Aston University

Some pages of this thesis may have been removed for copyright restrictions.

If you have discovered material in Aston Research Explorer which is unlawful e.g. breaches copyright, (either yours or that of a third party) or any other law, including but not limited to those relating to patent, trademark, confidentiality, data protection, obscenity, defamation, libel, then please read our Takedown policy and contact the service immediately (openaccess@aston.ac.uk)

MECHANISMS OF TUMOUR-INDUCED CACHEXIA

HELEN DAWN MULLIGAN

Doctor of Philosophy

THE UNIVERSITY OF ASTON IN BIRMINGHAM

APRIL 1991

This copy of the thesis has been supplied on condition that anyone who consults it is understood to recognise that its copyright rests with its author and that no quotation from the thesis and no information derived from it may be published without the author's prior, written consent.

The University of Aston in Birmingham
Mechanisms of tumour-induced cachexia
Helen Dawn Mulligan

A thesis submitted for the degree of DOCTOR OF PHILOSOPHY - 1991

SUMMARY

The effect of cancer cachexia on host metabolism has been studied in mice transplanted with either the MAC16 adenocarcinoma which induces profound loss of host body weight and depletion of lipid stores or, the MAC13 adenocarcinoma which is of the same histological type, but which grows without an effect on host body weight.

Oxidation of D-[U-^{14}C]glucose was elevated in both tumour-bearing states irrespective of cachexia, when compared with non tumour-bearing controls. Both the MAC16 and MAC13 tumours *in vivo* utilised glucose at the expense of the brain, where its use was partially replaced by 3-hydroxybutyrate, a ketone body.

Oxidation of both [U-^{14}C]palmitic acid and [1-^{14}C]triolein was significantly increased in MAC16 tumour-bearing animals and decreased in MAC13 tumour-bearing animals when compared with non tumour-bearing controls, suggesting that in cachectic tumour-bearing animals, mobilisation of body lipids is accompanied by an increased utilisation by the host.

Weight loss in MAC16 tumour-bearing animals is associated with the production of a lipolytic factor. Injection of this partially purified lipolytic factor induced weight loss in recipient animals which could be maintained over time in tumour-bearing animals. This suggests that the tumour acts as a sink for the free fatty acids liberated as a result of the mobilisation of adipose stores.

Lipids are important as an energy source in cachectic animals because of their high calorific value and because glucose is being diverted away from host tissues to support tumour growth. Their importance is further demonstrated by the evidence of a MAC16 tumour-associated lipolytic factor. This lipolytic factor is the key to understanding the alterations in host metabolism that occur in tumour-induced cachexia, and may provide future alternatives for the reversal of cachexia and the treatment of cancer itself.

KEY WORDS: CANCER CACHEXIA, MAC16 ADENOCARCINOMA, WEIGHT LOSS, HOST METABOLISM, LIPOLYTIC FACTOR.

TO DAD

ACKNOWLEDGEMENTS

I gratefully acknowledge the help and support of my supervisor, Professor M. J. Tisdale. I wish to express my appreciation to Mr. M. Wynter for his expert technical assistance, and I would like to thank Dr. Sue Beck for her help and friendship throughout this project. I also gratefully acknowledge Dr. J. Griffith for the kind provision of facilities necessary to perform the NMR study. I wish to extend my thanks to all members of the Aston CRC group for their help throughout this research.

I acknowledge and thank the Royal Pharmaceutical Society of Great Britain for their financial support of this project.

Finally, I would like to thank my husband, William, for his encouragement and patience throughout these studies.

CONTENTS

PAGE

SUMMARY 2

DEDICATION 3

ACKNOWLEDGEMENTS 4

CONTENTS 5

LIST OF FIGURES 13

LIST OF TABLES 18

ABBREVIATIONS 21

CHAPTER 1: INTRODUCTION

1.0 Cancer and weight loss 24

1.1 Cachexia and anorexia 25

1.2 Anorexia 25

1.2.1 Metabolic effects of anorexia and starvation 29

1.3 Cachexia 33

1.4 Host and tumour metabolism in cancer cachexia 40

1.5 Tumour metabolism 40

1.5.1 Glucose utilisation by the tumour 41

1.5.2 Lactate production by tumour cells 41

1.5.3 Protein metabolism by tumour cells 42

1.5.4 Lipid metabolism in tumour cells 42

1.6 Host metabolism in cancer cachexia 44

1.6.1 Alterations in carbohydrate metabolism 44

1.6.2	Alterations in protein metabolism	47
1.6.3	Alterations in lipid metabolism	49
1.7	Catabolic factors implicated in the pathogenesis of cancer cachexia	52
1.7.1	Tumour necrosis factor	53
1.7.2	MAC16 tumour-associated lipid mobilising factor	56
1.8	Nutritional support in cancer patients	58
1.9	Aims of the investigation	61

CHAPTER 2: MATERIALS

2.1	Animals	64
2.2	Chemicals	64
2.3	Buffers	70

CHAPTER 3: METHODS

3.1	*In vivo* characterisation of MAC16 and MAC13 adenocarcinomas in mice	76
3.1.1	Transplantation of MAC16 and MAC13 solid tumours	76
3.1.2	Characterisation of MAC16 cell line *in vivo*	76
3.1.3	Characterisation of MAC13 adenocarcinoma *in vivo*	77
3.1.4	Body composition analysis	77
3.1.5	Determination of organ weights	78
3.1.6	Energy intake reduction and its effect on	78

	body weight, tumour growth and body composition in MAC13 tumour-bearing animals and non tumour-bearing controls	
3.1.7	Other experimental tumours studied	79
3.1.7.1	MAC16 cell line	79
3.1.7.2	MAC13 cell line	79
3.1.7.3	L1210 cell line	80
3.1.7.4	K562 cell line	80
3.2	Glucose utilisation by MAC16 and MAC13 cell lines _in vitro_	80
3.2.1	MAC16 and MAC13 cell lines	80
3.2.2	Lipogenesis _in vitro_	81
3.2.3	Respiration from glucose _in vitro_	81
3.2.4	Glucose utilisation and lactate production _in vitro_	82
3.2.4.1	Determination of lactate	83
3.2.4.2	Determination of glucose	83
3.3	Glucose utilisation by tumour and non tumour-bearing animals _in vivo_	84
3.3.1	Lipogenesis from glucose _in vivo_	84
3.3.1.1	Treatment of animals	84
3.3.1.2	Preparation of tissues	84
3.3.1.3	Extraction of lipids	85
3.3.2	Lipogenesis from water _in vivo_	85
3.3.3	$^{14}CO_2$ production from ^{14}C-labelled glucose	86
3.3.4	Glucose utilisation _in vivo_	86
3.3.4.1	Treatment of animals	87

3.3.4.2 Blood glucose concentration and the decay of radioactivity (Cp^*) in the blood 88
3.3.4.3 Accumulation of radioactive 2-deoxyglucose -6-phosphate by tissues (Cm^*) 89
3.3.5 Phosphofructokinase assay 91
3.3.5.1 Sample collection and preparation 91
3.3.5.2 Enzyme assay 91
3.3.6 Determination of hepatic glucose-6-phosphatase activity in tumour and non tumour-bearing animals 92
3.3.6.1 Preparation of tissues 92
3.3.6.2 Enzyme assay 92
3.3.6.3 Protein determination 94
3.4 Lipid utilisation by tumour and non tumour-bearing animals 94
3.4.1 Palmitic acid utilisation _in vivo_ 94
3.4.2 Lipid oxidation and accumulation 95
3.4.2.1 Lipid accumulationin liver and tumour of MAC16 tumour-bearing animals 96
3.4.3 Direct injection of lipid 96
3.4.3.1 Complex of lipid with albumin 96
3.4.3.1.1 Collection of serum samples 96
3.4.3.1.2 Formation of soap solution 97
3.4.3.2 Preparation of animals 97
3.4.3.3 Determination of lipid accumulation 98
3.4.4 Liver Acetyl CoA carboxylase activity 98

3.4.4.1 Preparation of crude extract 98
3.4.4.2 Gel filtration 99
3.4.4.3 Activation 99
3.4.4.4 Enzyme assay 99
3.4.5 Determination of liver citrate levels 100
3.4.5.1 Tissue preparation 101
3.4.5.2 Assay system 101
3.4.6 Administration of lipid lowering drugs 102
3.4.6.1 Administration of bezafibrate to MAC16 tumour-bearing animals 102
3.4.6.2 Administration of bezafibrate to MAC13 tumour-bearing animals 103
3.4.6.3 Effect of bezafibrate *in vitro* 103
3.4.6.4 Effect of bezafibrate on lipid accumulation in MAC16 tumour-bearing animals 104
3.5 Ketone body utilisation by tumour and non tumour-bearing animals *in vivo* 104
3.5.1 $^{14}CO_2$ production from ^{14}C-labelled 3-hydroxybutyrate 104
3.5.2 Determination of plasma 3-hydroxybutyrate and acetoacetate levels 105
3.5.2.1 Preparation of blood samples 105
3.5.2.2 Analysis of 3-hydroxybutyrate levels 105
3.5.2.3 Analysis of acetoacetate levels 106
3.5.3 3-Oxoacid CoA transferase activity 106
3.5.3.1 Preparation of tissues 106

3.5.3.2	Enzyme assay	107
3.5.4	Tissue slice metabolism	108
3.6	NMR methods	108
3.7	The effect of MAC16 tumour-associated lipolytic factor *in vivo*	109
3.7.1	Measurement of glycerol production	109
3.7.1.1	Preparation of isolated adipocytes	109
3.7.1.2	Lipolysis assay	110
3.7.1.3	Glycerol determination	111
3.7.2	Chromatographic techniques	112
3.7.2.1	Sephadex gel filtration exclusion chromatography	112
3.7.2.2	Biogel column	113
3.7.2.3	Sep-pack C18 column	114
3.7.3	*In vivo* administration of partially purified lipolytic factor	114
3.7.3.1	Subcutaneous administration	114
3.7.3.2	Intraperitoneal administration	115
3.7.3.3	Administration of lipolytic factor to non-cachectic MAC16 tumour-bearing animals	115
3.7.3.4	Oral administration of lipolytic factor	116
3.7.3.5	Administration of L1210 and K562 medium	116

CHAPTER 4: RESULTS AND DISCUSSION

4.1	*In vivo* characterisation of the MAC16 and MAC13 colon adenocarcinomas	118

4.1.1 Introduction 118

4.1.2 Results 119

4.1.3 Discussion 130

4.2 Glucose utilisation by the MAC16 and MAC13 cell lines *in vitro* 131

4.2.1 Introduction 131

4.2.2 Results 133

4.2.3 Discussion 137

4.3 Alterations in glucose metabolism *in vivo* 138

4.3.1 Introduction 138

4.3.2 Results 139

4.3.3 Discussion 154

4.4 Lipid utilisation by tumour and non tumour-bearing animals *in vivo* 156

4.4.1 Introduction 156

4.4.2 Results 157

4.4.2.1 Lipogenesis in tumour and host tissues in mice bearing colonic adenocarcinomas 157

4.4.2.2 Production of $^{14}CO_2$ from [U-^{14}C]palmitic acid 163

4.4.2.3 Lipid oxidation and tissue lipid accumulation 163

4.4.2.4 Lipid mobilisation from direct injection of tracer into epididymal fat pads 169

4.4.2.5 The effect of lipid lowering drugs on tumour growth and weight loss 172

4.4.3 Discussion 178

4.5 Ketone body utilisation by tumour and non tumour-bearing animals 183
4.5.1 Introduction 183
4.5.2 Results 185
4.5.3 Discussion 194
4.6 Monitoring lipid depletion using NMR spectroscopy 195
4.6.1 Introduction 195
4.6.2 Results 196
4.6.3 Discussion 203
4.7 The effect of a MAC16 tumour-derived factor *in vivo* 203
4.7.1 Introduction 203
4.7.2 Results 205
4.7.3 Discussion 221

SECTION 5: CONCLUSION 223

REFERENCES 227

APPENDIX

Publications 247

LIST OF FIGURES

FIG		PAGE
1.1	Factors influencing weight loss in cancer cachexia	26
1.2	Fuel sources in anorexia	30
1.3	Formation of ketone bodies from acetyl CoA	32
1.4	Role of ketone bodies in prolonged starvation	34
1.5	The Cori cycle	37
1.6	Fuel sources in cachectic patients	51
1.7	The role of lipoprotein lipase in lipid metabolism	54
1.8	Relationship between weight loss in male NMRI mice bearing the MAC16 tumour and tumour burden	57
4.1	The relationship between carcass weight loss and tumour weight in MAC16 tumour-bearing female NMRI mice	120
4.2	The effect of MAC16 and MAC13 tumours on host organ weights	123
4.3	The effect of MAC16 and MAC13 tumours on percentage organ weights	124
4.4	Glucose utilisation and lactate	134

	production by the MAC16 and MAC13 cell lines *in vitro*	
4.5	Oxidation of U-[^{14}C]glucose by the MAC16 and MAC13 cell lines *in vitro*	135
4.6	Lipogenesis from U-[^{14}C]glucose in the MAC16 and MAC13 cell lines *in vitro*	136
4.7	Blood glucose concentration in tumour-bearing animals and non tumour-bearing controls during the 60min time period of study and after an overnight fast	141
4.8	Measurement of the disappearance of [^{3}H]-2DG and [^{14}C]-2DG from the blood of tumour and non tumour-bearing animals	142
4.9	The effect of tumour burden on tissue glucose metabolic rate (Rg) in tumour and host organs of male NMRI mice	143
4.10	The effect of tumour burden on organ glucose utilisation rate in male NMRI mice	144
4.11	The effect of tumour burden on the ^{3}H/^{14}C ratio of 2-DGP in the tissues of male NMRI mice	147
4.12	The effect of weight loss on the activity of liver glucose-6-phosphatase	149
4.13	Oxidation of U-[^{14}C]glucose *in vivo* by MAC16 and MAC13 tumour-bearing animals and non tumour-bearing controls	153

4.14 Lipogenesis from U-[^{14}C]glucose in various organs in tumour and non tumour-bearing male NMRI mice 158

4.15 Lipogenesis from $^{3}H_2O$ in various organs in tumour and non tumour-bearing male NMRI mice 159

4.16 Oxidation of U-[^{14}C]palmitic acid in vivo by tumour and non tumour-bearing male NMRI mice 162

4.17 Absorption of an oral dose of [1-^{14}C]-triolein 164

4.18 Oxidation of [1-^{14}C]triolein in vivo by tumour and non tumour-bearing male NMRI mice 165

4.19 Tissue lipid accumulation in tumour and non tumour-bearing male NMRI mice 166

4.20 Accumulation of [1-^{14}C]triolein in liver and tumour over time in MAC16 tumour-bearing animals 168

4.21 The rate of loss of radioactivity from [U-^{14}C]palmitate labelled epididymal fat pads 170

4.22 The effect of bezafibrate on weight loss in MAC16 tumour-bearing animals 173

4.23 The effect of bezafibrate on MAC16 tumour growth in vivo 174

4.24	The effect of bezafibrate on MAC13 tumour growth *in vivo*	175
4.25	The effect of bezafibrate on MAC16 cell growth *in vitro*	176
4.26	*In vitro* utilisation of glucose, lactate and 3-hydroxybutyrate by liver, brain and tumour from MAC16 and MAC13 tumour-bearing animals and non tumour-bearing controls	186
4.27	Plasma acetoacetate and free fatty acids in MAC16 tumour-bearing animals and the relationship to weight loss	190
4.28	Plasma 3-hydroxybutyrate levels in MAC16 tumour-bearing animals and the relationship to weight loss	191
4.29	The activity of 3-oxoacid CoA transferase in various organs in MAC16 and MAC13 tumour-bearing animals and non tumour-bearing controls	192
4.30	Oxidation of [3-^{14}C]hydroxybutyrate in MAC16 and MAC13 tumour-bearing animals and non tumour-bearing controls	193
4.31	^{1}H-NMR spectroscopy traces of leg muscle of non tumour-bearing animals	198
4.32	^{1}H-NMR spectroscopy traces of leg muscle of MAC13 tumour-bearing animals	199

4.33	^{1}H-NMR spectroscopy traces of leg muscle of MAC16 tumour-bearing animals	200
4.34	The effect of weight loss on the water:fat ratio of the leg muscle of MAC16 tumour-bearing animals	201
4.35	The water:fat ratio of leg muscles in tumour and non tumour-bearing animals	202
4.36	Purification of the MAC16 tumour-associated lipolytic factor	208
4.37	The effect of intraperitoneal administration of lipolytic factor on weight loss in non tumour-bearing mice	210
4.38	The effect of intraperitoneal administration of lipolytic factor on weight loss in non cachectic MAC16 tumour-bearing animals	213
4.39	The effect of an oral dose of lipolytic factor on weight loss in MAC13 tumour-bearing animals	216
4.40	The effect of MAC16 tumour-associated lipolytic factor and purified K562 tissue culture medium on weight loss in MAC13 tumour-bearing animals	217
4.41	The effect of MAC16 tumour-associated lipolytic factor and purified L1210 tissue culture medium on weight loss in MAC13 tumour-bearing animals	218

LIST OF TABLES

TABLE		PAGE
4.1	Characterisation of the MAC16 cell line in female NMRI mice	121
4.1a	Doubling times of the MAC16 tumour	121
4.1b	Effect of MAC16 tumour on Food and water intakes in female NMRI mice	121
4.1c	Effect of MAC16 tumour on body compositions of female NMRI mice	122
4.2	Energy intake in tumour-bearing animals and non tumour-bearing controls	126
4.3	The effect of food reduction on tumour weight and host body weight in MAC13 tumour-bearing animals and non tumour-bearing controls	127
4.4	The effect of food reduction on food and water intakes in MAC13 tumour-bearing animals and non tumour-bearing controls	128
4.5	The effect of food reduction on host body composition in MAC13 tumour-bearing animals and non tumour-bearing controls	129
4.6	Hepatic glucose-6-phosphatase activity in MAC16 and MAC13 tumour-bearing animals and non tumour-bearing controls	150

4.7	Inhibition of phosphofructokinase activity by sera from MAC16 and MAC13 tumour bearing animals and non tumour-bearing controls	151
4.8	Hepatic citrate levels and the activity of acetyl CoA carboxylase in the livers of MAC16 and MAC13 tumour-bearing animals and non tumour-bearing controls	161
4.9	The distribution of [U-^{14}C]palmitate 60min after direct injection into the epididymal fat pads	171
4.10	The effect of bezafibrate on the accumulation of an oral dose of [1-^{14}C]-triolein in MAC16 tumour-bearing animals	177
4.11	Plasma ketone body levels in tumour and non tumour-bearing animals	189
4.12	The effect of subcutaneous administration of lipolytic factor on body weight in non tumour-bearing animals	209
4.13	The effect of intraperitoneal administration of lipolytic factor on food and water intakes in non tumour-bearing mice	211
4.14	The effect of intraperitoneal administration of lipolytic factor on levels of plasma metabolites in non tumour-bearing animals	212

4.15	The effect of intraperitoneal administration of lipolytic factor on food and water intakes in non-cachectic MAC16 tumour-bearing animals	214
4.16	The effect of intraperitoneal administration of lipolytic factor on body composition of non-cachectic MAC16 tumour-bearing animals	215
4.17	The effect of MAC16 tumour-associated lipolytic factor and purified L1210 medium on food and water intakes in MAC13 tumour-bearing animals	219
4.18	The effect of MAC16 tumour-associated lipolytic factor and purified L1210 medium on body composition of MAC13 tumour-bearing animals	220

Abbreviations

Abs	Absorbance
ACTH	Adrenocorticotropic hormone
ADP	Adenosine 5'-diphosphate
APP	Acute-phase proteins
ATP	Adenosine 5'-triphosphate
BSA	Bovine serum albumin
CL	Citrate lyase
Cm	Tissue glucose concentration
Cp	Blood glucose concentration
2DG	2-Deoxyglucose
2DGP	2-Deoxyglucose-6-phosphate
EDTA	Ethylenediaminetetraacetic acid
FFA	Free fatty acids
GK	Glycerokinase
GDH	Glycerolphosphatedehydrogenase
h	Hour
3-HB	3-Hydroxybutyrate
IL-6	Interleukin-6
i.m.	Intramuscular
i.p.	Intraperitoneal
i.v.	Intravenous
LC	Lumped constant
LDH	Lactic dehydrogenase
LMF	Lipid mobilising factor
LPL	Lipoprotein lipase

min	Minutes
mRNA	Messenger ribonucleic acid
NAD	Nicotinamide adenine dinucleotide
NADH	Nicotinamide adenine dinucleotide reduced form
OA	Oxaloacetate
p	Probability
PBS	Phosphate buffered saline
PTH	Parathyroid hormone
r	Correlation coefficient
Rg	Glucose metabolic rate
s.c.	Subcutaneous
S.E.M.	Standard error of the mean
sp. act.	Specific activity
TCA	Trichloroacetic acid
TG	Triglyceride
TNF	Tumour necrosis factor
UV	Ultraviolet
VLDL	Very low density lipoproteins

SECTION 1: INTRODUCTION

1.0 Cancer and weight loss

The majority of patients with progressive malignant disease lose weight (Nixon et al, 1980), and a proportion become emaciated to the extent that they appear to die of starvation (Warren 1932, Inagaki et al 1974). This syndrome is known as cancer cachexia and is a significant contributory factor to the severe morbidity and mortality in cancer patients (De Wys 1985). In patients with cancer, weight loss indicates a poor prognosis and a shorter survival time (De Wys 1985). The incidence of weight loss varies with tumour type and stage of disease. Pancreatic and gastric cancer have the highest frequency of weight loss (83 - 87%) due to their anatomical location, while only 31 - 40% of patients with leukaemias, breast cancer and sarcoma lose weight at some time during their illness. The precise mechanisms by which cachexia may cause death in some patients and contribute to it in others, is not completely understood. Numerous metabolic studies have indicated that weight loss associated with cancer cachexia is different from that in simple starvation (Brennan, 1977), and although anorexia may be apparent, this often develops once weight loss is established (Rich, 1987). Also, the degree of debility and malnutrition which frequently accompanies cancer cachexia, often seems to far exceed that which might be expected on the grounds of the extent of the neoplastic process (Tisdale, 1986).

