosa gains weight [86,93,169,211]. When food is excluded from a bypassed segment of ileum of suckling rats, however, normal longitudinal and mucosal growth of the bypassed ileum do not occur, despite normal somatic growth and normal growth in the remainder of the small bowel [191,198,199]. When young growing rats or puppies are fed parenterally for 10 days or even less, normal growth of the intestinal mucosa in this period does not occur [114,138].

Although TPN initially inhibits post-resectional somatic growth and intestinal mucosal growth, our study shows that both the final body weight and the intestinal length are augmented after resumption of oral diet. Similar long term responses are also observed in rats initially on elemental diet. These results support previous observations that both long term somatic growth and intestinal growth are improved by early parenteral feeding after massive intestinal resection in growing animals [215]. In addition, our study shows that an orally administered elemental diet consisting of a mixture of amino acids and carbohydrates is equally effective.

The increase in mucosal DNA-content and RNA to DNA ratio in young rats fed an elemental diet or chow after intestinal resection demonstrates that the mucosal response to intestinal resection includes both cellular hyperplasia and hypertrophy.

In weanling rats on TPN after intestinal resection our study showed marked inhibition of mucosal sucrase activity while those fed by mouth had an early rise in jejunal sucrase activity. The long term sucrase activity in the jejunum reached similar levels after 4 weeks of chow feeding in all rats that had undergone intestinal resection, regardless of the initial dietary regimen. In the distal ileum an increase in sucrase activity was observed in all rats in response to intestinal resection.

Thus, TPN inhibits intestinal adaptation normally following proximal resection in young, growing rats. However, the inhibition is reversed by a normal diet, while differences in mucosal growth that persist do not affect somatic growth.

CHAPTER VII, Experiment 4

TUMOR GROWTH AND NUTRITIONAL ADEQUACY IN HEPATOMA-BEARING RATS: EFFECT OF TOTAL PARENTERAL NUTRITION WITH AND WITHOUT FAT

7.1 Introduction

An unresolved clinical dilemma is the extent to which a cachectic patient with cancer can be given nutritional support without stimulating growth of the cancer [19,43,74,206].

In human gastrointestinal cancers, total parenteral nutrition (TPN) does not alter the rate of protein synthesis [139]. In animal experiments designed to elucidate the effect of a single nutrient solution, results indicate stimulated growth of Morris hepatoma No. 777 [32,33], and of mammary carcinoma [183] in rats given TPN without fat and high in carbohydrate. Other authors demonstrate no effect of nearly the same TPN mixture on methylcholanthrene-induced sarcoma in rats [79]; they showed that the resultant hyperglycemia decreased the ^{3}H-TdR-labeled specific activity of DNA in the sarcoma.

In another study where TPN with a mixture of carbohydrate and fat was given to rats bearing mammary adenocarcinoma, stimulation of the tumor was least when fat was used as the single non-nitrogenous energy substrate [30]. However, hepatic dysfunction associated with infusion of large doses of fat emulsion has been observed in infants [154], adults [2] and non-tumorbearing rodents [14,31].

In the experiment presented here we will examine the effects of TPN with equal admixture of carbohydrate and fat as energy substrates on the growth of ACI-N rats and a transplantable hepatocarcinoma No. 3294A. Furthermore, we will consider in this experimental setup the effects of TPN on hepatic structure and function. The Morris hepatocarcinoma No. 3294A was chosen for its rapid growth, minimal cell necrosis, low metastatic potential and because knowledge of its carbohydrate and nucleic acid metabolism is extensive [208].

7.2 Material and Methods

Adult male ACI-N rats (10 weeks old), weighing 180-210 g., were used. Approximately 15 x 10^{6} cells of Morris hepatocarcinoma no. 3924A in 0.5 ml 0.9% NaCl solution were innoculated subcutaneously into the flank of

the rat. A palpable tumor in the flank was evident between days nine and ten while rats were maintained on Purina Laboratory Rat Chow and water ad libitum. Serial measurements of the size of the tumor was assessed by a caliper. On day 19, rats bearing tumor measuring 16 cm^3 or more (range 16-68 cm^3) were randomly assigned to one of six nutritional regimens (Table 7.I).

Table 7.I Nutritional Regimen

Chow	Rat chow and water ad libitum
Fasting	0.9% NaCl i.v.
Carbohydrate	Dextrose 25% i.v.
Amino acids	FreAmine ® III 4.25% i.v.
Carbohydrate-TPN	Dextrose 25%, FreAmine ® III 4.25% i.v.
Fat/Carbohydrate-TPN	3.9% Intralipid ® 20%, Dextrose 12.5%, FreAmine ® III4.25%i.v.

All rats were harnessed in metabolic cages. With the exception of the chow-group, all rats had a silastic catheter placed into the superior vena cava under ether anesthesia as described in section 3.3. Intravenous solutions were administered continuously at a rate of 2 ml per hour. Rats on intravenous regimen received equal fluid volumes and intravenous additives including multivitamins and trace elements. The carbohydrate-TPN regimen and the fat/carbohydrate-TPN regimen were isocaloric and isonitrogenous. In the latter regimen 50 percent of the nonprotein calories were from fat and 50 percent from carbohydrate (Table 7.II).

Table 7.II Composition of TPN solution

ml	Carbohydrate-TPN ml	Fat-TPN
8.5% Freamine ® III*	500	500
50 % Dextrose	500	250
20 % Intralipid ®	---	193
Sterile water	---	57

Additives (per liter)

K Acetate	34.6 meq
K Cl	26.8 meq
Ca Gluconate	9.3 meq
MgS04	1.7 meq
NaCl	20.6 meq
Na Phosphate	23.0 meq
Multivitamins**	0.4 ml
Trace elements***	1.0 ml
Penicillin	1 x 10^6 I.U.
Gentamicin	16.0 mg

*Solution content see Table 4.I
**MVI (USV Laboratories, Tuckahoe, NY)
***Each ml contains zinc 1 mg, copper 0.5 mg, manganese 0.2 mg, chromium 0.005 mg, iodide 0.028 mg

Rats were given the prescribed nutritional regimen for five days during which body weight, tumor size, caloric intake and urine volumes were recorded daily. Urine was collected in hydrochloric acid and urine nitrogen was determined as described in section 3.5. After five days of nutritional regimen, surviving animals were injected intraperitoneally with 100 uCurie/100 g of 300 uCi/mmole of tritiated methyl thymidine (New England Nuclear, Boston, MA) one hour before sacrifice under ether anesthesia by the method of exsanguination. Blood was collected for biochemical determinations; liver and paired tibialis anterior muscles were excised and weighed. Representative samples of tumor, liver and muscle were weighed and either immediately frozen in liquid nitrogen for biochemical analysis at a later time or placed in 10% formalin for histological examination as described in chapter III.