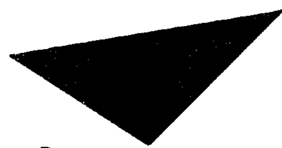
1.1 Cachexia and Anorexia

The relationship between cancer and weight loss is complex and by no means fully understood. Cancer cachexia has been recognised since 1932 (Warren, 1932) as a syndrome characterised by anorexia, weight loss, satiety, anaemia and asthenia (Lindsey 1986) (fig 1.1). Two thirds of cancer patients experience cachexia, and it is the major cause of death in many patients (Harnet, 1952). Although anorexia is a common feature in many cachectic patients, it is possible for cancer cachexia to occur when anorexia is not present (Brennan 1977 and Lawson, et al, 1982), and even with forced-feeding and hyperalimentation of cancer patients, the process is only temporarily reversed (Terepika and Waterhouse, 1956).

1.2 Anorexia

Loss of appetite leading to a decreased food intake is the syndrome known as anorexia. The regulation of food intake is under central nervous system control in the hypothalamic region of the brain, which either stimulates or suppresses the feeding response (Shamberger, 1984). Several receptor sources affect sensory input to the appetite control centre, including visceral receptors in the stomach and the intestine sensitive to osmotic, volumetric and chemical properties of ingested materials. Other mechanisms rely on

Figure 1.1



Aston University

Illustration has been removed for copyright restrictions

Factors influencing weight loss in cancer cachexia

(Lindsey, 1986)

monitoring nutrients in the blood giving rise to glucosensitive, liposensitive and amino acid-sensitive control mechanisms.

The pathogenesis of anorexia is complex and cannot be attributed to any single factor. It has been proposed that sustained stimulation of receptors in the gastrointestinal tract may occur in cancer patients. This would lead to prolonged satiety and decreased stimulation of appetite (Knox, 1983).

Other theories to explain anorexia propose that the tumour itself is the main cause of anorexia (Theologides, 1972). Theologides hypothesised that the tumour secretes peptides, oligonucleotides and other small metabolites that produce anorexia through a peripheral effect on neuroendocrine cells and neuroreceptors and through a direct effect on the hypothalamus. Hypotheses of alterations in feeding control at the hypothalamic level due to tumour metabolites have not however been substantiated in human studies.

Tumour necrosis factor (TNF), or cachectin, has been implicated in the pathogenesis of the anorexia/cachexia syndrome. TNF is produced by activated blood monocytes and tissue macrophages (Matthews and Watkins, 1978, Mannel et al, 1980, and Ruff and Gifford 1981). It was originally isolated as the mediator of wasting in chronic diseases

(Cerami et al, 1985), but is now thought to play a major role in the pathophysiology of anorexia rather than cachexia (Mahony and Tisdale, 1988, and Stovroff et al, 1988). In addition, no correlation between wasting and levels of TNF have been reported.

Numerous other factors have been suggested as contributing to the anorexia of cancer. These include changes in food perception and hence food aversion (Carson and Gormican, 1977, Gorshein, 1977, De Wys, 1978 and Williams and Cohen, 1978), and early satiety. Insulin response to glucose is both depressed and delayed in cancer patients (Kisner et al, 1978), and, since increased insulin stimulates appetite, this blunted insulin response in cancer patients may lead to a decreased appetite. Increased blood lactate levels (Baille et al, 1970, Weinhouse, 1972) and fatty acid levels (Waterhouse et al, 1964) in cancer patients may also lead to anorexia. The anorexia of cancer may be further complicated by the presence of a mechanical obstruction preventing the entry of food into the gastrointestinal tract. This often occurs in malignant diseases affecting the head and neck, oesophagus or upper gastrointestinal tract and by malabsorption, which appears to represent not only local but also distant systemic effects of some tumours (Blackwell, 1961, Schein et al, 1975, Kelley et al 1961). Finally, pain, fever, chemotherapy, radiotherapy and anxiety linked to a cancer

diagnosis, may all exacerbate decreased food intakes (Donaldson, 1977, Ohnuma and Holland, 1977, Donaldson and Lenon, 1979).

1.2.1 Metabolic effects of anorexia and starvation

Anorexia or decreased food intake leads to the condition of starvation or fasting. In this state, the length of survival depends not only on the amount and nature of the energy reserves in the body, but also on the metabolic adaptations that control the use of these reserves. Anorexia is a hypometabolic condition in which there is a decrease in the turnover of carbohydrate, lipid and protein stores. The fuel sources available during anorexia are summarised in fig. 1.2. During the acute stages of anorexia or starvation, blood glucose levels fall as a result of the decline in energy intake. Glucose levels are maintained by the mobilisation of amino acids (alanine) derived from muscle protein, gluconeogenesis initially occurring mainly in the liver, but progressing to involve the renal cortex as anorexia is prolonged. The first hormonal response to starvation is a fall in plasma insulin levels and a rise in plasma glucagon (White, et al, 1984), triggered by the falling blood glucose level. This leads to the rapid proteolysis characteristic of the initial acute phase of starvation, and helps maintain blood

Figure 1.2

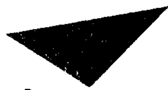
Fuel sources in Anorexia

The formation of ketone bodies in prolonged anorexia results in:

1. The sparing of glucose oxidation by the brain due to cerebral ketone body metabolism.

2. A decrease in the rate of FFA release from adipose tissue.

3. The inhibition of protein breakdown in muscle.



Aston University

Illustration has been removed for copyright restrictions

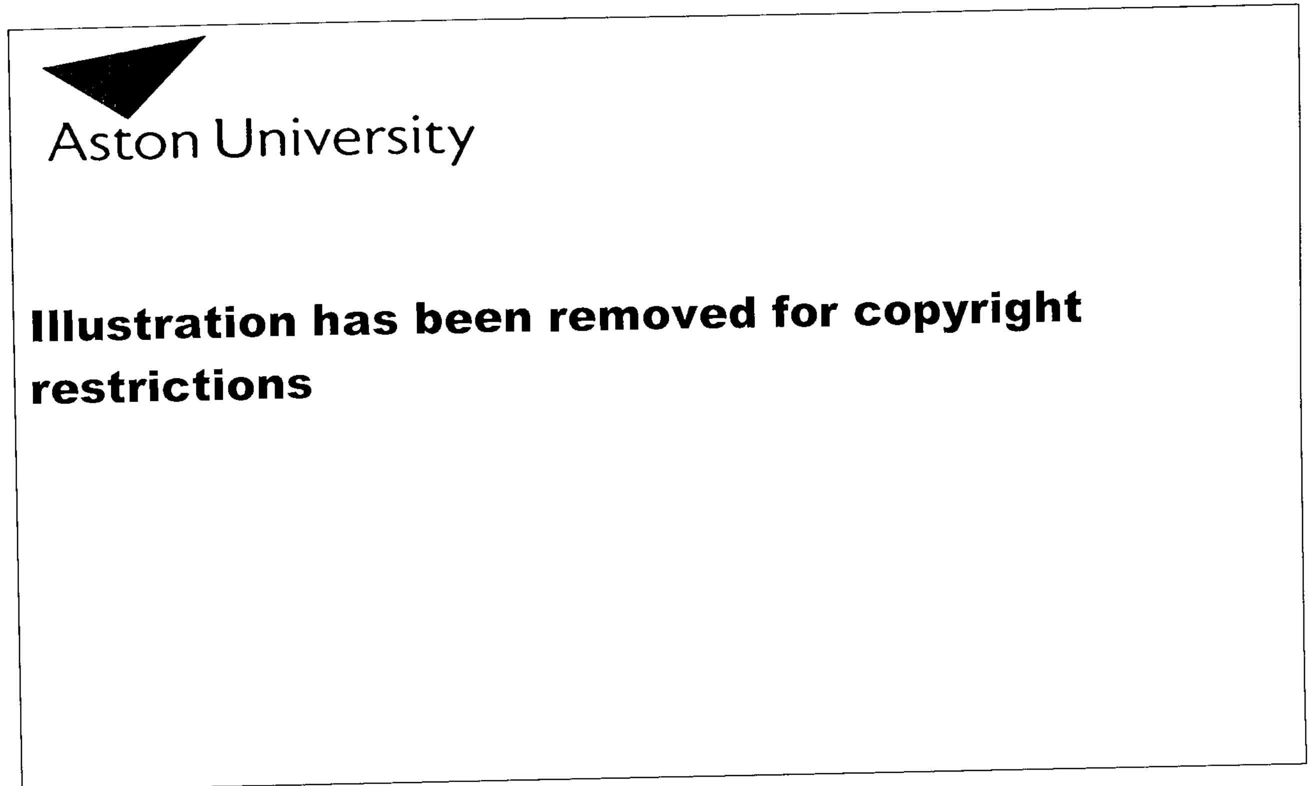
(Tisdale, 1982)

glucose levels within normal ranges. This maintenance of blood glucose levels is essential for normal brain metabolism to continue. The rise in intracellular protein

mobilisation causes an accompanying increase in the urinary losses of urea (Tisdale, 1982), calcium, magnesium and potassium. This also results in an increase in water loss from the body, which is mainly responsible for the initial rapid weight loss which occurs during the acute stage of starvation.

Prolonged protein breakdown would rapidly lead to the death of the starving patient. Body tissues must therefore adapt to utilise alternative energy sources. As a result of falling insulin levels, free fatty acids (FFA) are mobilised from adipose stores, and these can be utilised as a source of energy by peripheral tissues. Excess FFA are converted into ketone bodies (acetoacetate and 3-hydroxybutyrate), (fig 1.3) in the liver. Ketone bodies can be utilised by various tissues in the body, including the brain. During prolonged starvation, the brain is therefore able to adapt to utilise ketone bodies as an energy source, thus decreasing glucose demands and sparing the mobilisation of lean body tissues for energy (Owen, et al, 1967). Ketone bodies themselves are also able to inhibit protein breakdown in prolonged starvation, decreasing plasma levels of alanine and urinary nitrogen

Figure 1.3



Formation of ketone bodies from acetyl CoA

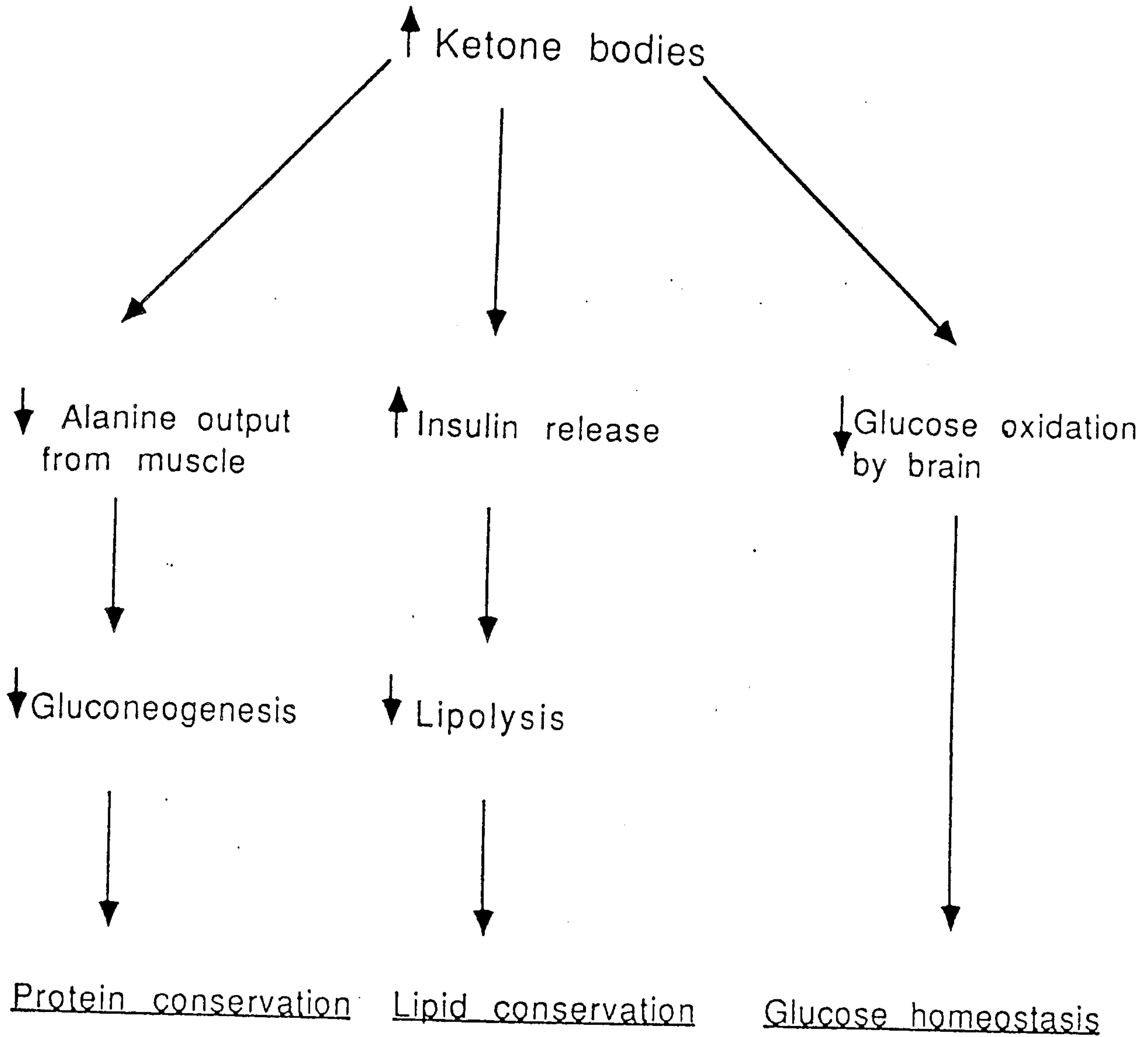
(Bowman and Rand, 1980)

excretion (Sherwin, et al, 1975). This process is thought to occur by inhibition of the oxidation of branched chain amino acids (Buse, et al, 1972). High ketone body levels also stimulate insulin secretion which decreases the breakdown of lipid stores. It has been suggested that ketone bodies can also directly reduce lipolysis in adipose tissue (Bjorntorp, 1966 and Hawkins, et al, 1971). Therefore, in chronic starvation, excess protein mobilisation is inhibited by an adaptation of tissues to utilise FFA and ketone bodies as an energy source. In turn, excess breakdown of lipid stores is inhibited by the production of ketone bodies, both by a production of insulin (an antilipolytic hormone) and by a direct inhibition of lipid mobilisation (fig 1.4). Once these metabolic adaptations have taken place, the body can survive for long periods of time in the fasting state (Brennan, 1977).

1.3 Cachexia

Progressive weight loss is a characteristic feature of malignant diseases and some studies suggest that nearly 90% of patients are affected (Laszlo 1986). Anorexia is frequently present in cancer patients, (Warnold et al 1978), but numerous studies have documented abnormalities of host metabolism that indicate that cancer cachexia is not the same as simple starvation (Bernstein, 1986). The

Figure 1.4



Role of ketone bodies in prolonged starvation

(adapted from White, Middleton and Baker, 1984)

degree of cachexia experienced by cancer patients does not correlate directly with food intake or with the size, type or site of the tumour. Cachexia can occur with small primary tumours and may actually be the first symptom that leads to a diagnosis of cancer. Cachexia is characterised by the following conditions;

1. anorexia and nausea
2. weight loss
3. anaemia
4. altered host metabolism
5. muscle weakness

Many theories have been suggested as to the causes of cancer cachexia and these can be divided into three groups;

1. Anorexia and defects in absorption
2. Increased energy expenditure and alterations in host metabolism
3. Circulating tumour by-products

Anorexia plays a part in the weight loss experienced by many cachectic cancer patients. In anorexia alone, animals and humans respond to the state of starvation by decreasing their metabolic rates and hence reducing their energy requirements. In cancer cachexia, this protective

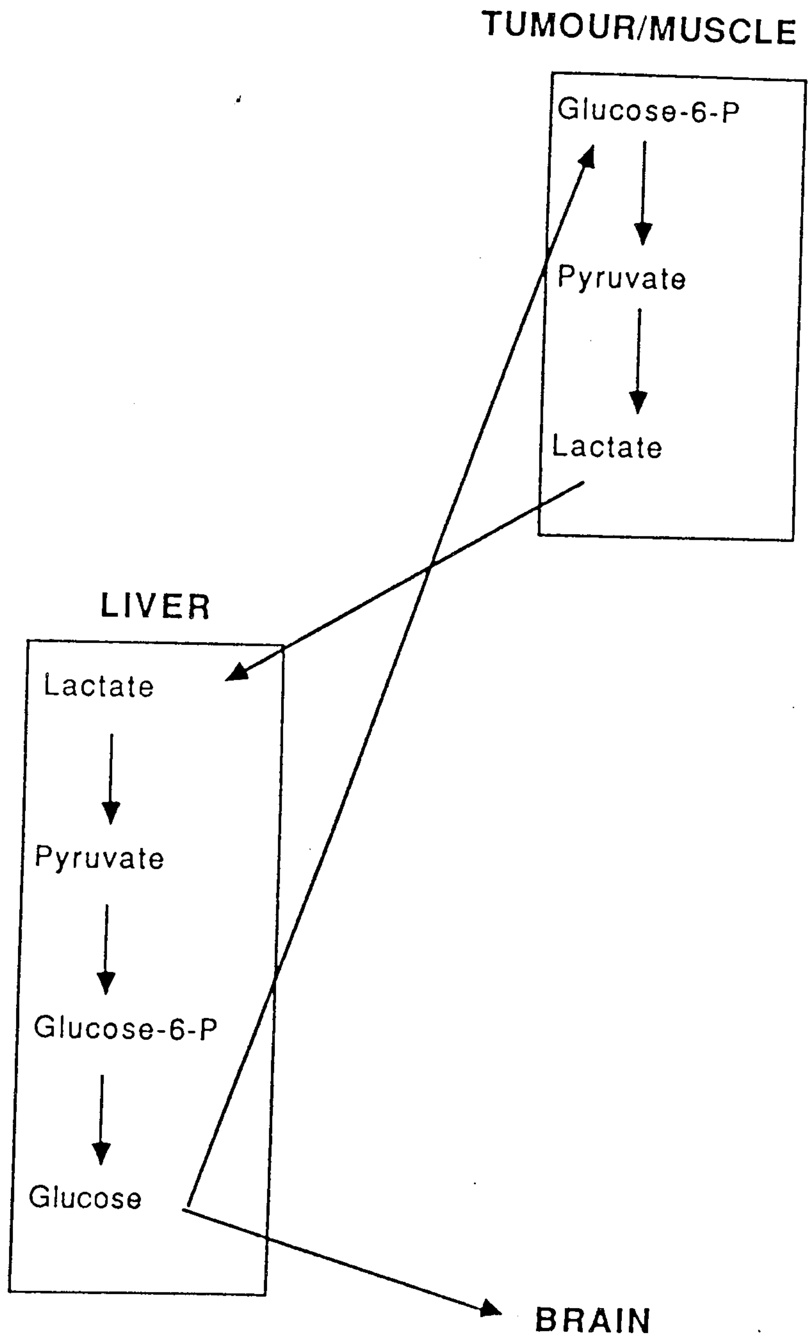
mechanism is not observed and many cachectic patients may actually have an inappropriately high energy expenditure despite a decrease in calorie intake (Young, 1977). That cachexia is unlikely to be the result of starvation alone is confirmed by the results of nutritional supplementation of cachectic patients (Hardy et al, 1986). When weight gain is achieved in this way, it is usually due to fat deposition rather than a repletion of vital lean body mass (Cohn et al, 1982). In addition, concern has been raised that hyperalimentation may accelerate tumour growth by selectively feeding the tumour (Balducci and Hardy, 1985).

Gold in 1968 suggested that increased energy expenditure in the cachectic host occurred as a result of excessive gluconeogenesis by the host in order to support the glucose requirements of the tumour. The Cori cycle (fig. 1.5) is a particularly inefficient process which consumes 6 ATP's per Cori lactate-glucose cycle. As the tumour grows, it consumes increasing amounts of glucose, and the energy resources of the host are depleted as it attempts to maintain normal blood glucose levels. This theory does not, however, explain how small tumours can produce cachexia. In addition, some tumours can produce cachexia in the host without an increased Cori cycle activity (Aisenberg, 1961).

Figure 1.5

The Cori cycle

Gluconeogenesis from lactate is a particularly inefficient process consuming 6 ATP's per futile Cori lactate-glucose cycle.



Other reports have explained cachexia as being caused by a competition for nutrients between the tumour and the host, with the tumour's needs being preferential to the hosts (Stein, 1978). This again cannot explain how cachexia occurs in patients with small tumour burdens, some less than 0.01% of the total body weight of the host (Lindsey, 1986).

Evidence that both anorexia and cachexia are the result of circulating tumour by-products can be shown by parabiotic experiments, in which it was demonstrated that cachexia can be conferred on to a non-cachectic animal from a cachectic one via the circulation (Norton et al, 1985). Other workers (Nakamura et al, 1986) have shown that sera from patients with advanced primary lung and gastric cancers can suppress the glucose consumption of cultured murine macrophages. The susceptible enzyme was found to be phosphofructokinase, one of the enzymes involved in glycolysis.

It has been hypothesised that the synthesis of peptides by the tumour results from impairment of repression of selected genes (Cox et al, 1978). This could explain why some patients do not experience cachexia. This theory has not yet been proven.

Attention has been focused on the role of tumour-necrosis factor (TNF) and other cytokines in cancer cachexia, although TNF has been shown in animal studies to be involved in the production of anorexia, rather than cachexia (Mahony and Tisdale, 1988) (section 1.7.1). Interleukin-6 (IL-6) is at present one of the cytokines receiving the most attention. As part of the inflammatory response to the presence of a tumour, various cytokines are thought to be produced by the host, resulting in the induction of hepatic synthesis of acute-phase proteins (APP). Patients with advanced cancer, and an on-going APP response, secondary to hepatic metastasis from colorectal cancer, have been shown to have elevated circulating IL-6 concentrations and accelerated whole-body protein kinetics (Fearon, et al, 1988c). It has been proposed that IL-6 may induce the APP response observed in cancer patients, although its effect on the distribution of hepatic protein synthesis is not known (Fearon, et al, 1990). Various cytokines, e.g. TNF, are capable of inducing IL-6 production in both tumour and non tumour-bearing animals, although the response is increased in the presence of a tumour (McIntosh, et al, 1989). The role of IL-6 in cancer cachexia does, however, remain unclear, since elevated levels of IL-6 have been found in tumour-bearing animals where weight loss did not accompany tumour growth (McIntosh, et al, 1989)

1.4 Host and Tumour metabolism in cancer cachexia

In order to understand the weight loss in cancer patients and to attempt to counteract it, it is necessary to understand both tumour metabolism and the changes in host metabolism elicited by the tumour. Metabolically, the tumour and host must be considered as two separate systems (Costa, 1963).

1.5 Tumour Metabolism

The tumour cell has a very distinctive metabolism and it acts as a metabolic trap for host nutrients. The tumour can in fact be considered as a parasite that feeds itself at the expense of the host. In the last few years, there has been considerable controversy concerning the preferred substrates required to maintain tumour growth and the control of their utilisation by cancer cells (Argiles and Azcon-Bieto, 1988). Tumour cells have been shown to be able to utilise a variety of substrates : glucose (Sauer and Dauchy, 1983, Lazo, 1981a and Lundholm et al, 1982), lipids (Thomson and Koons, 1981, Baker et al, 1977 and Ookhtens and Baker, 1979) and amino acids (Furst et al, 1981 and Carrascosa, et al,1984). The relative importance of each substrate varies according to the type of tumour and its state of development (Pederson, 1978).

1.5.1 Glucose utilisation by the tumour

Warburg (1930), showed that many tumours metabolise glucose via glycolysis at a high rate even in the presence of normal oxygen availability. In normal cells this pathway is usually reserved for anaerobic conditions. Recent evidence (Bhargava, 1977) suggests that altered glucose metabolism in the tumour cell is associated with altered membrane transport processes leading to enhanced glucose transport.

1.5.2 Lactate production by tumour cells

Large solid tumours often have a poor blood supply and consequently large areas of hypoxic cells which are only capable of utilising glucose via the Embden-Meyerhof pathway. Increased glycolytic activity results in a large production of lactic acid by the tumour. In the Walker 256 rat carcinoma, the pH decreases from 7.3 to 6.2 with increasing tumour mass (Jain et al, 1984). Ehrlich ascites cells show a high activity of the enzyme lactate dehydrogenase (Lazo and Sols, 1980). This may be the reason for their increased glycolytic activity. The physiological consequences of this altered metabolic activity are clear. The tumour generates a high degree of metabolic inefficiency in the host by enhancing energy wasting processes, such as the Cori cycle (fig 1.5) between

the tumour and the liver (Young, 1977).

1.5.3 Protein metabolism in tumour cells

Certain amino acids are essential for tumour growth in some cancer bearing states. Tumour cells have the ability to concentrate amino acids from the plasma pool in preference to normal cells. In this way, the tumour acts as a nitrogen trap competing with the host for nitrogen compounds (Mider, 1951). This would lead to a negative nitrogen balance which has been shown in a variety of animal and human tumours (Theologides, 1972).

1.5.4 Lipid metabolism in tumour cells

Fat depletion in cancer seems to be directly related to the increasing tumour mass, thus progressively contributing to the sustaining of both the normal cell types and the tumour. Fatty acids can reach the tumour associated in a complex with serum albumin, or in the form of lipoproteins. Levels of lipoprotein lipase, (the key enzyme for triglyceride rich lipoprotein metabolism) after starvation were elevated in mice bearing a non-metastatic tumour when compared to controls (Thomson and Koons, 1981). Enhanced lipoprotein lipase activity would account for the entry of fatty acids into the tumour cells. Recent studies using Ehrlich ascites tumour cells show that practically all the

esterified fatty acids in these cells are derived from *de novo* synthesis (Ookhtens et al, 1984). This implies that in some tumours, lipogenesis is an important pathway within the cancer cell. It has been reported that slowly growing hepatomas do not utilise glucose as an energy source, but rather rely upon fatty acids, as do normal hepatocytes, (Weinhouse et al, 1973), whereas rapidly growing tumours do not use fatty acids as a major energy source (Pederson, 1978). Fields et al (1981) found that hepatoma 7800 (a well differentiated tumour) was capable of fatty acid oxidation, whereas a more malignant cell type, hepatoma 7777, had low levels of fatty acyl CoA and carnitine palmitoyl transferase. Colon cancer cells have been reported to have very low amounts of coenzyme A which may explain their lowered ability to oxidise fatty acids (Willson et al, 1983).

Little information concerning the utilisation of ketone bodies by cancer cells is available, although ketonaemia has been reported by several workers, (Wagle et al, 1963, and Mueller et al, 1961). Fenslau et al (1975) reported that rapidly growing hepatomas can oxidise acetoacetate, since the mitochondria of these cells apparently contain acetoacetyl-CoA transferase. D-3-hydroxybutyrate dehydrogenase activity has been found in Morris hepatomas (Ohe, et al, 1967). The role of ketone bodies in tumour growth is not as yet very clear, but they may prove to be

important substrates for cell growth in some tumours.

1.6 Host metabolism in cancer cachexia

Although it is known that tumours can obtain substrates and energy necessary for their synthesis from the host at a significant energy cost, it is still not quite clear whether the resultant cachexia can be fully accounted for by inadequate energy intake and increased energy expenditure. The tumour can affect the metabolism of the host in two ways (Argiles and Azcon-Bieto, 1988). As a result of competition between the tumour and host tissues for important metabolites, the tumour produces changes in the metabolism and hormonal environment of the host. Secondly, the tumour can influence some host tissues by changing enzyme characteristics and sensitivity to hormones and by disturbing negative feedback systems. Although the tumour appears to place the host tissues in a state of accelerated starvation, the metabolic response which is generated is quite different from that which occurs in starvation.

1.6.1 Alterations in carbohydrate metabolism

Many tumours have a large requirement for glucose and this constant demand imposes a strain on the host's ability to maintain normal blood glucose levels. To counterbalance

this constant tendency towards hypoglycaemia in tumour-bearing animals, gluconeogenesis from non-carbohydrate precursors is enhanced (Shapot and Blinov, 1974). Despite this tendency towards hypoglycaemia, normal blood glucose levels have been observed in most tumour-bearing animals and patients (Tisdale, 1986). Gluconeogenesis from glycerol is increased in cancer patients, but this only contributes 3% of the glucose turnover rate in both cancer and control patients (Lundholm et al, 1982). This increased gluconeogenesis is not significant in terms of energy utilisation but the increased turnover of glucose may contribute to the high energy expenditure in cancer patients. Holroyde et al in 1984 observed elevated rates of glucose production and re-cycling in a group of weight losing patients with metastatic colorectal cancer. Eden et al (1984) also observed an elevated glucose flux in cancer patients.

Clofibrate is a lipid lowering drug, which lowers the blood triglyceride levels and may have the potential for making less glycerol available for conversion to glucose. This drug has been shown to reduce the growth of Walker 256 carcinosarcoma in rats (Gold, 1978)

The increased production of lactate by tumour cells as a result of increased glycolysis results in increased Cori cycle activity in the liver (fig 1.5). A study of Cori

cycle activity in patients with metastatic carcinoma has shown that patients without weight loss have normal glucose metabolism, but Cori cycle activity is increased in patients with progressive weight loss (Holroyde et al, 1975). This suggests that lactate production rates are increased in cachectic patients. Conversion of lactate to glucose is a particularly inefficient process and it utilises 6 ATP's per futile Cori lactate-glucose cycle. Waterhouse et al (1979) reported increased gluconeogenesis from alanine in cancer patients in the overnight fasting state when compared with a control group. This was not, however, regarded as significant in terms of energy expenditure in such patients.

Under some circumstances, the host may be unable to meet the glucose demands of the tumour. This was demonstrated in a study of rats bearing the MCA-induced sarcoma, in which serum glucose levels decreased with increasing tumour burden (Roh et al, 1984). Endogenous glucose production from both alanine and lactate was 27% higher in tumour-influenced hepatocytes.

Glucose intolerance in cancer cachexia may be due to abnormal insulin production and insulin resistance, without a reduction in insulin receptors in peripheral tissues (Kisner et al 1978). A high proportion of cancer patients display an abnormal or diabetic type of glucose tolerance

curve than normal controls (Glicksman and Rawson, 1956). Postabsorptive levels of insulin are decreased in the serum of rats bearing the Walker 256 carcinoma (Goodlad et al 1975) accompanied by lowered serum glucose levels and an elevation in serum unesterified fatty acids. The use of insulin in the nutritional management of cancer cachexia has been suggested (Schein et al, 1979) although resistance to exogenous and endogenous insulin and the problems associated with insulin treatment makes such therapy difficult to undertake. Recent animal studies (Beck and Tisdale, 1989a) have shown that insulin was effective in preventing weight loss in a mouse model of cachexia, but its administration was associated with an enhanced rate of tumour growth.

1.6.2 Alterations in Protein metabolism

Protein catabolism and a subsequent loss of skeletal muscle mass and negative nitrogen balance is a common feature in cancer cachexia (Clark and Goodlad, 1971 and Goodlad and Clark, 1972). Tumour cells can concentrate amino acids more efficiently than do normal cells (Wiseman and Ghadially, 1955 and Copeland et al, 1979) and this will inevitably lead to competition between host and tumour for nitrogen compounds. In tumour-bearing rats, skeletal muscle protein synthesis is reduced and protein synthesis is depressed by 70% in muscle and 40% in the liver of

cachectic tumour-bearing mice (Emery et al, 1984). The rates of whole body protein turnover were shown to be increased in children with newly diagnosed leukaemia or lymphoma (Kien and Camitta, 1983) and was significantly increased in non-cachectic lung cancer patients when compared with a control group (Heber et al 1982). In addition, cancer patients show abnormal plasma amino acid patterns (Feldman and Plonk, 1976), possibly arising from the tumour removing one or more of the amino acids from the amino acid pool of the host. Stein (1978) suggested that abnormal gluconeogenesis in cancer patients occurs as a result of the tumour removing only certain amino acids from the host's amino acid pool so leaving the host with the problem of disposing of the remainder of the amino acids. Amino acid imbalances in the host may lead to the depression in food intake observed in cachectic patients, since animal experiments have shown reduced intakes when feeding amino acid imbalanced diets (Anderson et al, 1968). There is an increase in the uptake of leucine by the tumour as tumour size increases, which significantly increases the host requirement for leucine (Lazo, 1981b). Branched chain amino acids, especially leucine are thought to play a unique physiological role, as a substrate and as a regulator of carbohydrate metabolism. Branched chain amino acids were found to be as least as effective as a complete amino acid mix in maintaining nitrogen balance in post-operative patients (Freund et al, 1979). Leucine

appears to have a role in maintaining homeostasis during starvation, and its removal by a tumour may give rise to some of the symptoms of cachexia.

1.6.3 Alterations in lipid metabolism

A progressive decrease in carcass lipids due to the mobilisation of free fatty acids from the host's adipose tissue is an important component of cancer cachexia. This depletion of adipose tissue can occur with small tumour burdens and is often associated with an increase of plasma lipids, probably as a result of decreased fractional clearance of triacylglycerols (Redgrave et al, 1984). Frederick and Begg (1956), demonstrated that tumour-associated weight loss occurred as a result of a decrease in body fat which was directly associated with an increase in plasma free fatty acids. Insulin resistance in adipose tissue may favour decreased lipid synthesis and increased lipolysis. Mobilisation of host free fatty acids may occur as the result of secretion of a lipolytic factor or factors by the tumour. These factors will be discussed in detail in section 1.7. Breakdown of adipose tissue may be related to an increase in basal metabolic energy expenditure (Waterhouse, 1981). Pyruvate dehydrogenase is the key enzyme for protecting lean body mass in hypocaloric states. During periods of starvation, fatty acid oxidation in host tissues leads to inactivation of pyruvate

dehydrogenase. This prevents loss of pyruvate precursors which would have to be replaced by protein breakdown. A tumour in which pyruvate dehydrogenase activity remains high in the fasting state would cause a loss of lean body mass in the host (Fields et al, 1982).

Ketosis is a common phenomenon in starvation and it causes a number of metabolic changes that result in the sparing of lean body tissues allowing the host to survive periods of chronic food deprivation (section 1.2.1). Although extensive weight loss and mobilisation of free fatty acids from adipose tissue occurs in cancer cachexia, ketosis is a very rare phenomenon in cancer patients. Ketonuria is uncommon in cancer patients (Conyers et al, 1979a) and in tumour-bearing rats (Mider, 1951). If cachectic patients are given an exogenous supply of fatty acids, ketonaemia is observed. This suggets that there is no impairment of the liver's ability to synthesise ketone bodies (Magee et al, 1979 and Conyers et al, 1979b). The absence of ketosis in cancer patients may explain some of the characteristics of cancer cachexia; loss of muscle protein and low protein synthesis, prolonged breakdown of adipose tissue and decreased insulin secretory capacity (fig 1.3). Many tumours have been shown to be deficient in enzymes required for the utilisation of ketone bodies as an energy source (Fields et al, 1981, and Tisdale and Brennan, 1983). The majority of tumours might be expected therefore to utilise

Figure 1.6

Fuel sources in cachectic patients

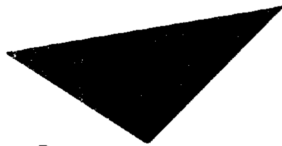


Illustration has been removed for copyright restrictions

(Tisdale, 1982)

ketone bodies at a much lower rate than host tissues. Studies have shown that diabetes mellitus decreases tumour incidence and size (Pavelic and Slijepcevic, 1978, and Demetrakopoulos and Brennan, 1982) and since diabetes is associated with ketosis, the ketone bodies may be responsible for the antitumour effects. It may therefore be possible to selectively starve the tumour and also prevent catabolism of lean body mass by using a ketogenic regimen.

1.7 Catabolic factors implicated in the pathogenesis of cancer cachexia

A variety of host-derived and tumour cell products have been identified in the serum of cancer patients and many of these have been suggested to be capable of producing the catabolic alterations observed in cancer cachexia. The products of catabolism of host tissues could be used in two ways. First, they could provide nutrients for the tumour, either directly or indirectly after suitable metabolic conversion in the liver. Secondly, they could be used by the host to create a new metabolic environment that favours the neoplastic state.

Peptide hormones, including ACTH, PTH, cortisol, glucagon and prostaglandins, (Ellison and Neville, 1973, Benson et al, 1974, Mallinson et al, 1974 and Odell and Wolfsen,

1980) are secreted by some tumour cells and can all induce physiological changes that lead to weight loss.

In 1980, Kitada et al identified a lipid mobilising factor (LMF) in the serum of AKR mice. LMF was produced by tumour cells and secreted into the serum where it mobilised fatty acids from adipose tissue triglycerides. LMF was found to be a small, heat stable protein, molecular weight about 5000, but it was never isolated, nor was its molecular structure elucidated. In 1981, Masuno et al isolated Toxohormone-L from tumour extracts and body fluids of patients and animals with tumours. Injection of this factor into the lateral ventrical of rats caused lipid depletion and weight loss by causing anorexia in recipient animals (Masuno, et al, 1984).

1.7.1 Tumour necrosis factor

The catabolic factor receiving the greatest interest at present is tumour necrosis factor (TNF) or cachectin. TNF was initially suggested to be a mediator of the cachexia associated with Trypanosoma infection (Rouzer and Cerami, 1980). TNF has since been implicated in the pathogenesis of cancer-associated cachexia and cachexia associated with other chronic illnesses (Beutler and Cerami, 1986). The first line of evidence leading to the classification of TNF as a cachectic agent was the observation that TNF is a

Figure 1.7

The role of lipoprotein lipase in lipid metabolism

Lipoprotein triacylglycerol (TG) in chylomicrons or in very low density lipoproteins (VLDL) cannot be taken up intact by tissues but must first undergo hydrolysis by lipoprotein lipase, an enzyme situated in the capillary endothelium of extrahepatic tissues, prior to uptake of the released free fatty acids (FFA).

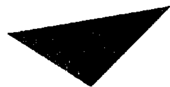


Illustration has been removed for copyright restrictions

(Harper et al, 1979)

potent inhibitor of lipoprotein lipase (LPL) activity both in cell culture and animals (Beutler et al, 1985, and Semb et al 1987). LPL activity has been found to be depressed in cancer patients (Vlassara, et al, 1986). LPL catalyses the hydrolysis of triglycerides in chylomicrons and very low density lipoproteins (VLDL) to form fatty acids and glycerol. The bulk of the released free fatty acids (FFA) are transported into the tissues and this process is essential for the normal utilisation of fat as an energy source (fig 1.7). Inhibition of LPL would lead to hypertriglyceridaemia similar to that observed by Rouzer and Cerami (1980) in Trypanosoma-infected rabbits. It was hypothesised that the TNF-induced inhibition of LPL was responsible for elevated serum triglyceride levels. This would decrease the amount of fat available for energy utilisation, leading to the loss of adipose stores and wasting seen in cachectic patients. Cerami et al in 1985 also showed that weight loss and anorexia could be demonstrated in animals receiving daily injections of dialysed conditioned medium from lipopolysaccharide-induced peritoneal macrophages. It was suggested that the macrophage secretory products contained impure TNF and that this was mainly responsible for the weight loss observed. More recently, Mahony and Tisdale (1988) have demonstrated a dose related weight loss in mice injected with human recombinant TNF. Weight loss was accompanied by, and directly proportional to, a decrease in both food and water

intake. The major weight loss was found to occur during the first 24 hours after injection and thereafter the weight of treated mice increased towards that of the controls.

Although TNF is capable of producing most of the changes associated with cachexia, such as weight loss, anorexia, muscle catabolism and hyperlipidaemia, both animal (Mahony et al 1988) and human (Socher et al, 1988) studies have failed to detect elevated levels of TNF in the cachectic state or elevated levels of TNF-mRNA in the tissues of cachectic animals (Lonnroth et al, 1990). Anti-TNF antibodies have been shown to delay, but not prevent, tumour-associated anorexia. (Sherry et al, 1989). These results suggest that if TNF is involved in cachexia, then other factors must also be present.