Analytical Method

The formula for a prolate spheroid was used to calculate tumor-volume at different time points of tumor growth: V = 1/6 *pi* AB, where A = long diameter, B = short diameter. The weight of the tumor was measured after sacrifice of the rat.

Tumor doubling time was calculated assuming uniform exponential growth during the five days of nutritioal regimen according to the relation: doubling time = 4 log2 : logR, where R is the ratio of final tumor volume to initial volume.

7.3. Results

7.3.1 *Initial Body-weight and Tumor-volume*

The mean body weight of the tumor-bearing rat and the mean tumor-volume calculated from the formula of a spheroid (V = 1/6 *pi* AB^2) were similar in the six study groups at the beginning of the five days of nutritional regimen (Table 7.III). The mean body-weight ranged from 237 g. to 245 g., and the mean tumor-volume from 31.3 cc to 39.9 cc.

Table 7.III Initial body-weight and tumor-volume

Group	No.	Weight (g)	Tumor Volume (cm^3)
Chow	12	239 ± 3.7	33.5 ± 4.0
Fasting	10	237 ± 5.1	31.3 ± 3.0
Amino Acids	13	243 ± 3.2	37.8 ± 3.6
Carbohydrate	11	245 ± 3.4	39.9 ± 3.7
Carbohydrate-TPN	12	242 ± 3.0	38.5 ± 3.3
Fat/Carbohydrate-TPN	12	238 ± 4.2	34.5 ± 4.0

7.3.2 *Calorie and Protein Intake*

Calorie and protein intake were similar in the carbohydrate-TPN group and the fat/carbohydrate TPN group that received an isocaloric and isonitrogenous nutritionally adequate intravenous regimen (Table 7.IV). As compared to the chow-fed rats, caloric intake was similar and protein intake was approximately 25 percent less in the rats receiving TPN with or without fat. Rats given amino acid only received a protein-adequate diet but approximately one-sixth of the calories received by the TPN groups. Rats given carbohydrate alone received a protein-free regimen with approximately 15 percent less calories compared with the TPN groups. Fasting rats received saline for hydration only.

Table 7.IV Caloric intake and Composition of Nutritional Regimen

Group	Caloric Intake (cal/100g/d)	Protein Intake (g/kg/d)	Non-Protein Calories % Carbohydrate	% Fat
Chow	22.5 ± 0.8	11.4 ± 0.4	88	12
Fasting	0	0	0	0
Amino Acids	3.2 ± 0.2	8.0 ± 0.2	0	0
Carbohydrate	17.0 ± 0.4	0	100	0
Carbohydrate-TPN	20.0 ± 0.4	8.4 ± 0.3	100	0
Fat/Carbohydrate-TPN	21.6 ± 0.7	9.1 ± 0.3	50	50

7.3.3 *Nitrogen Balance and Weight Change*

Consistent with the higher protein intake, nitrogen balance was best in chow-fed rats compared with others (Table 7.V). There was no difference in nitrogen balance between rats given TPN with or without fat. Better nitrogen balance was observed in rats given amino acid alone compared with rats given carbohydrate alone, despite the fact that the carbohydrate group received six times more calories.

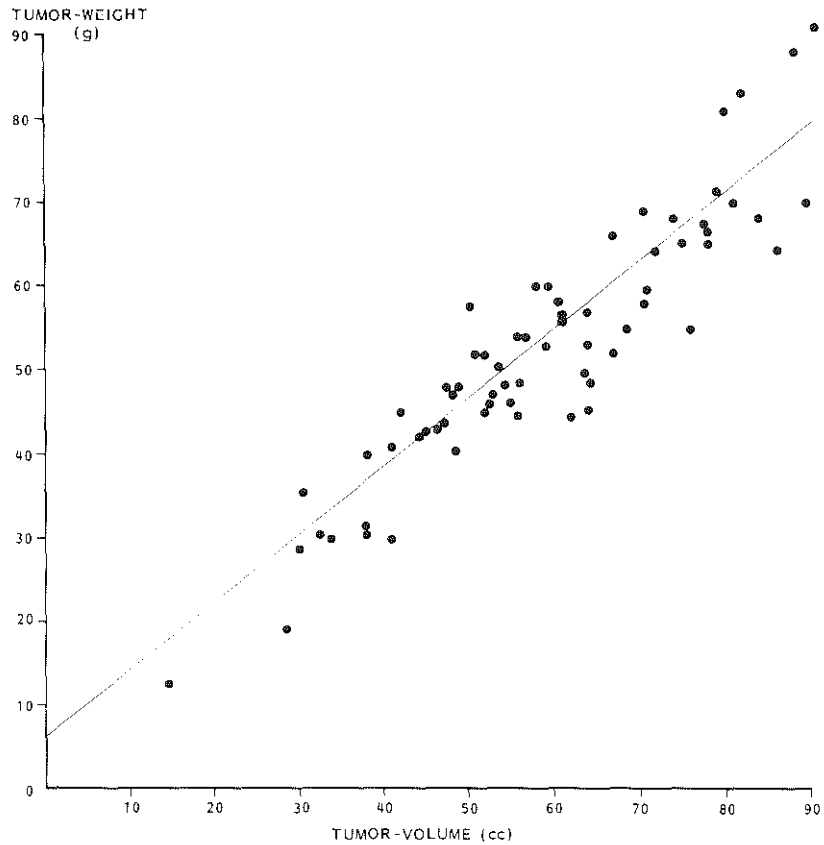
Table 7.V Nitrogen Balance and Weight Change After 5 Days Nutritional Regimen

Group	Nitrogen Balance (mg/kg/d)	Total Body Weight Change(g)	Host Weight Change(g)	Tumor Weight Change(g)
Chow	+1165 ± 71	+23.5 ± 4.9	- 0.6 ± 3.0	+26.8 ± 4.4
Fasting	- 627 ± 56a	-31.5 ± 4.0a	-38.2 ± 2.7a	+ 8.2 ± 1.9a
Amino acids	+ 72 ± 38a	-17.8 ± 1.9a	-33.5 ± 1.4a	+15.9 ± 2.0a
Carbohydrate	- 322 ± 26a	+ 7.6 ± 3.0a	-13.0 ± 2.9a	+20.3 ± 2.1
Carbohydrate TPN	+ 548 ± 76a	+30.7 ± 3.0	- 4.8 ± 3.4	+38.1 ± 10.5
Fat/Carbohydrate TPN	+ 597 ± 49a	+30.8 ± 5.5	- 2.8 ± 3.4	+26.3 ± 2.9

Relative to chow = a: p value < 0.05
Carbohydrate-TPN *vs.* Fat/Carbohydrate-TPN = no significant difference

Rats given TPN with or without fat and chow-fed rats had a similar 12 percent gain in total body weight (Table 7.V). In contrast, fasting rats had 12 percent reduction in total body weight, while rats that received hypocaloric regimens lost weight (aminoacid group) or gained less weight (carbohydrate group).
In the attempt to estimate tumor-weight during the course of the experiment, a relationship was derived by plotting calculated tumor-volume versus observed final tumor-weight (Fig. 7.2).