1.7.2 MAC16 tumour associated lipid mobilising factor

Attempts to characterise catabolic factors seem to be focused at present on macrophage-derived cytokines, but more recent studies have identified a low molecular weight peptide which is synthesised and released by tumours themselves (Tisdale, 1990). This tumour-derived factor is a lipid mobilising factor (LMF) produced by the experimental murine colon adenocarcinoma (MAC16). This is a useful model of cachexia, since it produces weight loss

Figure 1.8

Relationship between weight loss in male NMRI mice bearing the MAC16 tumour and tumour burden.

(Beck, 1989)

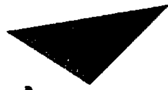


Illustration has been removed for copyright restrictions

with a small tumour burden and without any acompanying anorexia (Beck, 1989). The MAC16 tumour produces host body weight loss when the tumour burden exceeds 0.3% of the host body weight and the loss can reach 30 - 40% with a tumour burden of only 2.5% (Fig. 1.8, Tisdale and Beck, 1990). Tumour growth in animals bearing the MAC16 adenocarcinoma is associated with a decrease in both adipose and lean body mass. The MAC16 tumour-associated LMF resembles a factor found in the serum of cancer patients as regards charge and molecular weight (Beck, et al 1990). This factor may be related to the LMF described by Kitada et al in 1980 (section 1.7) although its stucture has not yet been determined.

1.8 Nutritional support in cancer patients

Clinically, it is very important to correct or preserve nutritional status in cancer patients (Terepika and Waterhouse, 1956) and this can only be achieved effectively by force supplementation of the patient's voluntary intake. It is difficult to increase voluntary intake in cachexia as anorexia is a common phenomenon in cancer patients (De Wys, 1979), and this may occur as a result of taste abnormalities (De Wys and Herbst, 1977 and Carson and Gormican, 1977). Nutritional support should theoretically prevent weight loss and in doing so, improve host tolerance of chemotherapy, radiotherapy and surgery. It appears,

however, that there are problems associated with nutritional support of cancer patients. Cachectic patients do not achieve the same response as malnourished patients when receiving nutritional supplementation (Nixon et al, 1981), suggesting again that cachexia is a complex problem which cannot be attributed to anorexia alone. When weight gain is achieved, it is usually due to fat deposition rather than lean body mass (Cohn et al 1982) or it occurs as a result of increases in extracellular fluids (Terepika and Waterhouse, 1956). The second major disadvantage of nutritional supplementation is that it may accelerate tumour growth. Buzby et al (1980) investigated the effects of various diets on rats bearing a transplantable mammary carcinoma. They found that amino acid supplementation improved host maintenance, but also stimulated tumour growth. Hyperalimentation has been shown to stimulate the growth of other experimental tumours (Balducci and Hardy, 1985 and Grubbs et al, 1979), and increased serum levels of lactic acid have been found by other workers during nutritional support (Holroyde and Reichard 1981) suggesting stimulation of tumour growth.

More recent work has concentrated on diets aimed at preferentially feeding the host whilst starving the tumour. Beck and Tisdale (1989a) showed that nitrogen excretion in cachectic mice could be modified by feeding a high fat diet and that ketone bodies could decrease weight loss and

reduce tumour volume in a mouse model of cachexia without any increase in calorie or protein intake (Beck and Tisdale, 1989b). The effectiveness of ketone bodies in reducing weight loss and tumour volume probably arises because most solid tumours are unable to utilise ketone bodies as an energy source (Tisdale and Brennan, 1983), and because 3-hydroxybutyrate may be acting as an inhibitor of a tumour-associated lipolytic factor (Beck and Tisdale, 1987). Other workers, (Fearon, and Carter 1988, and Fearon, et al, 1985) showed that dietary ketosis did not reduce tumour growth rate nor did it prevent weight loss in rats bearing the Walker 256 tumour. Dietary induced ketosis did not significantly alter nitrogen balance in cachectic patients (Fearon et al, 1988).

It appears that "aggressive" methods of hyperalimentation such as total parenteral nutrition (Dickerson, 1984) may not be as effective as expected in maintaining body weight in cancer patients and may actually be detrimental to the host in terms of growth of the tumour. Nutritional supplementation may have a role to play in cachexia, but careful selection of the nutrients to be administered is essential.

1.9 Aims of the Investigation

Cancer cachexia is a complex metabolic disorder which is as yet not fully understood. Most animal models of cancer cachexia utilise rapidly growing rodent tumours, which only produce weight loss when the tumour burden represents 30 - 40% of the total body weight (Strain et al, 1980). This is unlike the situation in humans in whom tumour growth is much slower and in which the tumour burden rarely exceeds 5% of total body weight (Costa, 1963). The MAC16 adenocarcinoma is a good animal model of cancer cachexia as it produces weight loss in recipient animals with small tumour burdens (section 1.7.2). The MAC16 tumour does not cause anorexia (Beck and Tisdale, 1987) and is therefore an ideal model for investigating the biochemical effects of cachexia without the complications of anorexia. This study aimed to investigate the effects of the MAC16 tumour on host metabolism and to compare its effects with those produced by the MAC13 tumour, which is of the same histological type as the MAC16 tumour, but which grows without inducing cachexia in the host. In order to determine any alterations in host and tumour metabolism which are involved in the production of cancer cachexia, glucose metabolism and lipogenesis were investigated in tumour and non-tumour bearing mice, as were alterations in lipid and ketone body metabolism. Substrate utilisation by the two tumours *in vivo* and by the corresponding cell lines

in vitro was investigated. The effect of lipid lowering drugs on tumour growth and weight loss was also examined.

A MAC16 tumour-derived lipolytic factor has recently been identified (Beck, et al, 1990). This lipid mobilising factor was partially purified from culture medium in which MAC16 cells had been growing by a series of exclusion chromatography methods. The effects of this partially purified factor were investigated *in vivo* in non-tumour bearing animals and in animals bearing the MAC16 and MAC13 tumours.

SECTION 2: MATERIALS

2.1 ANIMALS

Pure strain male/female NMRI mice were bred in a colony at Aston University. Rat and mouse breeding diet was purchased from Pilsburys Ltd, Birmingham, UK.

2.2 CHEMICALS

The following compounds were obtained from:

ALPHA LABORATORIES LTD, Hampshire, UK

Wako NEFA C kit

AMERSHAM INTERNATIONAL, Amersham, Bucks, UK

2-Deoxy-D-[2,6-^{3}H]-Glucose, specific activity 42Ci/mmol

2-Deoxy-D-[1-^{14}C]-Glucose, specific activity 56mCi/mmol

U-[^{14}C]-Glucose, specific activity 273mCi/mmol

$^{3}H_2O$, specific activity 5mCi/ml

L-[U-^{14}C]-Lactic acid, specific activity 169mCi/mmol

[^{14}C]-$NaHCO_4$, specific activity 56mCi/mmol

U-[^{14}C]-Palmitic acid, specific activity 828mCi/mmol

BDH LIMITED, Poole, England

Barium hydroxide

"Drierlite" anhydrous calcium sulphate

Ethanolamine (Scintran [R])

2-Ethoxyethanol (Scintran [R])

Ethylenediaminetetracetic acid,disodium salt

2-Mercaptoethanol

Potassium hydroxide

Sodium arsenite

Soda lime "Carbosorb" brand

Sodium hydrogen carbonate

Sodium hydroxide

Sucrose

Trichloroacetic acid

Tris

Zinc sulphate

BIO-RAD LABORATORIES, Richmond, CA

Biogel P_4

Bradford reagent

BOC LTD, London UK

Air : CO_2, 95% : 5%

Nitrogen

Nitrous oxygen

Oxygen

O_2 : CO_2, 95% : 5%

FSA LABORATORY SUPPLIES, Loughborough, UK

Acetic acid

Acetone

Ammonium molybdate

Ascorbic acid

Chloroform

Diethyl ether

Ethanol

Glucose

Hydrochloric acid

Magnesium sulphate

Methanol

Optiphase Hi-safe II

Optiphase Hi-safe 3

Perchloric acid

Petroleum ether 40-60^{0}C

Potassium dihydrogen phosphate

Sodium arsenite

Sodium citrate

Sodium chloride

Sulphuric acid

GIBCO LTD, Paisley, Scotland

Glutamine

RPMI 1640 medium (with 25mM HEPES and L-glutamine)

Trypsin

ICI CHEMICAL INDUSTRIES PLC, PHARMACEUTICAL DIV.,Macclesfield, Cheshire, UK

Halothane

IMPERIAL LABORATORIES LTD, Andover, Hants, UK

Foetal calf serum

JANSSEN PHARMACUETICAL LTD, Grove, Oxford, UK

Hypnorm (Fentanyl citrate/Fluanisone)

MACARTHY'S LTD, Romford Essex

Arachis oil

NEN

Carboxyl-[^{14}C]-Triolein, specific activity 114mCi/mmol

D-(-)-ß(^{14}C)-3-Hydroxybutyrate, specific activity 44.3mCi/mmol

OXOID LTD

Phosphate buffered saline tablets

PILSBURYS LTD, Birmingham, UK

Rat and Mouse Breeding diet

ROCHE PRODUCTS LTD, Welwyn Garden City, UK

Valium (Diazepam)

SIGMA CHEMICAL CO, Dorset, UK

Acetyl Coenzyme A, lithium salt

Acetoacetic acid, lithium salt

Adenosine-5-triphosphate, Na_3 salt

ASTON UNIVERSITY LIBRARY AND INFORMATION SERVICES

Bezafibrate

Bovine serum albumin

Bovine serum albumin, free fatty acid free

Cacodylic acid

Calcium carbonate

Calcium chloride

Citrate lyase

Clofibrate

Collagenase

Dithiothreitol

Evans blue

D-Fructose-6-phosphate

D-Fructose-1,6-diphosphate

Glucose-6-phosphate

Glucose-1,6-diphosphate

Glycerokinase

DL-ß-Hydroxybutyrate

3-Hydroxybutyrate dehydrogenase

Iodoacetamide

Lactate

Lactate dehydrogenase

Magnesium chloride

Maleate dehydrogenase

Nicotinamide adenine dinucleotide

Nicotinamide adenine dinucleotide reduced form

Ninhydrin reagent

O-toluidine kit

Palmitic acid sodium salt

Phenol red

Phosphoenol pyruvate

Phosphofructokinase

Potassium chloride

Potassium hydroxide

Pyruvate kinase

Sephadex G25

Sephadex G150

Sodium citrate

Succinyl Coenzyme A, sodium salt

Triethanolamine

Trifluoroacetic acid

Triglyceride kit

Zinc chloride

<u>WEDDEL PHARMACEUTICALS LTD, Wrexham, UK</u>

Multiparin (Heparin)

2.3 BUFFERS

2.3.1 Krebs-Ringer bicarbonate buffer (pH 7.6)

0.9% Sodium chloride	100ml
1.15% Potassium chloride	4.0ml
1.22% Calcium chloride	3.0ml
2.11% Potassium dihydrogen phosphate	1.0ml
3.82% Magnesium sulphate, hydrated	1.0ml
1.3% Sodium bicarbonate	21.0ml

On the day of the experiment, bovine serum albumin 30g/l and 0.55mM D-glucose were added to the above.

2.3.2 EDTA/Trypsin buffer (pH7.4)

Trypsin/EDTA*	10ml
Buffer**	10ml
Distilled water	up to 100ml

*Trypsin/EDTA contains:	Trypsin	5.0g/L
	EDTA	2.0g/L
	NaCl	8.5g/L

**Buffer contains:

Phosphate buffered saline tablets	20
EDTA	742mg
Phenol red	100mg
Distilled water	150ml

The buffer was adjusted to pH 7.4 with 1M KOH and made up to 200ml with distilled water. The buffer was autoclaved to produce a sterile solution.

2.3.3 0.4M Hydrazine - 0.5M glycine buffer (pH 9.0)

Hydrazine hydrate	25ml
Glycine	11.4g
Distilled water	200ml

The pH was adjusted to pH 9.0 with concentrated hydrochloric acid and the final volume was made up to 300ml with distilled water.

2.3.4 Tissue culture growth medium

RPMI 1640 tissue culture medium	500ml
Foetal calf serum	50ml
Glutamine(200mM)	5ml

2.3.5 Buffers for Acetyl CoA carboxylase determination

2.3.5.1 Buffer A

0.1M Tris - HCL (pH7.5)	50ml
Sodium citrate	0.588g
EDTA	0.0186g
2-Mercaptoethanol	0.035ml
Distilled water	up to 100ml

2.3.5.2 Column buffer

20mM Tris - HCL (pH 7.5)	2.424g
1mM Dithiothreitol	0.1542g
Distilled water	up to 1000ml

2.3.5.3 Activation buffer

0.1M Tris - HCl (pH 7.5)	50ml
Sodium citrate	0.588g
4.9M Magnesium chloride	0.408ml
Bovine serum albumin	50mg
Distilled water	up to 100ml

2.3.5.4 Assay buffer

0.4M Tris - HCl (pH7.5)	12.5ml

Dithiothreitol	0.008g
Acetyl CoA	0.008g
Citrate	0.294g
4.9M Magnesium chloride	0.204ml
Bovine serum albumin	25mg
ATP solution	5ml
Distilled water	up to 25ml

ATP solution:

ATP	0.5072g
Distilled water	10ml

The pH of the ATP solution was adjusted to pH 7.5 with 1M sodium hydroxide and the final volume was made up to 20ml with distilled water.

2.3.5.5 Homogenisation buffer

Tris 0.606g	
Mercaptoethanol	0.035ml
Distilled water	up to 500ml

The pH of the buffer was adjusted to pH7.1 with 1M sodium hydroxide.

Sucrose	42.79g
Tris - mercaptoethanol buffer	up to 500ml

2.3.6 0.01M Phosphate buffer (pH 8.0)

Solution A: Potassium dihydrogen orthophosphate (1.36g) was added to 1000ml of distilled water

Solution B: Dipotassium hydrogen orthophosphate, trihydrate (2.28g) was added to 1000ml of distilled water.

Solution B was added to 200ml of solution A until a pH of 8.0 was achieved.

SECTION 3: METHODS

3.1 In vivo characterisation of MAC16 and MAC13 colon adenocarcinomas in NMRI mice

3.1.1 Transplantation of MAC16 and MAC13 solid tumours

The MAC13 and MAC16 colon adenocarcinomas were originally induced with 1,2-dimethylhydrazine by Dr J. Double, Bradford University (Double, et al, 1975). Of these, only the MAC16 colon adenocarcinoma produces symptoms of cachexia in the host. The MAC16 adenocarcinoma was excised from donor animals, placed in sterile 0.9% saline and cut into small fragments, 1 - 2mm in size. Using a trocar, fragments were implanted subcutaneously into the flank of the right hind limb of male NMRI mice (24 - 26g), or female mice (20 - 22g). The doubling time of this tumour was 3 - 4 days (determined by Mr Mike Wynter, Aston University). Mice were weighed and food and water consumption were measured daily. Weight loss did not exceed 30% of total body weight at any time. Cachectic MAC16 tumour-bearing mice that had lost 2 - 4g in weight were used in the metabolic studies described in the rest of this section.

3.1.2 Characterisation of the MAC16 cell line in vivo

The MAC16 cell line is capable of producing cachexia when injected into NMRI mice. The doubling time of this tissue culture cell line derived tumour was determined after implantation of 2 - 3 x 10^6 MAC16 cells in PBS into the left hind leg muscle of female NMRI mice (20 - 22g). After

implantation, mice were weighed and food and water consumption was determined daily. When weight loss reached 30% of original body weight, the animal was sacrificed and body composition analysis was performed on the carcass.

3.1.3 Characterisation of the MAC13 colon adenocarcinoma in vivo

Fragments of the MAC13 adenocarcinoma (supplied by Dr J Double ,Bradford University) were implanted into the flank of male NMRI mice (24-26g). This tumour was found to have a doubling time of 2 - 3 days as determined by Mr Mike Wynter, Aston University. The MAC13 tumour grows without inducing cachexia in recipient animals. Food intake in MAC13 tumour-bearing animals was determined by housing mice in individual cages and monitoring energy intake daily for 7 days.

3.1.4 Body composition analysis

The gastrocnemius and thigh muscles were carefully dissected out from the left leg of the carcass and weighed. Carcass and muscles were placed in an oven at 80°C until a constant weight was reached. Dry weights of the carcass and muscles were recorded separately. The water content of the muscles and total carcass was then calculated from the wet and dry weights. Total fat content of the carcass was

determined using the method of Lundholm et al (1980). Each carcass was broken into small pieces and then extracted in turn with 25ml of acetone:ethanol (1:1 v/v), chloroform:methanol (1:1 v/v) and diethylether. The extracts were combined in a preweighed round-bottomed flask. The solvents were then removed using a Buchi rotary evaporator to leave a fatty residue. The flask was reweighed and the total fat content per carcass calculated.

3.1.5 Determination of organ weights

Various organs (brain, lungs, liver, kidneys, spleen, colon, fat and testes) were removed from male NMRI non-tumour bearing mice and from mice bearing the MAC16 and MAC13 tumours. Each organ was carefully rinsed in 0.9%w/v NaCl and gently blotted before weighing.

3.1.6 Energy intake reduction and its effect on body weight, tumour growth and body composition in MAC13 tumour-bearing animals and non tumour-bearing controls

MAC13 tumour-bearing male NMRI mice and non tumour-bearing controls (starting weight 24 - 26g) were housed individually and food and water intakes were monitored daily for 3 days. The average food intake per day was then calculated using these results. On day 4 of the experiment, energy intake was reduced by 10% in half of the

mice in each group, whilst the remainder continued to receive food as normal. Water intake was not restricted. On day 10 of the experiment, mice were sacrificed and body composition analysis was performed on the carcasses as described in section 3.1.4.

3.1.7 Other experimental tumour lines studied

3.1.7.1 MAC16 cell line

The MAC16 tissue culture line was derived by Drs J.A. Double and M. Bibby at the University of Bradford from the *in vivo* MAC16 tumour. It was capable of producing cachexia when re-injected into NMRI mice. Cells were grown by Prof. M. J. Tisdale, University of Aston, in suspension in RPMI 1640 culture medium with an atmosphere of 5% CO_2:95% air. Doubling time of the cells was approximately 38h.

3.1.7.2 MAC13 cell line

The MAC13 cell line was derived by Drs J. A. Double and M. Bibby at the University of Bradford from the *in vivo* MAC13 tumour. Cells were grown by Prof. M. J. Tisdale, Aston University using the conditions described in section 3.1.7.1. The cells grew as a monolayer attached to the plastic tissue culture flasks. Doubling time of the cells was 24h.

3.1.7.3 L1210 cell line

The L1210 cell line is a leukaemic cell line obtained from Flow laboratories (Glasgow). The cells were grown in RPMI 1640 medium (with 25mM HEPES) and gassed with 5% CO_2 in air. The cultures were initiated at a density of 0.3 - 4 x 10^5 cells/ml. Doubling time of the cells was 12 - 14 h.

3.1.7.4 K562 cell line

The K562 cell line is a human chronic myelogenous leukaemia cell line obtained from Dr. R. Hoffman, Charing Cross Hospital, London. The cell line was grown by serial subculture every 2 or 3 days in RPMI 1640 medium with 2mM L-glutamine, 25mM HEPES and 10% foetal calf serum in 10% CO_2 in air. The cells grew in suspension and had a doubling time of 24h (Watson, 1990).

3.2 Glucose utilisation by the MAC16 and MAC13 cell lines in vitro

3.2.1 MAC16 and MAC13 cell lines

MAC16 and MAC13 cell lines were derived by Drs. Double and Bibby at the University of Bradford from the corresponding in vivo tumours. Cells were grown in RPMI 1640 tissue culture medium +10% foetal calf serum with an atmosphere of

5% CO_2 : 95% air.

Since MAC13 cells grow as a monolayer attached to the tissue culture flask, cells were allowed to incubate overnight before the start of any experiment. MAC13 cells were removed from the flask by trypsinisation. 5ml of EDTA/trypsin buffer was added to each flask for 5min followed by 15ml RPMI 1640 medium to neutralise the buffer. MAC16 cells grow in suspension, so trypsinisation was not necessary.

3.2.2 Lipogenesis in vitro

MAC16 cells (1.5×10^6 cells/flask) and MAC13 cells (0.75×10^6 cells/flask) were grown in the above tissue culture medium (section 3.2.1), which contained 0.2µCi/ml of U-^{14}C-glucose. At specified time intervals, (3, 6, 24 and 48 h), cells from each cell line were counted using a Coulter electronic particle counter and the lipids extracted as described in section 3.3.1.3.