Fig. 7.2. TUMOR: VOLUME VERSUS WEIGHT



These estimations permitted the analysis of changes in total body weight minus the changes in tumor-weight i.e. changes in host-weight. Table 7.V shows the changes in host-weight obtained by deducting the changes in tumor-weight from changes in total body weight.

It became apparent that host weights were barely maintained in rats given TPN or fed per orally, while approximately 15-20 percent reduction in host-weight was observed in fasting rats and rats given amino acid alone. Weight loss in rats given carbohydrate alone was approximately half of that observed in fasting rats.
Therefore, nitrogen balance in rats given amino acid alone has a poor correlation with body weight change. The apparent positive nitrogen balance in rats given amino acid alone was not associated with actual gain in body weight. In all six groups of rats, the apparent change in total body weight could be attributed to an increase in tumor weight.

7.3.4 *Tumor Growth*

Tumor growth after five days of nutritional regimen as measured by tumor volume, weight, doubling time, DNA specific activity, nucleic acid content and protein content is shown in Table 7.VI.
Changes in tumor weight at the end of the 5 day period parallelled changes in tumor volume. Rats given TPN with or without fat and chow-fed rats had a similar, near 100 percent increase in tumor weight and tumor volume. Fasting rats showed approximately 35 percent increase in tumor volume which was about 1/3 of the increase in tumor volume in rats given TPN or chow-fed. Rats given amino acid alone showed 50 percent increase in tumor volume and rats given carbohydrate alone showed 67 percent increase in tumor volume. Therefore, tumor doubling times were about 75 percent longer in fasting and semi-fasting rats compared with chow-fed rats and rats given TPN.

Table 7.VI Effect of Five Days of Nutritinal Regimen on Tumor

Group	Chow	Fasting	Amino acid	Carbo-hydrate	Carbo-hydrate TPN	Fat/Carbo hydrate TPN
Volume (cc)	32 ± 3.3	10.9 ± 2.9a	20.0 ± 2.7a	26.9 ± 2.4	35.3 ± 3.4	34.0 ± 3.0
Weight (g)	26.8 ± 4.4	8.2 ± 1.9a	15.9 ± 2.0a	20.3 ± 2.1	38.1 ± 10.5	26.3 ± 2.9
Doubling Time (d)	4.2 ± 0.2	7.2 ± 0.8a	6.3 ± 0.5a	5.8 ± 0.7a	4.7 ± 0.5	4.1 ± 0.3
DNA (mg/g)	4.7 ± 0.2	4.9 ± 0.2	5.9 ± 0.2a	4.9 ± 0.2	4.8 ± 0.2	5.8 ± 0.3b
RNA (mg/g)	9.8 ± 0.5	8.0 ± 1.0	14.4 ± 0.8a	9.9 ± 0.4	15.1 ± 1.3a	15.4 ± 1.0a
RNA:DNA	2.1 ± 0.1	1.6 ± 0.2a	2.5 ± 0.2a	2.0 ± 0.1	3.1 ± 0.3a	2.7 ± 0.1a
DNA specif. activity (cpm/ug DNA)	373 ± 27	391 ± 100	360 ± 70	492 ± 100	511 ± 92	290 ± 65
Protein (mg/g)	142 ± 9	124 ± 10	139 ± 4	128 ± 4	143 ± 7	143 ± 8

Relative to chow = a: p value < 0.05
Carbohydrate-TPN *vs.* fat/carbohydrate-TPN = b: p value < 0.05

Tumor DNA showed a 25 percent increase in rats given amino acid alone and in rats given fat/carbohydrate-TPN as compared with chow-fed rats. Likewise, tumor RNA showed 47 percent increase in rats given amino acid and either TPN regimen. Tumor DNA specific activities were similar in all six groups of rats and appeared independent of the amount of caloric or protein intake.

As compared with chow-fed rats, the ratio of RNA to DNA showed a 25 percent decrease in fasting rats, a 25 percent increase in rats given amino acid and 30 percent increase in rats given either of the two TPN regimens. Tumor protein contents were similar in all six study groups suggesting that changes in the ratio of RNA to DNA were not directly related to changes in cell size (Table 7.VI).

Histological examination of tumors harvested from fasting and semi-fasting rats (amino acid and carbohydrate alone) showed increased areas of tumor necrosis compared with chow-fed rats and rats given either TPN regimen. The presence of increased tumor necrosis in fasting and semi-fasting rats explained the observation of a smaller tumor size without actual reduction in the rate of DNA synthesis by the viable portion of the tumor.

7.3.5 *Liver Changes*

As Table 7.VII shows rats given TPN without fat had a 17 percent increase in liver weight when compared with rats given chow. Rats given TPN with fat had a 42 percent increase in liver weight in comparison with chow-fed rats. The increase in liver weight was 21 percent in rats given TPN with fat compared to those given TPN without fat.