3.2.3 Respiration from glucose in vitro

MAC16 cells and MAC13 cells (4×10^4 cells/ml) were grown in the above medium (section 3.2.1) which contained 0.2µCi/ml of U-^{14}C-glucose. Each flask was fitted with a 1ml centre well and sealed with a rubber seal. At

specified time points (6, 24 and 48h), 300µl of 0.3M NaOH was injected into the centre well and 300µl of 5M perchloric acid was added to the medium to liberate the CO_2. The flasks were allowed to stand for 4h in order to collect the liberated CO_2. The radioactivity in the centre well was determined as follows; 50µl of 2M hydrochloric acid was added to a scintillation vial followed by 9ml of Optiphase MP scintillation fluid. The vial was shaken and the contents of the centre well were added plus a further 9ml of Optiphase MP scintillation fluid. The vial was shaken and the radioactivity was measured using a Packard TRI-CARB 2000CA liquid scintillation analyser. Duplicate flasks were set up to determine the cell number at each time point.

3.2.4 Glucose utilisation and Lactate production in vitro

MAC16 and MAC13 cells (3 - 4 x 10^4 cells/ml) were grown in RPMI 1640 medium as described previously (section 3.2.1). At specified time intervals, (6, 24 and 48h), cells were removed and counted and the mediums were tested for glucose and lactate content as described in sections 3.2.4.1 and 3.2.4.2. Tissue culture medium in which no cells had been growing was also tested in order to determine glucose utilisation and lactate production.

3.2.4.1 Determination of lactate

Lactate was measured using the method of Gutmann and Wahlefield (1974). This method depends on the oxidation of lactate to pyruvate. The increase in absorbance due to the formation of NADH was measured at 340nm.

$$\text{L-(+)-Lactate} + \text{NAD}^+ \text{-----------}> \text{Pyruvate} + \text{NADH} + \text{H}^+$$

Each cuvette contained 0.43M glycine, 0.34M hydrazine hydrate buffer pH9.0, 2.75mM NAD and 50µl of sample in a total volume of 3.0ml. A blank was set up containing 1N perchloric acid in place of sample. All readings were calibrated against distilled water. The reaction was initiated by the addition of 20µl of lactate dehydrogenase (19units/ml) and the increase in absorbance due to the formation of NADH was measured at 340nm for 30min at 37°C in a Beckman DU7 spectrophotometer.

3.2.4.2 Determination of glucose

Glucose levels were determined on de-proteinised whole blood or tissue culture medium using a Sigma O-toluidine kit (no. 635). This diagnostic kit uses a method based on the work of Hyvarinen and Nikkila (1962). In the presence of heat and acid, o-toluidine reacts readily with glucose to form a coloured (blue-green) complex. The intensity of

the colour produced was measured using a Beckman DU70 spectrophotometer at 635nm.

3.3 Glucose utilisation by tumour and non tumour-bearing animals in vivo

3.3.1 Lipogenesis from glucose in vivo

3.3.1.1 Treatment of animals

D-[U-^{14}C]Glucose in 200µl of 0.9% NaCl was administered by intraperitoneal injection (250µCi/Kg) to male NMRI mice. Cachectic MAC16 tumour bearing mice (22-23g) and MAC13 tumour bearing mice (26-27g) were compared with non-tumour bearing controls (26-27g).

3.3.1.2 Preparation of tissues

Blood was removed by cardiac puncture from animals under anaesthesia, 2 hours after injection of the D-[U-^{14}C]-glucose. It was transferred to microfuge tubes on ice and the volume noted. The livers, spleens, epididymal fat pads, colons, brains and tumours were dissected out and weighed.

3.3.1.3 Extraction of lipids

Lipids were extracted from the organs and the blood by the method of Stansbie et al (1976). Briefly, the organs and blood samples were transferred to glass scintillation vials containing 3ml 30%w/v potassium hydroxide and heated to 70^{0}C for 15 min. 3ml of 95%w/v ethanol was then added to each vial and heating at 70°C was continued for a further 2h. Vials were then cooled and the saponified material was acidified with 3ml of 9M sulphuric acid. Acid soluble lipids were extracted three times by shaking with light petroleum ether (b.p. 40-60^{0}C), centrifuging at 3000rpm in a Beckman bench centrifuge for 15 min and removing the supernatants. The three petroleum fractions were combined and evaporated to dryness under a stream of nitrogen. The lipid extracts were then redissolved in 10ml of Optiphase Hi-safe II scintillation fluid and the radioactivity was measured using a Packard TRI-CARB 2000CA liquid scintillation analyser.

3.3.2 Lipogenesis from water in vivo

Lipogenesis from water was determined using the method of Evans and Williamson, (1988). Mice were injected i.p. with 10mCi/Kg of ^3H_2O and lipids were extracted from various organs as described in section 3.3.1.3.

3.3.3 $^{14}CO_2$ Production from ^{14}C-labelled glucose

Mice were injected i.p. with 50μCi/Kg of D-[U-^{14}C]-glucose in 200μl of 0.9% NaCl. Animals were then placed in airtight metabolic cages into which air was pumped through solid calcium carbonate to absorb any CO_2. Metabolically produced CO_2 was trapped in glass test-tubes containing 20ml of a mixture of ethanolamine : ethoxyethanol (1 : 4). At specified time intervals (0.5, 1, 2, 4 and 8h), 500μl aliquots were taken and transferred to scintillation vials containing 10ml Optiphase Hi-safe II. The vials were shaken and the radioactivity was measured in a Packard TRI-CARB 2000CA scintillation analyser. Cachectic MAC16 tumour bearing male NMRI mice (22-23g) and mice bearing the MAC13 tumour (25-26g) were compared with non-tumour bearing controls (25-26g).

3.3.4 Glucose Utilisation in vivo

The extent of glucose utilisation by different tissues was investigated using the method of Sokoloff et al (1977), which involves the in vivo administration of radioactive 2-deoxy-D-glucose (2DG). The transport, cellular uptake and phosphorylation by hexokinase of this analogue correlate with those of glucose, but, because 2-deoxyglucose-6-phosphate (2DGP) cannot readily be metabolised further, it can be detected in tissues

containing little or no glucose-6-phosphatase activity, such as brain and muscle (Lackner et al, 1984 and Jenkins et al, 1986). Glucose utilisation was calculated according to the following equation:

$$Rg = \frac{Cm^*\ (T)}{LC \int_0^T \frac{Cp^*\ dt}{Cp}}$$

where Rg is the tissue glucose metabolic rate (nmol/g/min); Cm*(T) is the concentration of phosphorylated metabolites of 2-deoxyglucose in the tissue (dpm/g) at t=60min; Cp is the blood glucose concentration (nmol/ml); Cp* is the concentration of radioactive 2-deoxyglucose in the blood (dpm/ml) and LC is the lumped constant which was determined using the method of Ferre et al (1985). Previous workers (Mahony, and Tisdale 1988) have reported an LC value of 0.46 for NMRI mice. This figure was used in the following determinations of rates of glucose utilisation.

3.3.4.1 Treatment of animals

Non-tumour bearing, male NMRI mice (26-27g) and MAC13 tumour bearing NMRI mice (26-27g) were compared with

cachectic (22-23g) and non-cachectic (26-27g) MAC16 tumour bearing mice. Mice were starved overnight and throughout the experiment, but given water ad libitum. The following morning the mice were injected i.v. with 50µCi/Kg 2-deoxy-D-[2,6-^{3}H]-glucose in 200µl of 0.9% NaCl. In order to determine the retention of 2-deoxyglucose-6-phosphate by the different tissues, a second i.v. injection of 5µCi/Kg 2-[1-^{14}C-]deoxy-D-glucose in 200µl of 0.9% NaCl was administered 35min after the injection of the tritiated deoxyglucose.

3.3.4.2 Blood glucose concentration and the decay of radioactivity (Cp*) in the blood

Blood was removed by cardiac puncture from animals under anaesthesia at specified time intervals (2, 10, 20, 37, 45 and 60min) after injection of the 2-deoxy-D-{2,6-^{3}H]-glucose as described previously. Blood glucose concentration was measured by the o-toluidine method. The decay of radioactivity in the blood was measured by a modification of the method of Meszaros et al (1987); immediately after collection, 100µl blood was transferred to a microfuge tube containing 100µl ice cold 0.5M perchloric acid and, after thorough mixing, the deproteinised sample was centrifuged in a Beckmann microfuge for 30 sec. The supernatant was carefully transferred to a clean microfuge tube and the pH was

adjusted to 7 with 1N potassium hydroxide solution. The volume of supernatant present after neutralisation was measured and 20µl was transferred to a scintillation vial containing 10ml Optiphase Hi-safe scintillation fluid. The radioactivity in each sample was read in duplicate using a dual $^{14}C/^{3}H$ channel analyser by means of a Packard TRI-CARB 200CA liquid scintillation analyser.

3.3.4.3 Accumulation of radioactive 2-deoxyglucose-6-phosphate by tissues (Cm*)

The accumulation of phosphorylated metabolites of 2-deoxyglucose was measured in selected tissues 60min after the injection of 2-deoxy-D-[2,6-^{3}H]-glucose. Mice were killed by cervical dislocation and the following tissues were carefully dissected out and weighed; liver, brain, spleen, kidneys, lungs, colon and tumour. Each tissue was homogenised in 0.4ml/100mg tissue weight of ice-cold 0.5N perchloric acid using a CAMLAB 563C homogeniser (speed 8) fitted with a teflon pestle. The homogenate was then transferred to a centrifuge tube and centrifuged at 3000rpm in a Beckmann bench centrifuge for 15min. The supernatant was carefully transferred to a clean centrifuge tube, neutralised to pH7 with 10%w/v potassium hydroxide solution, re-centrifuged at 3000rpm for 5min and the final volume of neutral supernatant was measured. 100 - 500µl of this neutral extract was then transferred to scintillation

vials containing 10ml Optiphase scintillation fluid and the radioactivity was measured in duplicate using a dual $^{14}C/^{3}H$ channel analyser by means of a Packard TRI-CARB 2000CA liquid scintillation analyser. This gave a measure of the total radioactivity of 2-deoxyglucose and its metabolites present in the tissue.

Removal of 2-deoxyglucose-6-phosphate from the neutral extract was accomplished by use of the Somogyi (1945) reagent; 200ul of the neutral extract was transferred to a clean microfuge tube and 100ul of 0.175M zinc sulphate solution, followed by 100ul of 0.15M barium hydroxide , were added, yielding a neutral mixture. The resulting precipitate, which absorbed any 2-deoxyglucose-6-phosphate, was then sedimented in a Beckmann microfuge for 30 sec. 100ul of the supernatant was transferred to a scintillation vial containing 10ml Optiphase scintillation fluid and the radioactivity was measured in duplicate. This gave a measure of the free 2-deoxyglucose content of the tissue.

The difference between the total radioactivity of the neutral extract and the radioactivity present after the zinc sulphate / barium hydroxide treatment represented the 2-deoxyglucose-6-phosphate content of the tissue.

3.3.5 Phosphofructokinase assay

Inhibition of phosphofructokinase by serum from non-tumour bearing controls and MAC13 and MAC16 tumour bearing mice was determined using the method outlined in Methods of Enzymatic Analysis (Bergmeyer, 1974).

3.3.5.1 Sample collection and preparation

Blood was collected from non-tumour bearing control animals and also from tumour bearing mice by cardiac puncture. Blood samples were collected in heparinised tubes and plasma was obtained by centrifuging the samples in a Beckmann centrifuge for 30 sec. 20μl of each plasma sample was used in the assay.

3.3.5.2 Enzyme Assay

The above samples were added to cuvettes containing the following; Tris-HCl buffer pH8.5 70mM, $MgSO_4$ 1.4mM, Phosphoenolpyruvate 0.71mM, Fructose-1,6-biphosphate 0.64mM, fructose-6-phosphate 1.8mM, ATP 1.1mM, NADH 0.4mM and LDH 4.2U/ml. The samples were read at 340nm until a steady reading was obtained. 0.02ml of phosphofructokinase (2.0M, pH7.5) was added and the reaction was allowed to run for 30min. Inhibition of the activity of phosphofructokinase was calculated by reading a control

(water in place of sample) for each assay.

3.3.6 Determination of hepatic Glucose-6-phosphatase activity in tumour and non tumour-bearing animals

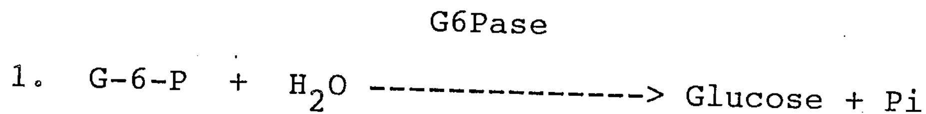
3.3.6.1 Preparation of tissues

Livers from control and tumour bearing animals (MAC16 cachectic and MAC13) were excised and quickly homogenised in 1ml of ice-cold 0.25M sucrose solution . Each sample was centrifuged at 11 000g in a Pegasus 65 Ultracentrifuge for 30 min. The resultant precipitate was discarded and the supernatant was centrifuged at 105 000g for 1h. The resulting precipitate (microsomes), was homogenised in 1ml of ice-cold EDTA/sucrose solution (pH 7.0) (1mM / 0.25M) and assayed for activity.

3.3.6.2 Enzyme assay

The enzyme preparation was assayed according to the method outlined in Methods of Enzymatic analysis (Baginski, et al, 1974). Briefly, 100µl of the sample was incubated with 100µl of a 0.1M glucose-6-phosphate solution in the presence of a cacodylate buffer (0.1M, pH6.5) for 5min. 2ml of an ascorbic acid/TCA buffer was added (2% / 10% w/v) and the sample was centrifuged for 3min. at 3000g. 1ml of the resultant supernatant was pipetted into a

clean test tube and 0.5ml of an ammonium molybdate solution (1%w/v) was added and the sample was mixed. 1ml of an arsenite / citrate buffer (2% / 2% w/v) was added and the sample was mixed and allow to stand for 15min at room temperature. The absorbance of each sample was read at 840nm in a Beckman DU70 spectrophotometer. The reaction sequence is illustrated below:



$$1.\quad G\text{-}6\text{-}P + H_2O \xrightarrow{G6Pase} Glucose + Pi$$

Where: G-6-P = glucose-6-phosphate and G6Pase = glucose-6-phosphatase

The activity of the enzyme sample was determined using a phosphate standard and the equation shown below:

$$\text{Volume activity} = \frac{\text{Abs sample} - \text{Abs control}}{\text{Abs standard}} \times \frac{1500}{t}$$

where volume activity refers to the activity of glucose-6-phosphatase in U/L; Abs control is the absorption of the sample without the colour reaction and Abs standard

is the absorption of a phosphate standard (1.5mM KH_2PO_4) and t=time (min).

3.3.6.3 Protein determination

Protein concentration was determined by the method of Bradford (1976) using Bio-rad reagent. Dilutions of protein samples (1:200 to 1:1000) were made. 0.8ml of diluted protein sample was mixed with 0.2ml of dye reagent and allowed to stand for 5min. The absorbance was read on a Beckman DU7 spectrophotometer at 595nm. All samples were calibrated against a control containing distilled water in place of the protein sample. A standard curve was constructed using bovine serum albumin and used to determine protein concentrations.

3.4 Lipid utilisation by tumour and non tumour-bearing animals

3.4.1 Palmitic acid utilisation in vivo

Mice were injected i.p. with 50µCi/Kg of D-[U-^{14}C]-palmitic acid in 200µl of 0.9% NaCl. Animals were then placed in airtight metabolic cages into which air was pumped through solid calcium carbonate to absorb any CO_2. Metabolically produced CO_2 was trapped in glass test-tubes containing 20ml of a mixture of ethanolamine : ethoxyethanol (1 : 4).

At specified intervals, (0.5, 1, 2, 4 and 8h), 500µl aliquots were taken and transferred to scintillation vials containing 10ml of Optiphase Hi-safe II. The vials were shaken and the radioactivity was measured in a Packard TRI-CARB 2000CA scintillation analyser. MAC16 tumour bearing mice (22 - 23g) were compared to control non-tumour bearing mice (25 - 26g) and MAC13 tumour bearing mice (25 - 26g).

3.4.2 Lipid oxidation and accumulation

The absorption, accumulation and oxidation of an oral dose of triolein was determined using the method of Oller do Nascimento and Williamson (1986). ^{14}C-Triolein (0.33µCi in 100µl normal saline) was administered by intragastric intubation to MAC16 and MAC13 tumour bearing mice and non-tumour bearing controls. Immediately after administration of the oral dose, the mice were placed in airtight metabolic cages and metabolically produced CO_2 was collected at the following time points (0.5, 1, 2, 3, 4 and 5h), using the method described in section 3.3.3. After 5h, the mice were anaesthetised and blood was collected by cardiac puncture. The complete gastrointestinal tract was removed and homogenised in 5ml of 3% perchloric acid. The liver, adipose tissue and tumour were removed. Lipids were extracted from the above organs, the blood and the gastrointestinal tract as described in section 3.3.1.3.

Triolein absorption was calculated by subtracting the total gastointestinal tract radioactivity from that administered.

3.4.2.1 Lipid accumulation in liver and tumour of MAC16 tumour-bearing animals

Male NMRI mice bearing the MAC16 tumour (22 - 23g), were given an oral dose of [1-^{14}C]-triolein as described in section 3.4.2. At specified time intervals, (1, 2, 3, 4, and 5h), mice were sacrificed and lipids were extracted from the livers and tumours using the method described in section 3.3.1.3. The radioactivity was then determined using a Packard TRI-CARB 2000CA liquid scintillation analyser.

3.4.3 Direct Injection of Lipid

3.4.3.1 Complex of lipid with albumin

3.4.3.1.1 Collection of serum samples

Male NMRI mice were fed with a 60% glucose : 40% rat and mouse breeding diet (section 2.1) overnight in order to lower their plasma free fatty acid level. The mice were anaesthetised and blood was removed by cardiac puncture. The blood was allowed to clot at room temperature, and serum was obtained by centrifuging the samples for 30

seconds in a Beckman microfuge.

3.4.3.1.2 Formation of soap solution

[U-^{14}C]Palmitic acid (50µCi) in toluene was dried under nitrogen. To this was added 500µl KOH (30%w/v) and the resultant soap was dried under nitrogen. The dried soap was dissolved in 200µl of 0.9%w/v NaCl and heated to 70°C until a clear solution was obtained. The soap solution was added dropwise to 200µl of the serum prepared in section 3.4.3.1.1 and shaken well. A few drops of Evans Blue dye was added to the tracer to aid visual inspection after injection into the mice.

3.4.3.2 Preparation of animals

Non-tumour bearing male NMRI mice (24 - 26g) were compared with mice bearing the MAC16 tumour (22 - 23g) and mice bearing the MAC13 tumour (24 - 26g). Mice were anaesthetised using a mixture of halothane (2.5%), oxygen (0.5cc/min) and nitrous oxide (0.7cc/min). A small incision was made in the lower abdomen and the left epididymal fat pad was gently externalised using a saline wetted probe. With the aid of a magnifying glass, 2µl of the above tracer was injected into the fat pad using a Hamilton microsyringe. The fat pad was examined to ensure no leakage of the tracer had occurred. For time points

beyond time zero, the fat pad was gently returned to the abdominal cavity and the wound was clipped together. At specified time points, (0, 10min, 30min and 60min), the mice were sacrificed and the left epididymal fat pad was removed. At 60min, the liver, brain, tumour and thigh and gastrocnemius muscles were dissected out.

3.4.3.3 Determination of lipid accumulation

The concentration of labelled lipid in the epididymal fat pads over time was determined by the method described in section 3.3.1.3. The concentration of labelled lipid in the liver, brain, tumour and thigh and gastrocnemius muscles at t=60min was also determined using this method.

3.4.4 Liver Acetyl CoA Carboxylase activity

3.4.4.1 Preparation of crude extract

Mouse livers were prepared according to the method of Inoue and Lowenstein (1975). Briefly, livers were removed and homogenised in 2ml of buffer A (section 2.3.5.1) and then centrifuged at 2000g for 10min in a Beckmann centrifuge. The packed residue was then homogenised in 0.4ml of buffer A and the homogenate was combined with the supernatant obtained from the first step. The samples were centrifuged in a Pegasus 65 Ultracentrifuge at 14 000g for 45 min. The

resultant residue was washed with 0.6ml of buffer A and the supernatants and washings were combined and centrifuged at 105 000g for 45min.

3.4.4.2 Gel Filtration

The high-speed supernatant (0.5ml) was placed on a G-25 column (10cm) which had been equilibrated with column buffer (section 2.3.5.2). The column was eluted with column buffer and 0.5ml fractions were collected. The protein concentration of each fraction was determined using the method described in section 3.3.6.3, and the fractions containing the highest concentrations of protein were pooled together.

3.4.4.3 Activation

A sample of the eluted fractions (0.5ml) was activated by the addition of 0.5ml of activation buffer (section 2.3.5.3) for 30min at 37^{0}C.