Table 7.VII Effect of Five Days of Nutritional Regimen on Liver

Group	Chow	Fasting	Amino acid	Carbo-hydrate	Carbo-hydrate TPN	Fat/Carbo-hydrate-TPN
Weight (g)	8.8 ± 0.4	5. ± 0.3a	7.6 ± 0.2	7.8 ± 0.3	10.3 ± 0.3a	12.5 ± 0.5a,b
DNA (mg/g)	1.9 ± 0.1	2.4 ± 0.1a	3.0 ± 0.1a	2.1 ± 0.1a	2.3 ± 0.1a	2.6 ± 0.1a,b
RNA (mg/g)	13.4 ± 0.4	12.9 ± 0.6	20.4 ± 0.6a	13.9 ± 0.6	20.4 ± 0.5a	21.3 ± 0.7a
RNA/DNA	7.1 ± 0.2	5.4 ± 0.3a	6.0 ± 0.4	6.9 ± 0.4	9.1 ± 0.4a	8.2 ± 0.4
Protein (mg/g)	239 ± 10	216 ± 12	226 ± 3	180 ± 6a	224 ± 8	204 ± 7a

Relative to chow = a: p value < 0.05
Carbohydrate-TPN *vs.* fat/carbohydrate-TPN = b: p value < 0.05

Histological examination of liver showed a moderate amount of fatty infiltration in the liver of rats given carbohydrate alone and of rats given TPN with and without fat.
In comparison with chow-fed rats, liver DNA was increased by 10 percent in rats given carbohydrate alone, 21 percent in fasting rats and those given carbohydrate-TPN. In rats given amino acid alone a 58 percent increase of liver DNA was recorded and those given fat/carbohydrate TPN showed an increase of 37 percent.
Liver RNA was increased by approximately 50 percent in rats given amino acid alone and in rats fed either TPN with or without fat when compared to chow-fed rats. However, the ratio of RNA to DNA showed an increase of 28 percent in rats given carbohydrate-TPN only when compared to chow-fed rats. In contrast, the ratio of RNA to DNA in fasting rats decreased 24 percent relative to chow-fed rats consistant with a decrease in cell size in the liver of fasting rats.
Liver protein was reduced by 25 percent in rats given carbohydrate alone and by 15 percent in rats given TPN plus fat as compared with chow-fed rats. However, liver protein was similar between rats given TPN with or without fat (Table 7.VII).

7.3.6 *Serum Biochemistry*

At the time of animal sacrifice, hyperglycemia was present in rats given TPN with fat. When compared to chow-fed rats, serum glucose decreased 35 to 45 percent in fasting and semi-fasting rats. Serum lactate level of 6.31 $\pm$ 0.39 mmoles/l in chow-fed tumor-bearing rats was similar to non tumor-bearing rats (6.83 $\pm$ 1.14 mmoles/l, n = 12, unpublished data). However, serum lactate levels were elevated by 29 percent in rats given carbohydrate alone, 53 percent in rats given TPN without fat and 64 percent in rats given TPN with fat compared to chow-fed rats. Serum cholesterol was increased by approximately 18 percent in rats given TPN with fat when compared to chow-fed rats but the increase was not significant. Serum albumin levels were decreased by 28 percent in fasting and semi-fasting fats and in rats given TPN without fat compared to chow-fed rats, while rats given TPN with fat showed a smaller decline in serum albumin level. Serum SGPT and alkaline phosphatase were significantly elevated in rats given carbohydrate alone when compared to chow-fed rats (Table 7.VIII).

Table 7.VIII Serum Glucose and Liver Function Tests After 5 Days Nutritional Regimen

Group	Chow	Fasting	Carbo-hydrate	Amino Acid	Carbo-hydrate TPN	Fat/Carbo hydrate-TPN
Glucose (mmoles/l)	7.51 ± 0.73	4.50 ± 0.97a	4.11 ± 0.49a	4.74 ± 0.54a	6.38 ± 1.01	11.13 ± 1.12a
Cholesterol (mmoles/l)	3.67 ± 0.34	1.78 ± 0.24a	2.0 ± 0.11	3.45 ± 0.61	3.04 ± 0.30	4.45 ± 0.71
Albumin (g/l)	35.6 ± 1.2	25.2 ± 2.4a	27.1 ± 1.4a	23.2 ± 1.6a	24.6 ± 1.9a	30.4 ± 3.6
SGPT (IU/l)	24.6 ± 1.9	39.7 ± 8.5a	46.6 ± 11.8a	18.5 ± 2.1a	58.8 ± 36	26.2 ± 6.9
Alk. Phos. (IU/l)	151 ± 8	336 ± 41a	512 ± 91a	214 ± 28	262 ± 56	199 ± 43
Lactate (mmoles/l)	6.31 ± 0.36	4.39 ± 0.92	8.16 ± 0.45a	5.44 ± 0.45	9.67 ± 0.54b	10.35 ± 0.92c

Relative to chow = a: $p < 0.05$
b: $p < 0.001$
c: $p < 0.002$

7.3.7 *Muscle Changes*

Changes in the weight of individual skeletal muscle may reflect changes in the entire skeletal muscle mass [38]. The average weight of paired tibialis anterior muscle was decreased by 23 percent in fasting rats and in rats given amino acid alone, 10 percent in rats given carbohydrate alone and in rats given TPN with fat when compared to chow-fed rats (0.39 ± 0.01 g.). The weight of tibialis anterior muscles of rats given TPN with fat did not differ significantly from the weight found in rats on TPN without fat (Table 7.IX).

Table 7.IX Effect of Five Days of Nutritional Regimen on Muscle

Group	Chow	Fasting	Amino Acid	Carbo-hydrate	Carbo-hydrate TPN	Fat/Carbo-hydrate-TPN
Weight(g)	0.39 ± 0.01	0.30 ± 0.01a	0.30 ± 0.01a	0.35 ± 0.01a	0.37 ± 0.01	0.35 ± 0.01a
DNA(mg/g)	0.52 ± 0.04	0.50 ± 0.03	0.61 ± 0.04	0.44 ± 0.03	0.46 ± 0.03	0.49 ± 0.03
RNA(mg/g)	7 ± 0.5	5 ± 0.2a	6.5 ± 0.2a	6.2 ± 0.5	5.9 ± 0.3	6.2 ± 0.5
RNA/DNA	13.6 ± 0.3	10.7 ± 0.8a	11.3 ± 0.5a	14.4 ± 1.2	12.6 ± 1.1	13.4 ± 1.0
Protein (mg/g)	290 ± 14	268 ± 8	248 ± 9a	259 ± 11	257 ± 11	283 ± 16

Relative to chow = a: p value < 0.05

Muscle DNA showed insignificant variation among the study groups, and muscle RNA showed decreases in fasting rats and rats given amino acid alone. Muscle protein showed a significant decrease in rats given amino acid alone, perhaps reflecting the preservation of skeletal protein in early stages of nutritional deficiency.

7.3.8 Discussion

TPN, with or without fat, did not stimulate the growth of transplanted hepatocarcinoma in these ACI-N rats, despite an adequate supply of nutrient substrates to maintain host nutrition as well as chow-fed rats. Rats given TPN with and without fat showed similar changes in tumor weight, tumor volume, tumor doubling time, tumor protein content and incorporation of radioactive thymidine into tumor DNA [119] when compared to chow-fed rats. For rats given TPN without fat, similar findings were previously observed in methylcholanthrene-induced sarcoma [79]. However, the reduced incorporation of radioactive thymidine into DNA associated with hyperglycemia was not seen in this study. TPN without fat also did not stimulate growth of Walker 256 carcinosarcoma [111] and hepatoma 5123 [148]. In contrast, TPN without fat stimulated growth of transplantable mammary carcinoma [183] and Morris 7777 hepatoma [32]. In the case of TPN with fat, Buzby et al.[30] observed reduction in growth of mammary adenocarcinoma when fat was used to provide all non-nitrogenous calories. However, the administration of TPN with all calories from fat to ACI-N rats bearing hepatocarcinoma 3924A in a pilot study (unpublished data) resulted in a high mortality rate associated with marked hepatosplenomegaly consistant with fat overloading [31].

On the other hand, TPN with fat (equal calories from carbohydrate and fat) as used in this study maintained host body weight, nitrogen balance, serum albumin, liver protein and muscle weight as well as TPN without fat. The nutritional efficacy of TPN without fat in tumor-bearing rats has been observed by others [32,48,79,183]. Our similar results using TPN with fat are in agreement with the observation of Kishi et al.[111].

The nutritional deficit in fasting and semi-fasting rats inhibited tumor growth as shown by a 33 to 65 percent reduction in tumor weight when compared to adequately nourished rats given chow or TPN. However, the inhibition of tumor growth was associated with increase in tumor necrosis and parraled a marked reduction in the body weight of the host. Daly et al.[49] also noted reduction in growth of Walker 256 carcinosarcoma-bearing rats given protein-free diet. Whereas, tumor growth in rats bearing mammary adenocarcinoma [30] or sarcoma [78] was not inhibited by fasting. Therefore, variations in the biochemical constitution of different tumor types may result in minor differences in its responses to nutritional manipulation.

In this study, serum lactate levels in tumor-bearing rats fed TPN with and without fat and in rats receiving carbohydrate alone were elevated by 29 to 64 percent. It appears that hyperlactemia in tumor-bearing rats is associated with the infusion of a high glucose load.

Hepatomegaly is observed in tumor-bearing rats given TPN with and without fat. Increases in hepatic nucleic acids may represent hepatic injury which is suggested on histological examination by the presence of moderate hepatic steatosis. Serum SGPT and alkaline phosphatase were increased but not significantly when compared to chow-fed rats. It has been previously observed that hepatomegaly in rats was not reflected by SGPT increases until fat provided 100 percent of the non-nitrogenous calories [31]. In non tumor-bearing rats receiving TPN with fat, hepatomegaly is observed when fat contributed more than 50 percent of the calories [14,31].

Therefore, TPN with and without fat maintains the nutritional status of ACI-N rats bearing hepatocarcinoma 3924A equally well without stimulation of tumor growth albeit hepatomegaly limits excessive administration of TPN.

CHAPTER VIII

GENERAL DISCUSSION

Total parenteral nutrition (TPN) is a widely utilized therapeutic modality of documented efficacy in treating patients in whom enteral nutrition is impossible or undesirable. Although metabolic derangements and catheter-related complications were commonly encountered during the early experience with TPN, most of these are now well understood and are easlily avoided with proper monitoring, improved nutrient solutions, and technical availability of supplemental preparations to avoid or correct specific deficiencies (essential fatty acids, trace elements, etc).

Of continued concern, however, is the frequent occurrence of hepatic dysfunction during TPN. Since first described by Peden [151], abnormalities of liver structure and function in patients receiving TPN have been noted with increased frequency. This is particulary true in infants, occurring in as many as 42 percent of neonates receiving TPN [187].

This dysfunction is characterized anatomically as hepatomegaly, morphologically as cholestasis and parenchymal fat deposition, and biochemically by increased serum levels of alkaline phosphatase, liver transaminases, and by conjugated hyperbilirubinaemia.

Hepatic dysfunction during TPN has been related to numerous deficiencies and/or imbalances in parenteral nutrient solutions including essential fatty acid deficiencies, improper calorie to nitrogen ratio and specific amino acid or vitamin deficiencies.

However, in the absence of such specific abnormalities, hepatic dysfunction remains a common clinical problem in the parenterally alimented patient.

Healthy human beings ingesting the same quantities of nutrients as patients receiving parenteral hyperalimentation do not develop the above mentioned metabolic complications associated with parenteral hyperalimentation. Ordinarily, nutrients pass, via the intestinal tract, through the portal circulation to the liver and are then metabolized, stored, and released by the liver in an exquisitly controlled manner. With infusions of concentrated nutrient solutions into the general circulation, contrary to nutrients entering the body via the portal venous system, the infused substrates are first exposed to "peripheral tissues".

Amino acids delivered into the general circulation are brought to the liver only as a function of that portion of the cardiac output flowing through the hepatic artery and portal vein, and the amino acids are available to metabolically-active peripheral tissues at the same plasma concentration as in blood flowing to the liver. Thus, amino acids infused into the superior

vena cava may be squandered, with the carbon chains being oxidized, and part of the amino groups excreted as excess urinary nitrogen [152]. Therefore, release of amino acids from the periphery to the liver for hepatic utilization may cause an aminoacid imbalance, which in turn could be responsible for hepatic dysfunction.
Prehepatic infusion of TPN allows the preferential and selective hepatic modifications of amino acids that normally occur with passage of portal blood through the liver, an organ with a powerful role in amino acid metabolism.
The possibility that the route of administration of TPN (portal-venous compared with central-venous and gastrointestinal administration) is important, has been experimentally investigated with special emphasis on changes in liver function and structure.
Both groups of rats fed parenterally by either central-venous or prehepatic route, demonstrated hepatomegaly with increased total hepatic lipid content. The same basic liver function abnormalities encountered with systemic infusion of TPN were noted in the prehepatic-infused rats.
Our results are in agreement with previous reports [28] postulating a more effectively utilization of amino acids when administered through the gastro-intestinal tract rather than intravenously, suggesting that both the modulation of nutrient substrates by the gastro-intestinal tract and the associated postabsorptive hormonal interaction are important factors in minimizing the development of fatty infiltration of liver. Furthermore, it appears that in the absence of substantial benefit in improving the nutritional efficacy of TPN, the clinical usefullness of prehepatic TPN with its added risk of portal vein thrombosis is very limited.