3.4.4.4 Enzyme Assay

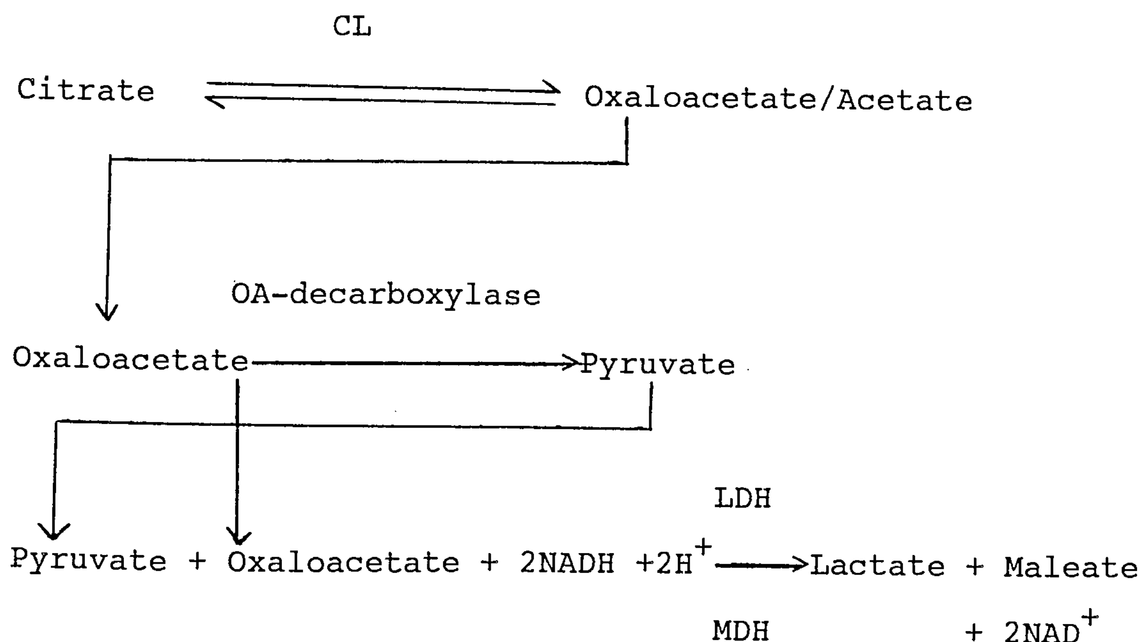
The activated enzyme mixture (20µl) was added to the assay buffer (section 2.3.5.4) in a final volume of 0.4ml. The mixture was incubated at 37°C for 5 min and the reaction was stopped by the addition of 0.1ml of 4N HCl. The mixture

was dried in air and the resulting residue was dissolved in 10ml of Optiphase Hi-safe II. The radioactivity was determined using a Packard TRI-CARB 2000CA liquid scintillation analyser.

Livers from cachectic MAC16 tumour-bearing mice and from mice bearing the MAC13 tumour were compared with livers from non-tumour bearing controls.

3.4.5 Determination of liver Citrate levels

The level of citrate in the liver was determined as shown below using the method outlined in Methods of enzymatic analysis (Dagley 1974):



where; CL = citrate lyase; MDH = maleate dehydrogenase and LDH = lactate dehydrogenase

3.4.5.1 Tissue preparation

Livers from male NMRI non-tumour bearing controls and livers from MAC16 and MAC13 tumour bearing mice were excised and placed on ice. Each liver was quickly homogenised in 2.5ml of ice-cold 0.6M perchloric acid. The sample was centrifuged in a Beckmann centrifuge at 3000g for 5min. The supernatant was neutralised with 2N KOH and the sample was alllowed to stand for 5min in an ice bath. The $KClO_4$ was filtered off, and if necessary, the supernatant fluid was adjusted to pH7 - 7.6.

3.4.5.2 Assay System

The sample (100µl) prepared in section 3.4.5.1 above, was added to a buffer containing $ZnCl_2$ 0.19M, triethanolamine 93mM and NADH 0.2M in a final volume of 2.96ml. The sample was mixed carefully, and the absorption of the sample (E1) was read at 340nm using a Beckman DU70 spectrophotometer. A solution containing 7U LDH/ml and 14U MDH/ml (0.02ml) was added to the sample and allowed to stand for 3min after mixing. The absorption of the sample at 340nm (E2) was again determined. Citrate lyase (0.5U/ml) was then added to the reaction mixture and the sample was allowed to stand

for 10min, after which time the absorption at 340nm (E3) was determined.

From this assay the concentration of citrate present in the liver can be calculated as follows:

Concentration of citrate $= (E2 - E3) \times 4.82$ (µmole/ml)

$= (E2 - E3) \times 0.927$ (mg/ml)

3.4.6 Administration of lipid lowering drugs

3.4.6.1 Administration of Bezafibrate to MAC16 tumour bearing animals

Mice bearing the MAC16 tumour were injected once daily by intraperitoneal injection with either 100mg/Kg of bezafibrate in 100µl of arachis oil or 100µl of arachis oil. Each group contained 5 male MAC16 tumour bearing NMRI mice (average weight loss 2g) randomised according to tumour volume. Weight loss and tumour volumes were monitored daily throughout the experiment.

3.4.6.2 Administration of bezafibrate to MAC13 tumour bearing animals

MAC13 tumour bearing mice were injected with bezafibrate as described in section 3.5.6.1. The control group were injected with 100µl of arachis oil. Each group was randomised according to tumour volume at the start of the experiment. Body weights and tumour volumes were determined daily for each group.

3.4.6.3 Effect of bezafibrate in vitro

The effect of bezafibrate on tumour cell growth was determined in vitro using MAC16 cells. Cells were grown in RPMI 1640 culture medium with 10% foetal calf serum and 1% glutamine in an atmosphere of 5% CO_2 : 95% air at a density of 4 x 10^4 cells/ml. To each 2ml cell sample was added 20µl of ethanol containing bezafibrate of various concentrations (1µM, 10µM, 0.1mM, 0.5mM and 1mM). Control samples (20µl of ethanol) were also investigated. Cells were incubated at 37^{0}C for 72h after which time cell number for each sample was determined using a Coulter electronic particle counter. Each concentration of bezafibrate was investigated in triplicate.

3.4.6.4 Effect of bezafibrate on lipid accumulation in MAC16 tumour-bearing animals

MAC16 tumour-bearing animals (weight loss 1 - 3g) were injected once daily by subcutaneous injection with either 200mg/Kg bezafibrate in 100µl arachis oil or 100µl arachis oil alone for 3 days. On day 3 of the experiment, 50µCi of [1-^{14}C]triolein was administered by intragastric intubation, 1h after the injection of bezafibrate or arachis oil. After 2h, organs were removed from the mice as described in section 3.4.2, and lipids were extracted using the method outlined in section 3.3.1.3. The accumulation of radioactivity was then determined for each organ.

3.5 Ketone body utilisation by tumour and non tumour-bearing animals in vivo

3.5.1 $^{14}CO_2$ Production from ^{14}C-labelled 3-Hydroxybutyrate

Mice were injected i.v. with 50µCi/Kg of D-[^{14}C]-3-Hydroxybutyrate in 100µl of 0.9% NaCl. Animals were then placed in airtight metabolic cages and metabolically produced CO_2 was collected as described in section 3.3.3. MAC16 tumour bearing male NMRI mice (22-23g) and mice bearing the MAC13 tumour (26-27g) were compared to non-tumour bearing controls (26-27g).

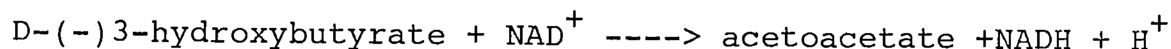
3.5.2 Determination of plasma 3-hydroxybutyrate and acetoacetate levels

3.5.2.1 Preparation of blood samples

A sample of whole blood (100µl) was transferred to a microfuge tube on ice and deproteinised by adding 100µl of ice cold 10% (w/v) perchloric acid. This was then mixed using a vortex whirlimixer and centrifuged in a Beckman microfuge for 1 min. The resultant supernatant was assayed for 3-hydroxybutyrate and acetoacetate concentration.

3.5.2.2 Analysis of 3-hydroxybutyrate levels

Levels of 3-hydroxybutyrate were determined using the method of Williamson and Mellanby (1974), which depends on the oxidation of 3-hydroxybutyrate by 3-hydroxybutyrate dehydrogenase and NAD.



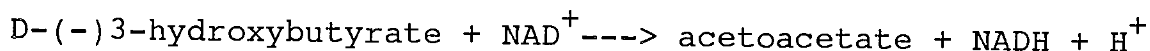
$$\text{D-(-)3-hydroxybutyrate} + NAD^{+} \text{ ----> acetoacetate} + NADH + H^{+}$$

The increase in extinction at 340nM due to the formation of NADH is a measure of the reaction. Assay cuvettes contained 16mM Tris, 0.32mM hydrazine hydrate buffer pH 8.4, 0.45mM NAD and 50ul of sample in a total volume of 3.1ml. The reaction was initiated by adding 10µl of 3-hydroxybutyrate dehydrogenase (150mU/ml). The increase

in absorbance was measured at 340nM for 40 to 60 min at a temperature of 25^{0}C in a Beckman DU7 spectrophotometer.

3.5.2.3 Analysis of acetoacetate levels

Levels of plasma acetoacetate were determined using the method of Mellanby and Williamson (1974) which depends on the oxidation of NADH at 340nM.



D-(-)3-hydroxybutyrate + NAD^+---> acetoacetate + NADH + H^+

Assay cuvettes contained 33mM phosphate buffer pH 6.8, 0.2mM NADH and 50μl sample in a total volume of 3.1ml. The reaction was initiated by the addition of 10ul of 3-hydroxybutyrate dehydrogenase (50 mU/ml). The decrease in absorbance was measured at 340nM for 20 minutes at a temperature of 25^{0}C in a Beckman DU7 spectrophotometer

3.5.3 3-Oxo-acid CoA Transferase activity

3.5.3.1 Preparation of tissues

Brains, kidneys, thigh muscles and gastrocnemius muscles were removed as quickly as possible from non-tumour bearing mice and from mice bearing the MAC16 and the MAC13 tumours. The organs were minced finely and homogenised at 4^{0}C in 4 volumes of ice-cold 0.25M sucrose in 1mM 2-mercaptoethanol,

10mM Tris-HCl buffer (pH 7.4). The resultant homogenate was exposed to ultrasonic vibration for 30 sec using a MSE 150 watt ultrasonic disintegrator Mk2 (amplitude 18 - 20 microns). The homogenate was then centrifuged at 30,000g for 20min in a Pegasus 65 ultracentrifuge. The supernatant so formed was retained.

3.5.3.2 Enzyme assay

The assay was based on a method by Williamson, et al, (1971). Cuvettes containing the following were read at 313nm : Tris-HCl 50mM, $MgCl_2$ 5mM, iodoacetamide 5mM, succinyl CoA 0.1mM and 200ul of prepared enzyme sample from above. Once a steady reading was obtained, the reaction was initiated by the addition of 5mM acetoacetate and the cuvettes were read for 2min at 25°C. The reaction rate was calculated from the linear portion of the curve using the equation below:

Change in absorption = E x C x pathlength

where E is the millimolar extinction coefficient (12cm^{-1}), C is the concentration of acetoacetyl-CoA produced in umol/min/mg protein and the pathlength is 1cm.
Protein concentration was determined using the method of Bradford (1976) as described in section 3.3.6.3.

3.5.4 Tissue Slice Metabolism

Slices of liver and brain from control and tumour-bearing animals, together with tumour slices were incubated individually in a Krebs-Ringer bicarbonate solution containing glucose, 2.2mM, DL-3-hydroxybutyrate, 0.1mM and lactate, 8mM, using the method of Shambaugh (1985). In each fuel mixture only one of the three substrates was radioactive. The radiolabelled compounds D-[U-^{14}C] glucose, D(-)ß-[3-^{14}C]hydroxybutyric acid and L-[U-^{14}C]lactic acid were added. The slices were incubated in 10ml of fuel mixture containing 2.5µCi of the labelled fuel in a sealed flask containing a centre well. Samples were gassed with a 95% O_2 : 5%CO_2 mix and incubated at 37^{0}C for 1h. At this time 0.3ml of 5M $HClO_4$ was added to the medium to liberate the CO_2, which was collected in 0.3ml of 0.3M NaOH added to the centre well. After standing for 4h to allow complete absorption, the contents of the centre well were added to 10ml of Optiphase Hi-safe 3 scintillation fluid and the radioactivity was determined.

3.6 NMR methods

Using ^{1}H-NMR spectroscopy, it is possible to detect signals from water and fat in the leg muscles of mice. Using this technique, water and fat signals were determined in the left hind leg muscles of anaesthetised MAC16 and MAC13

tumour bearing animals and non tumour-bearing controls. The water to fat ratios which can then be calculated, can be used as a measure of the amount of fat present in the leg muscle.

3.7 The effect of a MAC16 tumour-associated lipolytic factor in vivo

3.7.1 Measurement of glycerol production

3.7.1.1 Preparation of isolated adipocytes

BKW mice were killed by cervical dislocation and their epididymal adipose tissue removed and placed in isotonic saline. The adipose tissue was then minced and incubated at 37^0C for 2h in Krebs-Ringer bicarbonate buffer containing collagenase (1.5-2.0mg/ml). The cells were gassed prior to incubation with 95% O_2 : 5% CO_2. Digestion of the tissues was detected by the disappearance of intact pieces and an increased turbidity of the medium. Undigested tissue and non-adipose matter was removed by allowing the fat cells to float to the surface of the buffer. The infranatant was aspirated, and replaced with fresh buffer. The washing procedure was repeated five times to remove all collagenase, non-adipose cells and any endogenous hormones.

After washing, the cells were suspended in an appropriate amount of buffer, stirred to uniformity using a magnetic stirrer, and aliquots taken for cell counting. The cells were counted using a Neubauer haemocytometer, and the volume of buffer adjusted to give a cell density of $1\text{-}2x10^5$ adipocytes per ml.

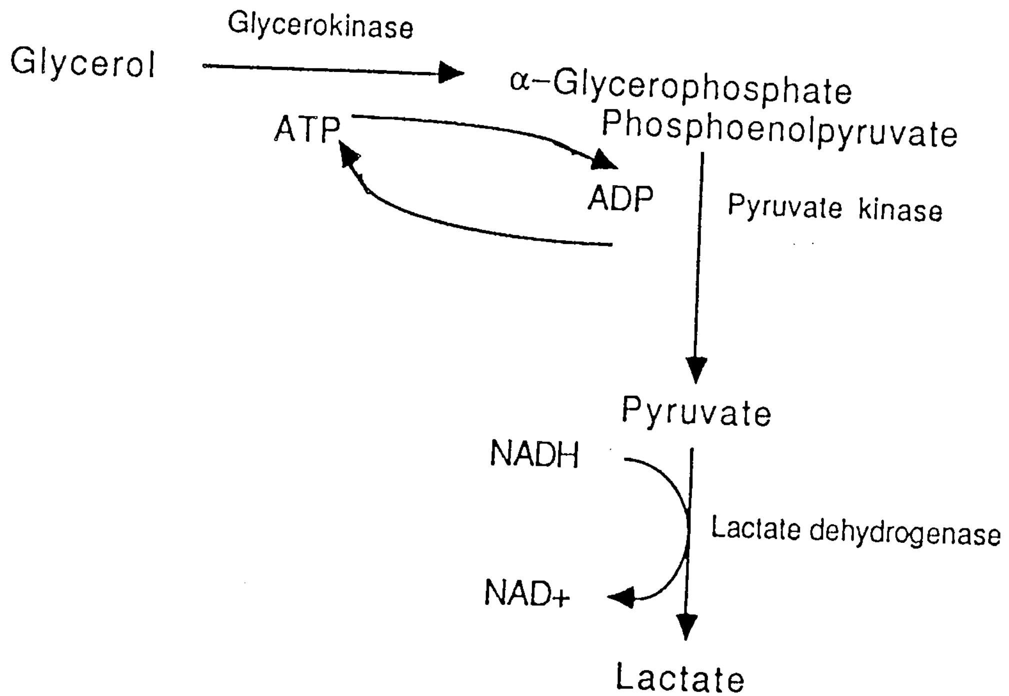
3.7.1.2 Lipolysis assay

Cell samples (1.0ml) were incubated with the appopriate test substance and gassed with 95% O_2 : 5% CO_2. The samples were incubated for 2h at 37^0C in a shaking water bath. Control samples containing adipocytes alone were also analysed to measure spontaneous glycerol release. At the end of the incubation period, 0.5ml of the incubation buffer was added to 0.5ml of perchloric acid (10% w/v) and the mixture was shaken to ensure deproteinisation. The precipitated protein was sedimented by centrifugation at 2000rpm for 10 min in a Heraeus labofuge 6000 centrifuge. The supernatant was aspirated using a pasteur pipette. The supernatant was then neutralised with KOH (20% w/v). The final volume was recorded and used to calculate the dilution factor. The potassium perchlorate precipitate was sedimented by centrifugation (2000 rpm for 10 min) and the supernatant was aspirated. Assays were then either performed immediately, or after storage at -20^0C for approximately 18 - 72h. The concentration of glycerol was

determined enzymatically by the method of Wieland (1974).

3.7.1.3 Glycerol determination

The level of glycerol in the samples was determined as indicated in the scheme below:



The reaction buffer contained triethanolamine (100mM), $MgSO_4$ (2.0mM), phosphoenolpyruvate (0.4mM), NADH (0.25mM), ATP (1.2mM), pyruvate kinase (1 U/ml) and lactic dehydrogenase (7 U/ml). In each cuvette 0.83ml of reaction buffer was added to 200µl of sample. The blank contained 0.83ml reaction mixture and 200µl of distilled water. All samples were calibrated against distilled water alone (no buffer). The optical density was read at 340nM for 2 min until stable, then 10µl of glycerokinase (1mg/ml) was added to each cuvette. The absorbance was read for a further 25 min. The change in absorbance was proportional to the concentration of glycerol present.

$$\frac{\Delta ABS}{6.22\text{(NADH extinction coefficient)}} \times \text{neutralising volume}$$

$$= \text{mM glycerol}/10^5 \text{ adipocytes}$$

3.7.2 Chromatographic techniques

3.7.2.1 Sephadex gel filtration exclusion chromatography

RPMI 1640 culture medium in which MAC16 cells were growing was centrifuged at 2000rpm and the cell-free supernatant was removed. 250 - 300ml of the cell free supernatant was

freeze-dried overnight and the resultant residue was dissolved in 2-4ml of distilled water. The concentrated medium was applied to a Sephadex G-150 column (size 1.6 x 30.0 cm). The column was equilibriated with 0.01M phosphate buffer pH8.0 and active material was eluted with the same buffer. The flow rate was 15ml/h and the effluent was collected in 5.0 ml fractions. The void volume of the column was 150ml. The lipolytic activity of each fraction was measured as described in section 3.7.1.2.

Using this assay of lipolytic activity, the most active fractions were pooled and freeze-dried overnight, then dissolved in 2ml distilled water.

3.7.2.2 Biogel column

Biogel columns were prepared using Biogel P4 which is suitable for the separation of proteins of molecular weights between 2.0k and 4.0kDa. The biogel was soaked overnight in distilled water, then packed into a column (size 1.0 x 14.0cm). The concentrated active fractions from the G-150 purification step were applied to the column and eluted with distilled water at a flow rate of approximately 15ml/h. Fractions of 5ml were collected. The void volume of the column was 22ml. The biogel was used primarily to separate salts such as NaCl and periodate. The fractions were then analysed for lipolytic

activity as described in section 3.7.1.2. The most active fractions were pooled and freeze-dried overnight. The resultant residue was dissolved in 1ml of distilled water.

3.7.2.3 Sep-pack C18 column

A Sep-pack C18 column attached to a 10ml syringe was wetted with 5ml methanol. The column was then washed with 2 x 5ml of distilled water. Active fractions from the biogel P4 fractionation were freeze-dried overnight and then dissolved in 1ml of de-ionised water. The sample was then applied to the Sep-pack column, and eluted with water. Fractions of 0.5ml were collected from the column and assayed for lipolytic activity as described in section 3.7.1.2. Samples producing at least 0.1mMolar glycerol release/100µl were pooled and stored at -20^{0}C.

Culture mediums in which MAC16 cells, L1210 and K562 cells had been growing were purified as defined in the steps outlined in sections 3.7.2.

3.7.3 In vivo administration of Partially Purified Lipolytic Factor

3.7.3.1 Subcutaneous administration

Partially purified factor (200µl) of known lipolytic

activity was administered to female NMRI mice (20 - 21g) once daily by subcutaneous injection. Control mice were injected with 200µl of 0.9%w/v NaCl. Body weights and food and water intakes were monitored daily.

3.7.3.2 Intraperitoneal administration

Partially purified factor (100µl) of known lipolytic activity was administered to female NMRI mice (19 - 20g) three times a day by intraperitoneal injection. Control mice were injected with 100µl of 0.9% NaCl. Body weights were recorded three times a day and food and water consumption was monitored daily. On day 5 of administration, mice were anaesthetised and blood was removed by cardiac puncture. Blood samples were assayed for glucose, lactate and acetoacetate levels. Body composition analysis was determined for each carcass as described in section 3.1.4.