Another possibility is that hepatic dysfunction during TPN may be related to fatty deposition within the liver, when the rate of glucose infusion exceeds the capacity of the hepatocytes. It has therefore become a common clinical practice to decrease the total parenteral carbohydrate load delivered to patients with abnormal liver function tests indicating hepatic dysfunction.
The mechanism proposed above, however, may suggest an alternative approach. If fatty deposition is the result of excess glucose delivery, then partial caloric provision by a non-gluconeogenic substrate like Intralipid® may permit a decreased glucose load while maintaining optimal total caloric delivery.
The purpose of the second experiment was to compare the effect of fat-free and fat-containing caloric substrates on the development of hepatic dysfunction during TPN. Although weight gain and positive nitrogen balance can be obtained by either fat-containing or carbohydrate-based

TPN [9,184], some studies reported that fat-based TPN may be less efficient compared with carbohydrate-based TPN in man [150] or in rats[71]. However, better weight gain and nitrogen utilization in rats given fat-based TPN have been observed [159].

The results of our study confirmed that carbohydrate-based and fat-based TPN are equally effective in maintaining bodyweight, positive nitrogen balance, muscle weight, liver and muscle protein in rats over a 7-day period.

However, in our study, hepatomegaly and hepatic dysfunction were not prevented by incorporating fat emulsion equivalent to 50 percent of the caloric source. Rats given 10 days of fat-based TPN (20% Intralipid ®, 9 g/kg/d) were previously observed to have hepatomegaly and hepatic dysfuntion [116].In puppies given 4-8 weeks of fat-containing TPN (10% Intralipid ®, 2-4 g/kd/d) the lipid content of their livers doubled [113]. However, fatty infiltration of liver and hepatomegaly were prevented in rats given fat-based TPN in which a smaller proportion of calories (25%) were from fat [31].

Elevated serum alkaline phosphatase activity and levels of bilirubin and cholesterol were observed in adult patients given fat-based TPN with 60 percent calories from 10% Intralipid ®
(2 g/kg/day)[165].

Liver biopsies in some of these patients showed no fatty infiltration, but the presence of periportal inflammation and bile duct proliferation in the portal triads with cannalicular bile plugs [165]. Both portal triad inflammation and fatty infiltration of liver were observed in our rats given fat-based TPN. Serum cholesterol levels and alkaline phosphatase activity were elevated two-fold in our rats given fat-based TPN as well.

Fouin-Forturet et al.[70] observed an increase in biliary lithocholic acid in patients with inflammatory bowel disease with elevated serumalkaline phosphatase and amino transferase activities after 2 weeks of TPN. Alteration in the composition of biliary bile acids by TPN may be a cause of hepatic dysfunction, since a similar pathological picture can be produced in the liver of animals ingesting lithocholic acid [65,101,104].

Allerdyce [2] recently observed that hypercholesterolemia and rise in alkaline phosphatase in patients on fat-based TPN (10% Intralipid ®, 2-3 g/kg/day) were early signs of hepatic cholestasis. However, in adults receiving the same dosage of fat emulsion no significant elevation of alkaline phosphatase or hepatic dysfunction was previously observed [88]. Similarly, hepatic dysfunction was not observed in home-TPN patients relying on 10% Intralipid ® to supply 40 percent of their caloric requirements over periods of months to years [107]. Available animal and human data suggest that a balanced TPN regimen with the fat moiety contributing not more than 50 percent of caloric requirements may be

optimal for avoidance of fatty infiltration of liver or cholestasis during TPN.

Total parenteral nutrition as a model for studying the consequences of withdrawing exogenous luminal nutrition from the intestine has been used by others [63,108,124]. Our results confirm these reports that luminal nutrition is essential for the development of both structural and functional adaptation in the residual intestine after resection. It could be argued that with continued intestinal cell shedding and the secretion of salivary, gastric and pancreatico-biliary secretions, the intestine is not totally deprived of luminal nutrition during intravenous feeding. However, the contribution to luminal nutrients from these sources is probably relatively small. Furthermore, it seems likely that both the epithelial cell proliferation rate and the volume of digestive secretions will be markedly reduced in the absence of a food stimulus. Evidence in support of this hypothesis has been delivered in a previous report from our laboratory [198,199]. When food is excluded from a bypassed segment of ileum, normal longitudinal and mucosal growth of the bypassed ileum is inhibited, despite normal somatic growth and normal growth in the remainder of the small bowel. Several authors showed that compensatory growth of the intestinal mucosa does not occur when TPN is given as the sole nutrient support to immature rats and dogs after resection [63,137]. However, we showed in experiment 3 that ten days of TPN does not permanently inhibit intestinal adaptation following small bowel resection in young growing rats. We found that a resumption of oral diet reversed the decreases in villus height, crypt depth, and mucosal DNA and RNA contents.Moreover, both the final body weight and the intestinal length, initially inhibited during TPN, were augmented when rats were allowed oral food intake for a further 4 weeks.

Despite evidence that in man, the nitrogen of an elemental diet like Vivonex-HN is said to have a low biologic value and is not efficiently used for anabolism [179], we observed that long term somatic growth as well as intestinal growth in the rat were improved after oral administration of an elemental diet consisting of a mixture of amino acids and carbohydrates. The increase in mucosal DNA content and RNA to DNA ratio in young rats fed an elemental diet or chow after intestinal resection demonstrates that the mucosal response to intestinal resection includes both cellular hyperplasia and hypertrophy. Hyperplasia requires food in the intestinal lumen [15,53,133]. Cellular hypertrophy (an increase in enterocyte cell size), however, is not a normal response seen in mature adult rats after intestinal resection [210] and may be peculiar to the suckling rat [69]. The decreased longitudinal growth of intestinal length when rats were fed chow continuously after intestinal resection may have acted as a stimulus to increase the size of enterocytes to compensate for a shorter length.

In both growing and mature rats luminal nutrition stimulates mucosal enzymic activity as well as mucosal growth [26,97,137,138]. Deprived of luminal nutrition and maintained on TPN after intestinal resection, the weanling rats in our experiment showed markedly decreased sucrase activity while those fed by mouth had an early rise in jejunal sucrase activity. The early rise in ileal sucrase activity seen only in rats fed 20% Dextrose by mouth may be a non-specific consequence of a high carbohydrate load, similar to that seen in weanling rats on a lactose-free diet [97].

The increased sucrase activity in the distal ileum 38 days after intestinal resection could be explained by the distal ileum coming into contact with a greater concentration of carbohydrate once a part of the proximal bowel is removed [53,97].

In conclusion, we have shown that TPN inhibits the adaptive response to intestinal resection in young, growing rats. However, reversal of this inhibiton occurs after resumption of an oral diet. Differences in mucosal growth that persist do not affect somatic growth. Despite evidence that an elemental diet does not lead to efficient nitrogen retention in adult human beings, it may stimulate intestinal adaptation and somatic growth in the maturating intestine provided that it is tolerated without diarrhea or other metabolic problems.

The importance of malnutrition as a major source of morbidity and mortality in the cancer patient is widely appreciated. As early as 1932 Warren [202] recognized that cachexia may be the most common cause of death in patients with a variety of malignant tumors. Despite this early recognition and widespread investigation into the mechanism underlying cancer cachexia, no widely applicable explanation is available. As shown in chapter II, many functional, biochemical, and metabolical alterations have been identified in patients with malignant disease [44,186,206]. However, no single mechanism appears to underlie the wasting seen in the majority of cancer patients [44,186].

Adjuvant nutritional repletion by force-feeding (parenteral or enteral) can substantially improve the nutritional status of the host and may increase the tolerance of cancer patients to radiotherapy [194] and chemotherapy [40,105]. However, recent double-blind prospective studies have failed to demonstrate an increase in survival of cancer patients receiving TPN [110,153,166]. In fact, decreased survival was observed in patients with adenocarcinoma of the colon receiving TPN [141]. On the other hand, the pre-operative use of TPN in malnourished patients with gastro-intestinal cancer was associated with significant decrease in morbidity and mortality rates [140].

Concern has arisen that provision of large quantities of exogenous nutrient substrates may stimulate tumor growth. Several authors [33,129,148,183] have documented increased tumor growth in animals

receiving adequate exogenous nutrients compared to animals on restricted diets. Our study confirms these findings.
Generally, in animal models, the degree of improvement in host nutritional status has been believed to outweigh the increase in tumor growth associated with administration of carbohydrate-based TPN solutions [32,183].
Nevertheless, in most clinical circumstances, possible stimulation of tumor growth must be considered undesirable. The data from our study indicate that nutritional repletion of the host, equivalent to that achieved in chow-fed rats, may be obtained utilizing TPN with and without fat. However, in contrast to other studies [30,32,183], TPN with or without fat did not stimulate tumor growth.
Variations in the biochemical constitution of different tumor types may result in differences in its responses to nutritional manipulation. Hence caution is necessary in the interpretation of significant findings arising from a particular animal-tumor study.
The growth of a sizeble tumor imposes certain metabolic demands on the fuel and substrate supplies of the host. It has long been recognized that tumors consume large amounts of glucose in order to produce energy by glycolysis [201], the end product of which is lactate.
Consisting with this concept is the finding of hypoglycemia in fasting and semi-fasting rats. However, hypoglycemia was not observed in nutritionally adequate chow-fed rats or rats given TPN with or without fat. Conceivably, glucose homeostasis remains operative in the maintenance of normoglycemia in the well-nourished tumor-bearing rats [178], although others have observed a lower blood glucose level in sarcoma-bearing rats with tumor to body weight ratios between 0.31 and 0.50 [178].
The provision of a high concentration of glucose substrate to tumor resulted in accelerated glycolysis with increased lactate production [84,167].
Increased resting lactate concentration in cancer patients is unusual but has been described previously [102,207]. Goodgame et al.[77] have reported a case of severe lactic acidosis in a patient with extensive cancer associated with hypertonic dextrose infusion. Therefore, careful monitoring of the acid-base status of patients with a bulky tumor receiving TPN is mandatory. In our animal-tumor experiment, the reduction of glucose load by using fat as calorie source did not alleviate hyperlactemia. In the hepatoma 3924A, butyrate oxidation to CO2 and to acetoacetate is known to be low or negligible; hence, it is unlikely that oxidation of fatty acids derived from fat emulsion contribute to hyperlactemia [13].
In conclusion, the administration of TPN with and without fat maintains the nutritional status of ACI-N rats bearing hepatocarcinoma 3924A equally well without stimulation of tumor growth.

SUMMARY

In Chapter I, the motives and objectives fot this study are given.

Chapter II describes the basic facts concerning the experimental design. With the availability of total parenteral nutrition (TPN) man as well as animals can be nutritionally supported for long periods of time. Although TPN has become a common therapeutic tool that can be life saving, there are, however, many potential dangers in its usage. Apart from the commonly encountered complications as septicaemia, line complications and venous thrombosis, it may also cause dysfunction of the liver. It has been suggested that with parenteral feeding via the central venous route amino acid imbalance may occur due to prior uptake of certain amino acids by metabolically-active peripheral tissues. Up till now it was not clear, whether TPN infused via the portal venous system, resembling the postabsorptive state after oral feeding, will minimize hepatic dysfunction associated with central venous infusion of nutrient substrates.
It has been suggested that fatty infiltration of liver associated with glucose-based TPN can be reversed by using fat as part of the energy source. There are, however, reports of fat accumulation in liver and lung when fat is given as a major caloric source. We compared the efficacy of TPN with and without fat and studied their effect on liver function and structure in the rat.
When TPN is given as the sole nutrient support after intestinal resection, the adaptive response in the residual intestine is inhibited. Because lack of luminal nutrients passing through the gastro-intestinal tract seems to be a major factor for maximal adaptive response, we studied the effect of TPN on the development and adaptive hyperplasia of the mucosa after intestinal resection in the young growing rat.
TPN has been reported to avert cancer cachexia and improve tolerance for chemotherapy and radiation. However, conflicting reports are present as to whether carbohydrate-based TPN stimulates tumor growth as well. An experiment was carried out to determine whether carbohydrate-based TPN or fat-based TPN maintains host weight without stimulating tumor growth.

Chapter III, "Experimental design and methods", gives a general description about the experimental animals, the surgical operations, the histological measurements, the biochemical estimations and the statistical analysis used in the different experiments. The biochemical assays of RNA and DNA content are discussed.

Chaper IV describes the first experiment. The nutritional efficacy and hepatic changes in rats given a total parenteral nutrition solution consisting of 4.25 percent of amino acid and 25 percent of dextrose by intragastric, central-venous or prehepatic route were studied over a 4-day period. Rats fed chow or given intragastric TPN maintained body weight and showed no appreciable fatty change of the liver.
In contrast, weight loss, hepatomegaly, and a 37 percent increase in total liver lipid content were observed in rats given intravenous TPN and a 60 percent increase in those with prehepatic TPN. Half the rats given central venous or prehepatic TPN developed minimal to mild fatty change of the liver. Serum SGOT-levels were significantly elevated in rats given prehepatic TPN only. Prehepatic TPN may result in further hepatic injury and offers no apparent benefit over conventional central-venous TPN.