3.7.3.3 Administration of lipolytic factor to non-cachectic MAC16 tumour bearing mice

Partially purified factor (100µl) of known lipolytic activity was administered to mice bearing the MAC16 tumour in which tumour growth was not accompanied by a reduction in body weight. Mice were injected three times a day by intraperitoneal injection and body weight and food and

water intakes were monitored. Tumour volumes were recorded daily. Control mice were injected with 100µl of 0.9% NaCl. At the end of the experiment, body composition analysis of the carcasses was performed as described in section 3.1.4.

3.7.3.4 Oral administration of lipolytic factor

Partially purified factor (200µl) of known lipolytic acivity was administered orally to mice bearing the MAC13 tumour by the use of an oral feeding tube attached to a 1ml syringe. Control mice were given 200µl of 0.9% w/v NaCl. Body weights were determined daily.

3.7.3.5 Administration of L1210 and K562 medium

In order to show that weight loss was not due to other factors present in the partially purified culture medium, mice were administered culture medium in which L1210 and K562 cells had been growing for 4 days. The culture medium was partially purified using the methods outlined in sections 3.7.2. The partially purified medium was administered to MAC13 tumour bearing mice.

SECTION 4: RESULTS

4.1 In vivo characterisation of the MAC13 and MAC16 colon adenocarcinomas

4.1.1 Introduction

Cancer cachexia is characterised by extensive loss of body weight and may occur in patients in which the tumour can compromise as little as 0.01% of their total body weight (Nathanson and Hall, 1974). Animal models of cachexia that have been reported in the literature often possess high tumour burdens before cachexia becomes apparent, and an anorectic response can often explain the observed weight loss (Morrison, 1973). In contrast, a transplantable colon adenocarcinoma (MAC16) passaged in NMRI mice has been shown to be more suitable as a model of human cancer cachexia (Beck, 1989). The MAC16 tumour becomes palpable approximately 7 days after transplantation, and weight loss occurs between days 14 and 21 after transplantation. Using this model, host weight loss is observed with tumour burdens as small as 0.3% of total body weight and it occurs without any effect on food and water intake throughout the period of weight loss. There is a progressive decrease in carcass weight as the MAC16 tumour size increases, and both carcass fat and muscle dry weight decrease in direct proportion to the weight of the tumour (Beck and Tisdale, 1987). The MAC13 adenocarcinoma was induced in the same way as the MAC16 tumour (Double, et al, 1975). It is histologically related to the MAC16 tumour but does not cause cachexia in the host (Double, et al, 1975). It is

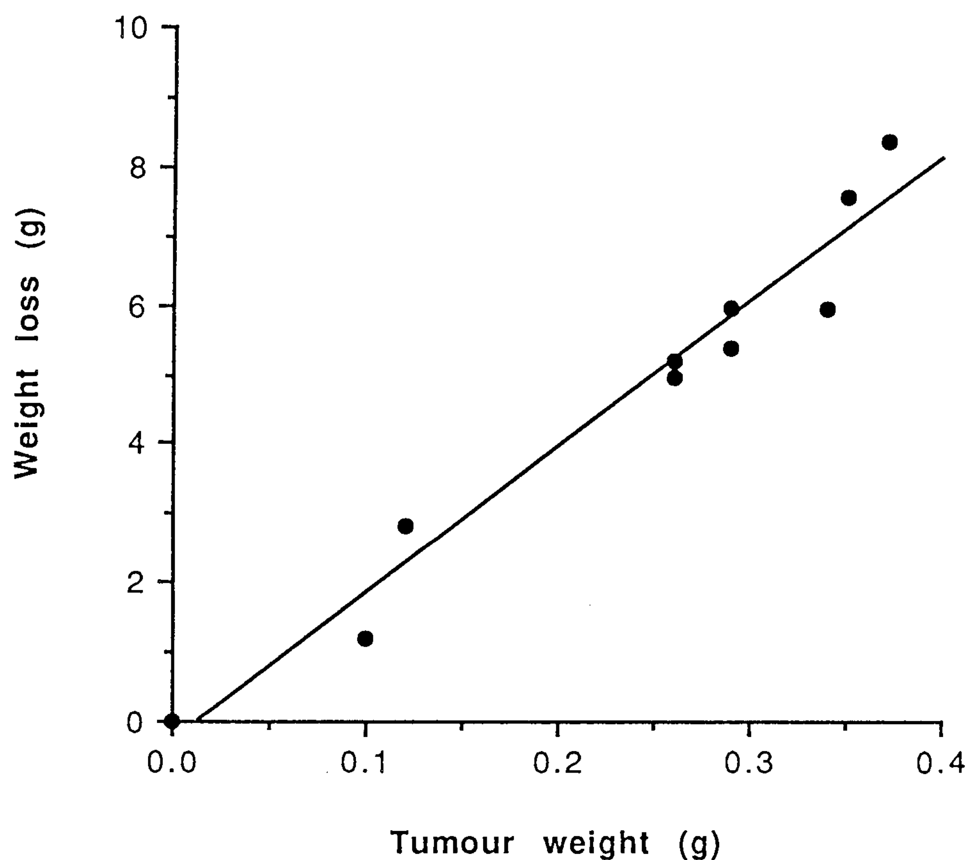
therefore useful as a control when investigating the metabolic effects of the MAC16 tumour *in vivo*. The MAC16 cell line has been derived from the corresponding *in vivo* tumour, and when injected into NMRI mice, it exhibits the same cachectic response as is seen in mice bearing the MAC16 tumour (Beck, 1989). Metabolic studies presented in this section were performed after transplantation of the solid MAC16 tumour.

4.1.2 Results

Female NMRI mice injected intramuscularly with the MAC16 cell line exhibited a cachectic response, and weight loss was proportional to tumour size (R=0.96), (fig 4.1). The doubling time of the induced tumour was 3 - 4 days (Table 4.1a). There was no significant difference in the food and water intakes of tumour-bearing animals when compared with non tumour-bearing controls (Table 4.1b), and there was a significant decrease in total body fat and lean body tissues in cachectic animals (table 4.1c). Percentage body water did not differ from control values. The effect of the MAC16 and MAC13 tumours on the organ weights of male NMRI mice is shown in fig 4.2. Animals bearing the MAC16 tumour showed a significant decrease in the wet weight of the brain, colon, kidneys and epididymal fat pads when compared with non tumour-bearing controls and animals bearing the MAC13 tumour. To allow for the decrease in total body weight of animals bearing the MAC16 tumour, organ weights have also been expressed as a percentage of the total body weights (fig 4.3).

Figure 4.1

The relationship between carcass weight loss and tumour weight in MAC16 tumour-bearing female NMRI mice



2 x 10^6 MAC16 cells were transplanted by intramuscular injection into each mouse.

The results were fitted to a linear model by a least squares analysis (r=0.96)

Each point represents the results from an individual animal

Table 4.1

Characterisation of the MAC16 cell line in female NMRI mice

Table 4.1a

Doubling time of the MAC16 tumour

MAC16 cell line	3 - 4 days
MAC16 solid tumour	3 - 4 days

Table 4.1b

Effect of MAC16 tumour on food and water intakes in female NMRI mice

Group	Food intake (Kcal/day)	Water intake (ml/day)
MAC16 tumour bearing	8.69 ± 0.67	3.7 ± 0.1
Non-tumour bearing	8.71 ± 0.49	3.8 ± 0.2

Results are expressed as mean ± S.E.M. of 9 - 10 animals per group

Table 4.1c

Effect of MAC16 tumour on body composition of female NMRI mice

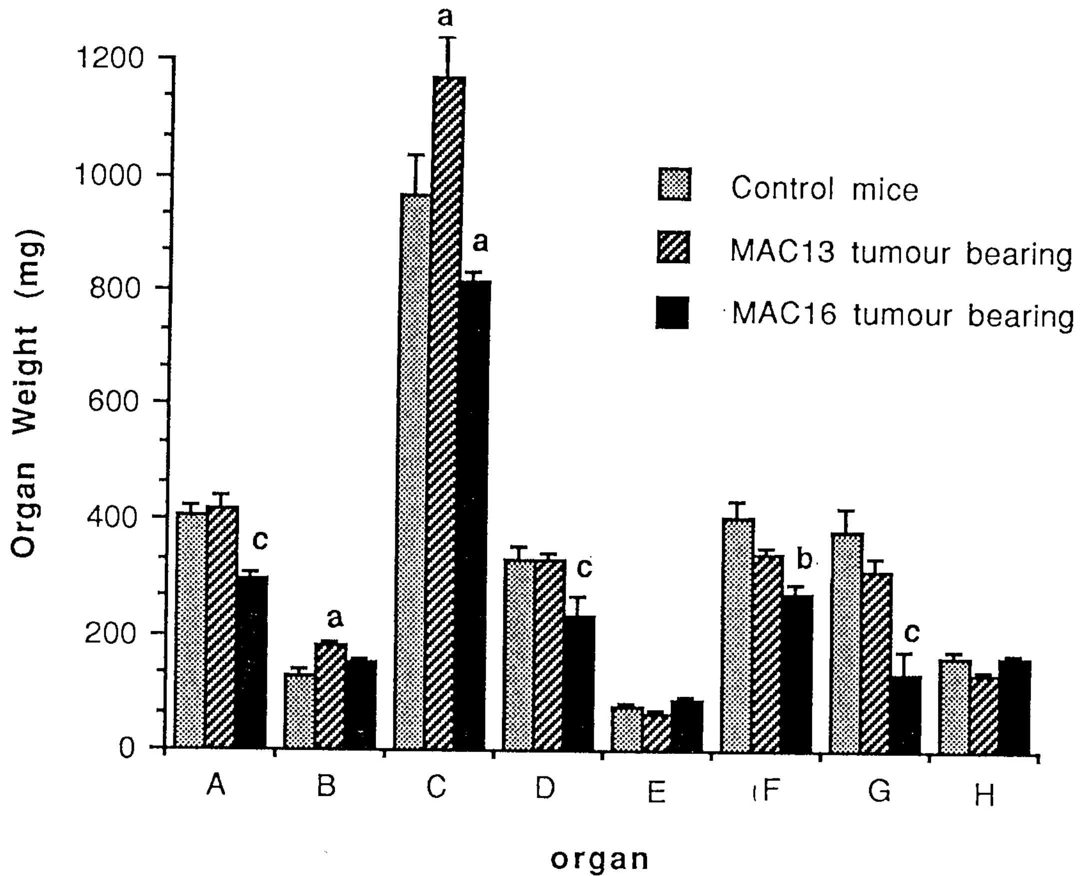
Group	Body water (%)	Body Fat (g)	Left thigh and Gastrocnemius Muscle (g)
MAC16 tumour bearing	67.6 ± 0.6	0.47 ± 0.10*	0.050 ±0.005*
Non-tumour bearing	69.1 ± 0.8	1.54 ± 0.16	0.065 ±0.002

Results are expressed as mean ± S.E.M. of 9 - 10 animals per group

*=p<0.01 from controls using the Students t-test

Figure 4.2

The effect of MAC16 and MAC13 tumours on host organ weights



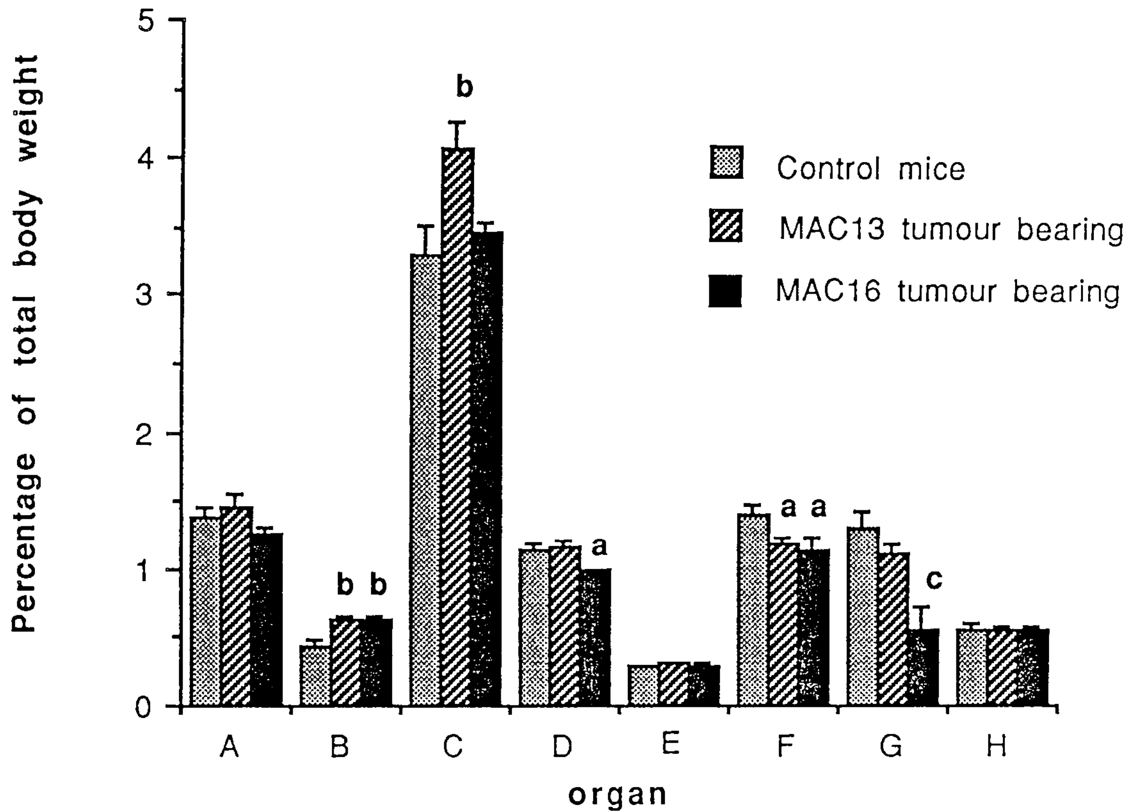
Values represent mean ± S.E.M. for 10 animals per group.

A=brain, B=lungs, C=liver, D=kidneys, E=spleen, F=colon, G=epididymal fat pads, H=testes

a=p<0.01, b=p<0.005, c=p<0.001 from control values, using Students t-test

Figure 4.3

The effect of MAC16 and MAC13 tumours on percentage organ weights



Values represent mean ± S.E.M. of 10 animals per group

A=brain, B=lungs, C=liver, D=kidneys, E=spleen, F=colon, G=epididymal fat pads, H=testes

a=p<0.05, b=p<0.01, c=p<0.005 from control values using Students t-test

In cachectic animals, the kidneys, colon and epididymal fat pads all showed a significant reduction in the percentage contribution of the total body weight, when compared with non tumour-bearing controls. The apparently increased contribution of the lungs in tumour bearing animals may be due to the inability to remove blood after extraction.

Although animals bearing the MAC16 tumour lost weight, the daily food intake per mouse did not differ from that of non tumour-bearing controls, while in animals bearing the MAC13 tumour, the daily food intake was significantly ($p<0.01$) increased (table 4.2). Reducing food intake by 10% in MAC13 tumour-bearing animals, i.e. to that of control and MAC16 tumour-bearing animals, had no effect on tumour weight (table 4.3). There was no difference between MAC13 tumour-bearing animals and non tumour-bearing controls with regard to weight loss caused by a 10% reduction in energy intake (table 4.3). Water intake was also increased ($p<0.01$) in MAC13 tumour-bearing animals especially in those receiving 10% less food ($p<0.005$) when compared with the corresponding control groups (table 4.4). Reducing food intake by 10% did not significantly alter the body composition in either the MAC13 tumour-bearing animals or the non tumour-bearing controls (table 4.5).

Table 4.2

Energy intake in tumour-bearing animals and in non tumour-bearing controls

Group	Energy Intake (Kcal/day)
MAC16 tumour bearing animals	15.1 ± 0.6
MAC13 tumour bearing animals	16.4 ± 0.3 *
Non-tumour bearing animals	15.3 ± 0.3

* = $p<0.01$ from control values

Values represent mean ± S.E.M. for 10 animals per group

*= $p<0.01$ from control value using Students t-test

Food intake was measured over the period of weight loss for MAC16 tumour-bearing animals (days 14 - 21 after transplantation of the tumour), and when the tumour burden was greater than 200mg for MAC13 tumour-bearing animals (days 7 - 21 after transplantation of the tumour)

Table 4.3

The effect of food reduction on tumour weight and host body weight in MAC13 tumour-bearing animals and non tumour-bearing controls

Group	Diet	Change in body weight (g)	Final tumour weight (g)
MAC13 tumour bearing	normal	+1.9 ± 0.4	0.66 ± 0.23
	10% reduction	-1.3 ± 0.2	0.47 ± 0.10
Non-tumour bearing	normal	+1.9 ± 0.5	-----
	10% reduction	-1.7 ± 0.4	-----

Values represent mean ± S.E.M. of 10 animals per group

Table 4.4

The effect of food reduction on food and water intakes in MAC13 tumour-bearing animals and non tumour-bearing controls

Group	Diet	Food intake (Kcal/day)	Water intake (ml/day)
MAC13 tumour	normal	16.44 ± 0.30	6.6 ± 0.2 *
	10% reduction	14.8	7.2 ± 0.2 **
Non-tumour bearing	normal	15.30 ± 0.30	6.2 ± 0.2
	10% reduction	13.8	5.9 ± 0.1

* = p< 0.01

**= p< 0.005 from control values using the Students t-test

Results represent mean ± S.E.M. of 10 animals per group

Table 4.5

The effect of food reduction on host body composition in MAC13 tumour-bearing animals and non tumour-bearing controls

Group	Diet	Water (%)	Thigh (mg)	Gastroc (mg)	Fat (g)	Fat (%)
MAC13 tumour	normal	70.2 ±0.4	43.8 ±3.0	49.4 ±5.7	1.26 ±0.1	15.32 ±1.5
	10% reduction	69.6 ±0.3	38.0 ±1.5	43.8 ±2.7	1.20 ±1.1	15.27 ±1.6
Non-tumour bearing	normal	69.6 ±0.5	45.0 ±2.6	43.0 ±3.9	1.26 ±0.1	14.75 ±1.2
	10% reduction	69.9 ±0.5	43.2 ±3.6	42.4 ±3.5	1.12 ±0.1	15.09 ±0.4

Results represent mean ± S.E.M. for 10 animals per group

4.1.3 Discussion

The cachectic response of mice injected with the MAC16 cell line resembled that caused by the passage of the solid tumour *in vivo*. Weight loss is proportional to tumour weight in both models and is characterised by a depletion of host body fat and lean body tissues. It appears therefore, that the MAC16 cell line *in vitro* retains its ability to induce cachexia in the recipient host and that the site of establishment of the MAC16 tumour (either subcutaneously or intramuscularly) does not affect its ability to cause cachexia.

Although food and water intake does not decrease in MAC16 tumour-bearing animals, there is a progressive loss of host body weight. This suggests that there is a significant energy cost associated with the process of tumour growth. This could explain why MAC13 tumour-bearing animals increase their energy intake with a resultant maintenance of host body weight.

Many workers have suggested that cachexia can be explained to a large extent by a decrease in food intake (section 4.12). Although a reduction in food intake in MAC13 tumour bearing animals did cause a reduction in host body weight, the weight loss did not resemble the massive loss of host tissues which occurs during cachexia induced by the MAC16

tumour. Using these results, it was concluded that the MAC16 tumour is a good model with which to study alterations in host metabolism in cachexia without the complications of anorexia. This will be investigated in the following sections.

4.2 Glucose utilisation by the MAC16 and MAC13 cell lines in vitro

4.2.1 Introduction

The tumour cell has a distinctive metabolism and it can act as a metabolic trap for host nutrients (Argiles and Azcon-Bieto 1988). Depending on the particular tumour growing pattern, cancer cells can use preferentially glucose, amino acids or fatty acids (section 1.5)

The establishment of a systemic energy-losing cycle during the interplay of tumour glycolysis and host gluconeogenesis has been reported (Gold and Syracuse, 1987). High rates of anaerobic glycolysis were first observed in 1930 by Warburg and is a very distinctive metabolic feature in a large number of tumours. The physiological consequences of this altered metabolic behaviour lead to the generation of metabolic inefficiency in the host, via the enhancement of energy wasting cycles, such as the Cori cycle (Fig 1.5), (Young, 1977 and Holroyde, et al, 1975). A high tumour

glycolytic activity would result in the generation of a large amount of pyruvate and an increase in the cytosolic $NADH/NAD^+$ ratio. This would favour the reduction of pyruvate to lactate through the action of lactate dehydrogenase. This is supported by the low mitochondrial content of some tumour cells (Pederson, 1978 and Cederbaum, et al, 1976) which would decrease the possibility of dissipating NADH through the action of the electron transfer chain and also the low levels of NADH-shuttle systems found in a number of tumours (Boxer and Devlin, 1961). The lactate produced by the tumour is released and transported to the host liver where it is used to re-synthesise glucose, in an energy consuming process. Consistent with this mechanism, increased glucose turnover has been reported in late-stage cancer patients (Chlebowski, et al, 1984).