In chapter V the role of fat-based TPN in preventing or ameliorating hepatic dysfunction during TPN was investigated. Adult rats were given fat-free carbohydrate-based TPN or isocaloric, isonitrogenous fat-based TPN for 7 days. Chow-fed rats and fasting rats served as controls. Abnormal hepatic function tests and fatty infiltration of the liver were observed in rats given both types of TPN, but were more marked in rats given TPN with fat. Both the TPN regimens were equally effective in maintaining body weight, positive nitrogen balance, muscle and hepatic protein content. Hepatic dysfunction in rats during TPN was not prevented when a fat emulsion was used providing 50 percent of the caloric requirements.

In chapter VI the possibility, that inhibition of intestinal adaptation by TPN is reversible, was tested in young-growing rats. After a 70 percent mid-small bowel resection 7-week old rats were given a TPN-solution parenterally or orally for 10 days. The adaptive response of the residual intestine was inhibited after 10 days of TPN. When fed a chow-diet for a further 4 weeks, rats given TPN achieved similar body weight and 15 percent greater intestinal length compared with rats fed chow throughout. The jejunal villus-height, ileal crypt-depth, and RNA to DNA-ratio were less in comparison with chow-fed rats. Therefore, the inhibition of intestinal adaptation after intestinal resection in young-growing rats by TPN is largely reversible. Furthermore, oral feeding of a high calorie, high protein liquid diet before a normal regular diet appears to promote adaptation after intestinal resection in weanling rats.

Chapter VII describes the last experiment. Tumor growth and nutritional adequacy were evaluated in hepatoma-bearing rats given TPN with and without fat as compared with chow-fed, fasting, and semi-fasting (amino acid or carbohydrate alone) rats over a 4-day period. Tumor growth, measured by volume, weight, doubling time, protein content, and specific activity of DNA of the tumor was similar in rats given TPN with and without fat compared with chow-fed rats. The TPN regimens with and without fat were equally effective in maintaining body weight, positive nitrogen balance, serum albumin, and liver and muscle protein. However, hepatomegaly, increased hepatic nucleic acid content, and hepatic steatosis were observed after TPN with and without fat as compared with chow-fed rats. Fasting and semi-fasting rats had slower tumor growth with increased tumor necrosis, which occurred in parallel with marked weight loss and nutritional depletion of the host. In this animal tumor model, TPN with and without fat maintained host nutrition equally well without stimulating tumor growth. Hepatic steatosis limited the administration of TPN.

The last chapter describes in a general discussion the results of these four experiments in relation to relevant data from the literature.

SAMENVATTING

De motivatie en de doelstellingen die tot dit proefschrift leidden zijn in het eerste hoofdstuk beschreven.
Toediening van calorieën langs enterale weg verdient immer de voorkeur boven parenterale voeding vanwege de geringere kans op complicaties. Bovendien blijkt deze reeds lang toegepaste enterale methode superieur vanwege het feit dat ze meer fysiologisch is. In geval van functiestoornissen van de tractus digestivus kan men zijn toevlucht nemen tot totaal parenterale voeding.
Na een kort historisch overzicht worden in het tweede hoofdstuk de verschillende aspecten van parenterale voeding besproken. Eveneens wordt een overzicht gegeven van de verschillende in de literatuur vermelde studies naar het ontstaan van leverfunctie stoornissen tijdens parenterale voeding en de invloed van deze wijze van voeden op de structurele en functionele adaptatie van de dunne darm na resectie. Tevens wordt melding gemaakt van verschillende theorieen, die betrekking hebben op de relatie tussen tumorgroei en voeding.
Hoofdstuk III, getiteld "Proefopstelling en gebruikte technieken" houdt een algemene introductie in betreffende de dieren die gebruikt zijn in de verschillende eigen experimenten. De verschillende operatie-technieken, de histologische metingen en de gebruikte biochemische onderzoekmethoden, o.a. voor de bepaling van DNA en RNA worden beschreven.De gebruikte statistische methoden worden vermeld.
In hoofdstuk IV wordt aangetoond, dat leverfunctiestoornissen, ontstaan tijdens centraal veneuze toediening van parenterale voeding, niet worden voorkomen door toediening via het portale systeem.
Vettige degeneratie van de lever tijdens parenterale voeding wordt in belangrijke mate veroorzaakt door het hoge glucose aanbod aan de lever, indien koolhydraten als voornaamste energiebron dienen. In hoofdstuk V wordt parenterale voeding, samengesteld uit gelijke hoeveelheid calorieën van Intralipid ® en glucose, vergeleken met parenterale voeding zonder Intralipid ®. Hoewel beide voeding regimes even effectief bleken voor het in stand houden van het lichaamsgewicht, de stikstofbalans en het eiwitgehalte van de lever, blijken vettige degeneratie en stoornissen in de functie van de lever van de rat niet voorkomen te worden door de toevoeging van Intralipid ®.
In het derde experiment, beschreven in hoofdstuk VI, wordt de invloed van parenterale voeding en van peroraal gegeven parenterale voedingsvloeistof op de adaptatie van de dunne darm na resectie bestudeerd bij de jonge, opgroeiende rat. Dieren na identieke

darmresectie met een normaal rattevoer-dieet dienden als contrôle, terwijl ook dieren zonder operatie bestudeerd werden. Het bleek, dat intraveneus zowel als peroraal toegediende voedings-oplossing de adaptatie van het resterende jejunumum en ileum onderdrukte. Echter, nadat deze ratten vervolgens vier weken lang werden gevoed met normaal rattevoer, bleek volledige compensatie op te treden van de tevoren in de darm ontstane adaptatie achterstand.
In het laatste experiment, beschreven in hoofdstuk VII, werd de invloed van parenterale voeding met en zonder Intralipid ® op tumorgroei en cachexie bestudeerd bij ratten met een subcutaan groeiend hepatocarcinoma. De tumorgroei, gemeten aan het volume, het gewicht, de verdubbelingstijd, het eiwitgehalte en de inbouw van radioactief thymidine in het DNA van de tumor, bleek niet te worden beinvloed door het geven van parenterale voeding. Evenmin bleek de toevoeging van Intralipid ® hierop van invloed te zijn. Ten aanzien van het lichaamsgewicht, de stikstof balans, het serum albumine-gehalte en het eiwitgehalte van lever- en spier-weefsel bleken beide parenterale voeding regimes even effectief. Echter, in vergelijking met normaal gevoede dieren ontstonden tijdens parenterale voeding significante veranderingen in de lever (hepatomegalie, steatosis, DNA en RNA stijging).
In hoofdstuk VIII worden de resultaten van de vier beschreven experimenten getoetst aan de bestaande literatuur.

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