Although a high glycolytic capacity is a common feature of many tumour cells, it is not a universal characteristic. In fact, some tumour cells have been shown to rely almost exclusively on fatty acids and amino acids (Weinhouse, et al, 1973 and Zielke, et al, 1976) and depletion of host lipid stores and lean body tissues is characteristic of MAC16 tumour-induced cachexia (Beck and Tisdale, 1987). Tumours require lipids for membrane synthesis and as an energy source. It is not clear whether tumours can synthesise lipids *de novo* or if they are obtained from the

host. Ehrlich ascites cells have been shown to have only limited ability to synthesise fatty acids and sterols from non-lipid precursors (Spector and Brenneman, 1973). The preferred substrate for maintenance of tumour growth is not clearly defined, and it appears to depend on the individual tumour type and its growth characteristics.

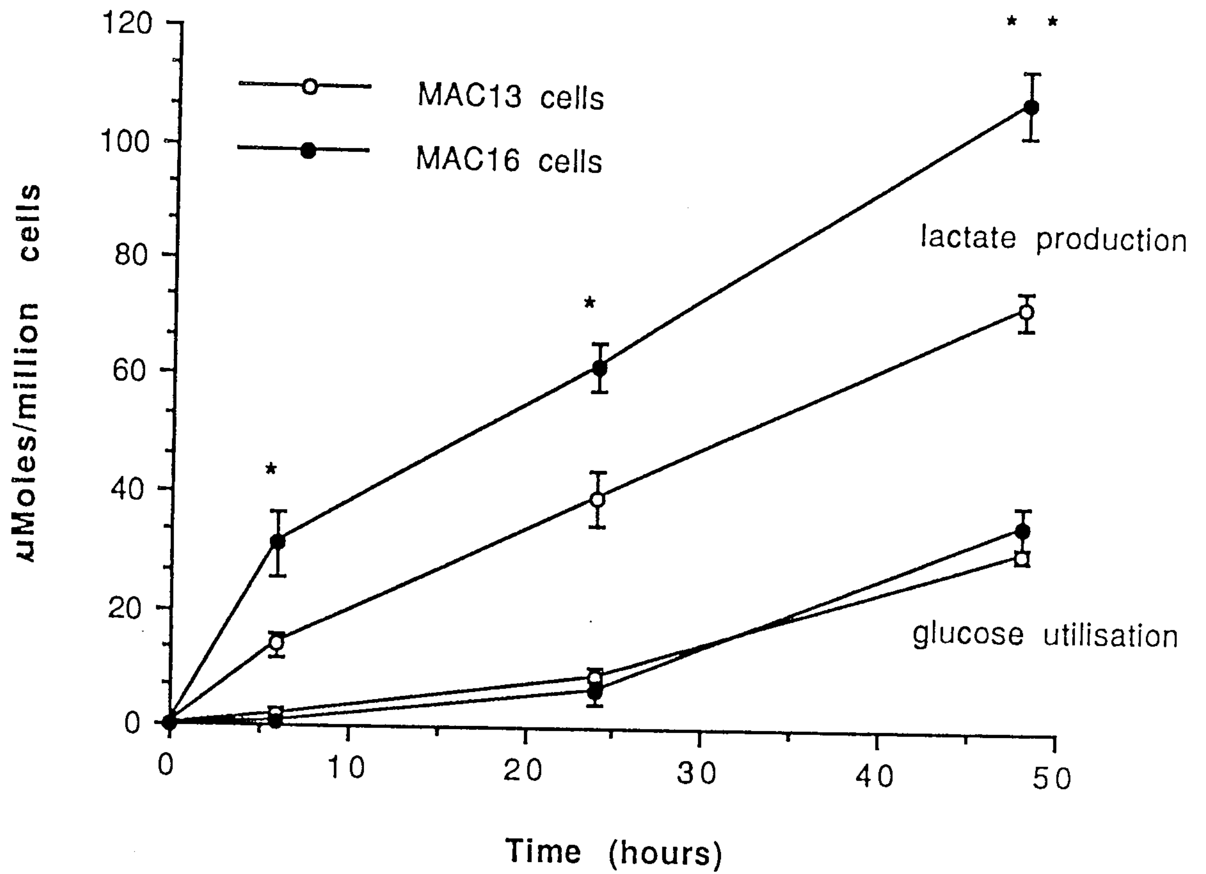
4.2.2 Results

We have studied glucose metabolism in the MAC16 and MAC13 cell line *in vitro*. These cell lines are derived from the corresponding solid tumours *in vivo* and are described in sections 3.2.1 and 3.2.2. Glucose utilisation *in vitro* did not differ between the 2 cell lines (fig 4.4), although lactate production by the MAC16 cell line was significantly greater than the corresponding MAC13 cell line at all time points studied (fig 4.4). In both cell lines, lactate production was high, in fact more than 2 moles of lactate were produced per mole of glucose consumed in both cases. This suggests the use of other substrates (e.g. pyruvate), by the cell lines *in vitro*.

The production of $^{14}CO_2$ from [U-^{14}C]glucose was investigated *in vitro* (fig 4.5). In both cell lines, glucose utilisation over the first 24h was low, corresponding to a lag period for growth of the cell lines. At 48h, respiration from glucose was significantly

Figure 4.4

Glucose utilisation and lactate production by the MAC16 and MAC13 cell lines in vitro

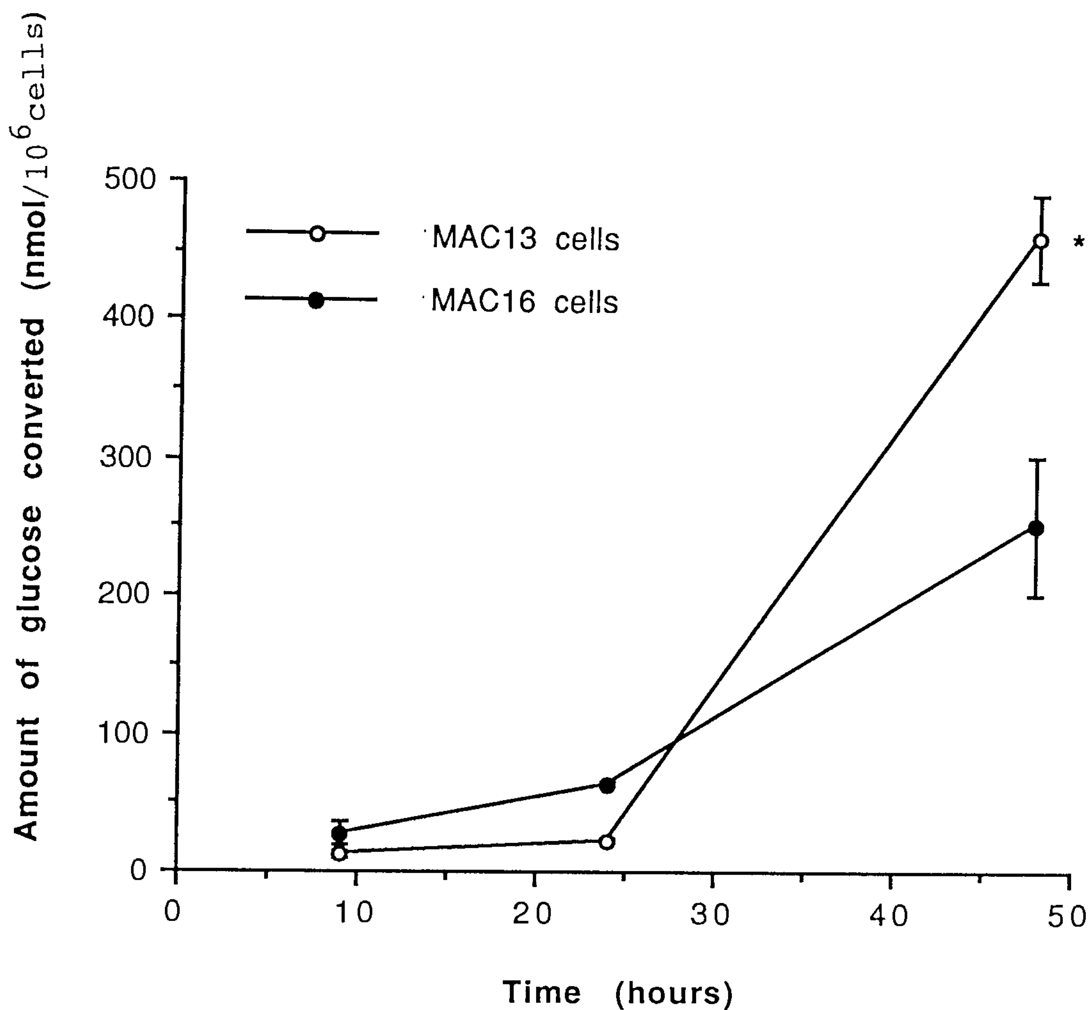


Glucose utilisation and lactate production is expressed as nmol/10^6 cells. Results represent mean ± S.E.M. of 7 determinations per group

*=p<0.005 and **=p<0.001 from the MAC13 cell line values using the Students t-test

Figure 4.5

Oxidation of U-[^{14}C]glucose by the MAC16 and MAC13 cell lines in vitro

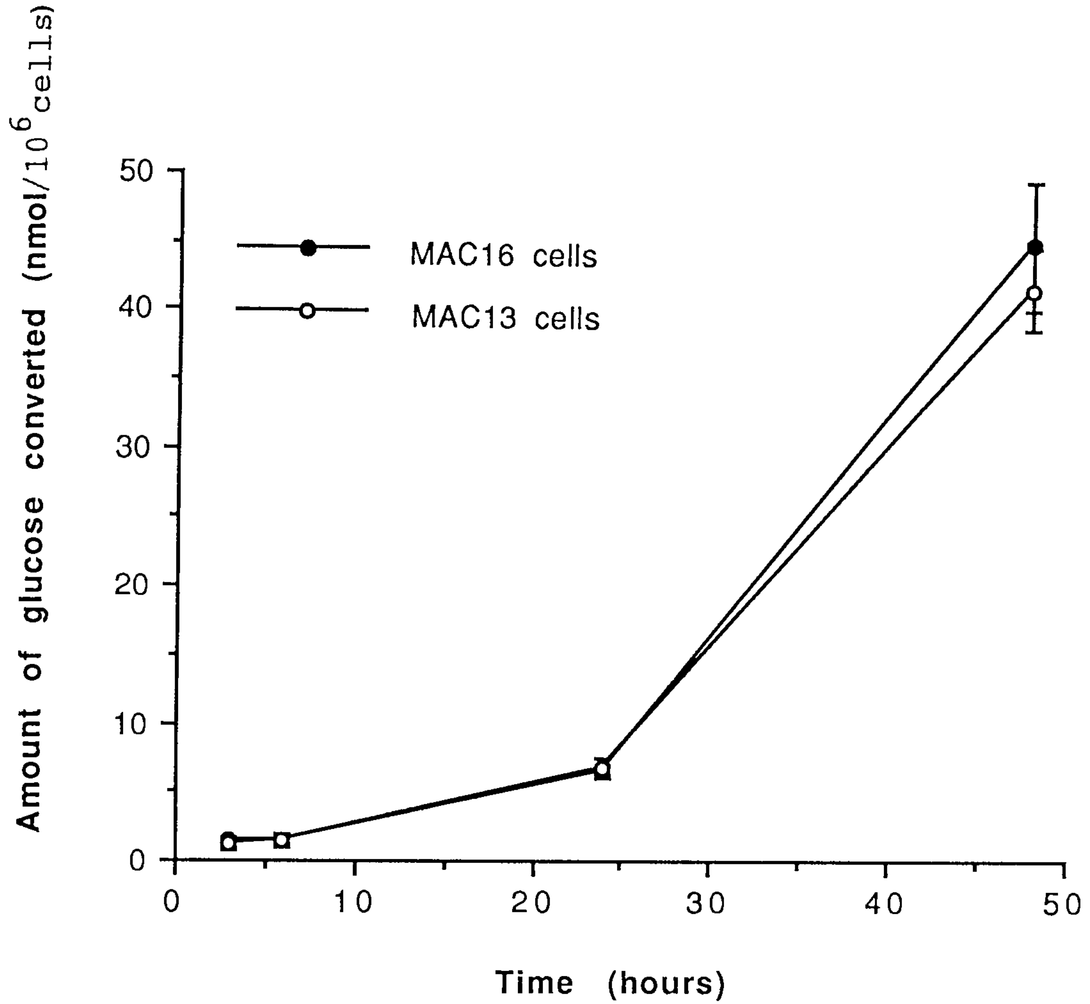


Glucose utilisation is expressed as nmol/10^6 cells. Results are expressed as mean ± S.E.M. of 3 determinations at each time point per group

*=$p<0.01$ from MAC13 cell line value using the Students t-test

Figure 4.6

Lipogenesis from U-[^{14}C]glucose in the MAC16 and MAC13 cell lines in vitro



Glucose utilisation is expressed as nmol/10^6 cells. Results are expressed as mean $\pm$ S.E.M. of 3 determinations at each time point per group

increased in the MAC13 cell line when compared with the MAC16 cell line.

The lipogenic capacity of the MAC16 and MAC13 cell lines was also investigated _in vitro_. There was no difference between the two cell lines with regards to their ability to synthesise lipids from [U-^{14}C]glucose (fig 4.6) and both cell lines appear to have the ability to synthesise lipids _de novo_.

4.2.3 Discussion

Consistent with the theory that all tumours display high rates of anaerobic glycolysis (Warburg, 1930), both the MAC16 and MAC13 cell lines produced large amounts of lactate _in vitro_, especially the MAC16 cell line. This may explain in part the weight loss observed in MAC16 tumour-bearing animals, since the energy consuming Cori cycle would also be expected to be high. The MAC13 cell line exhibited a higher rate of aerobic glycolysis when compared with the MAC16 cell line. Although glucose utilisation via anaerobic glycolysis by the MAC16 tumour was high, total glucose utilisation by the 2 cell lines was the same. The increased production of lactate and its subsequent conversion to glucose by the host could also contribute to the progressive weight loss observed in cachexia, but this requires further investigation. _De novo_

lipid synthesis was a feature of both the MAC16 and MAC13 cell lines *in vitro*. The ability of the tumour to synthesise its own lipids may be an indication that lipid requirements by these cell lines are high. This may be especially so in the MAC16 tumour which also induces excessive breakdown of host adipose stores in recipient animals (Beck and Tisdale, 1987). The increased breakdown of lipid stores is not due to a decreased ability to synthesise lipids by the MAC16 cell line, since its lipogenic capacity resembled that of the MAC13 cell line. Although these results may suggest that glucose is an important substrate in the growth of these cell lines, it is important to remember that growth *in vitro* may not resemble growth *in vivo*. The tumour and the host cannot be considered as separate entities, rather the interaction between host and tumour tissues must be examined as a whole system. This will be investigated in the following sections.

4.3 Alterations in glucose metabolism in vivo

4.3.1 Introduction

Alterations in host carbohydrate metabolism occur in animals and patients with cancer, but their contribution to the development of cachexia are not fully understood. Elevated glucose metabolism in cancer cells has been

reported and leads to an increased intracellular concentration of glucose (Hume and Weidemann, 1979). Increased glucose consumption depends on the tumour type and may be due to increased glucose transport through the cell membrane, (Hume and Weidemann, 1979, and Hatanaka, 1974), or an enhanced capacity for glycolysis because of increased activity of the key glycolytic enzymes, (Weber, 1977). Increased glucose utilisation may lead to an increased lactate production by tumour cells (Bygrave, 1976 and Sauer and Dauchy, 1986) which would result in increased Cori cycle activity. Decreased blood glucose levels have been reported in tumour bearing animals (Shapot, 1972), suggesting an increased glucose demand being imposed on the host. Reduced plasma insulin levels are often found in the tumour bearing host (Chance et al, 1983) along with elevated rates of gluconeogenesis from non-carbohydrate precursors (Waterhouse, et al, 1979).

4.3.2 Results

We have studied the changes in glucose utilisation in host and tumour tissues in animals with or without cachexia in order to determine if alterations in carbohydrate metabolism are related to the cachectic state.

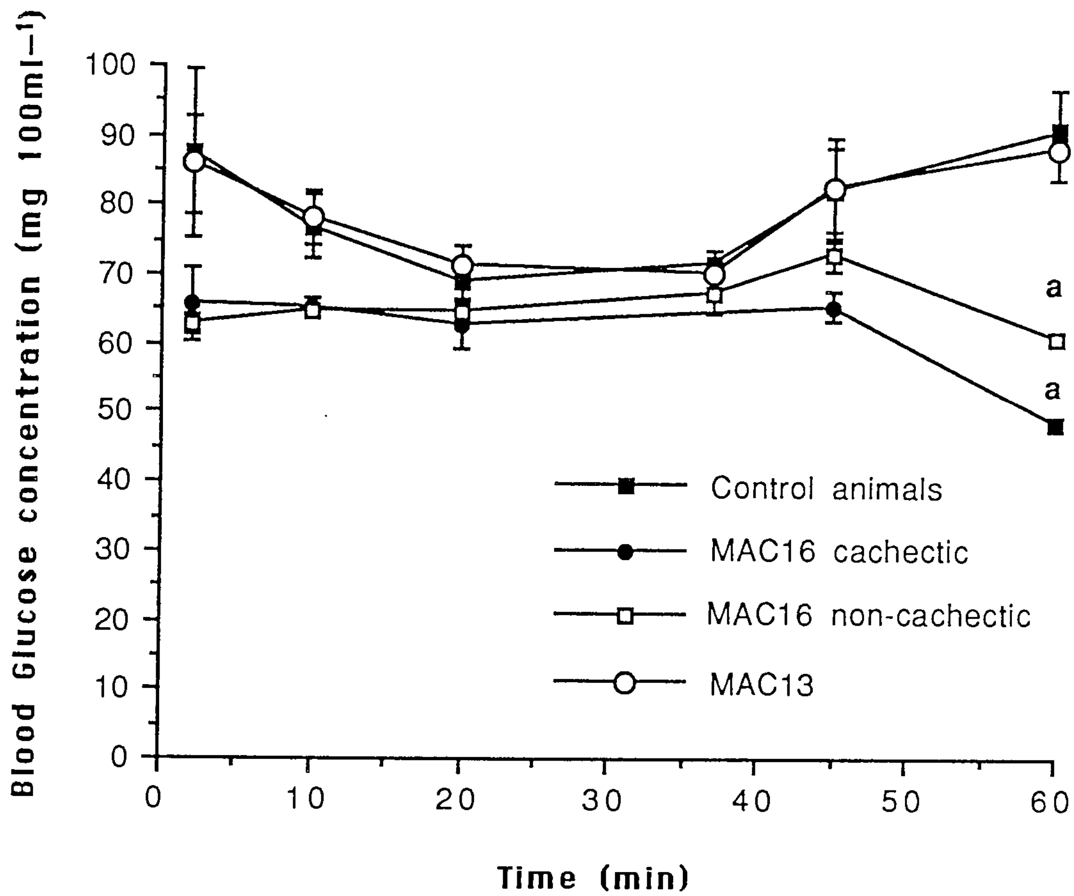
Glucose utilisation by host tissues and tumours was investigated using the 2-deoxyglucose (2DG) tracer

technique. The transport, cellular uptake and phosphorylation by hexokinase of 2DG correlate with those of glucose, resulting in the production of 2-deoxyglucose-6-phosphate (2DGP). Glucose-6-phosphate would normally undergo isomerisation to fructose-6-phosphate, but the absence of an oxygen atom on the 2 position of the pyranose ring of 2DGP hinders isomerisation. Metabolism of 2-DGP is therefore blocked at this stage, and it accumulates within the tissues. Previous workers have only applied the 2-DG tracer technique to the study of the brain (Sokoloff et al, 1977) and muscle (Meszaros, et al, 1987a). These tissues are deficient in the enzyme glucose-6-phosphatase which cleaves glucose-6-phosphate to form glucose. 2-DGP would therefore not be expected to accumulate in tissues in which glucose-6-phosphatase is abundant (liver, kidney, intestines). This method has however been successfully applied to the determination of glucose utilisation by other tissues using a sequential double-labelling technique (Meszaros, et al, 1987b).

Blood glucose levels in animals bearing the MAC13 tumour, that had been starved overnight, were similar to control, non-tumour bearing animals over a 60 min time period (fig 4.7), although both groups were reduced below that of fed animals (128.4 ± 5.1 mg/ml). Animals bearing the MAC16 tumour were hypoglycaemic at the 60min time point when

Figure 4.7

Blood glucose concentration in tumour-bearing animals and in non tumour-bearing controls during the 60min time period of study and after an overnight fast.

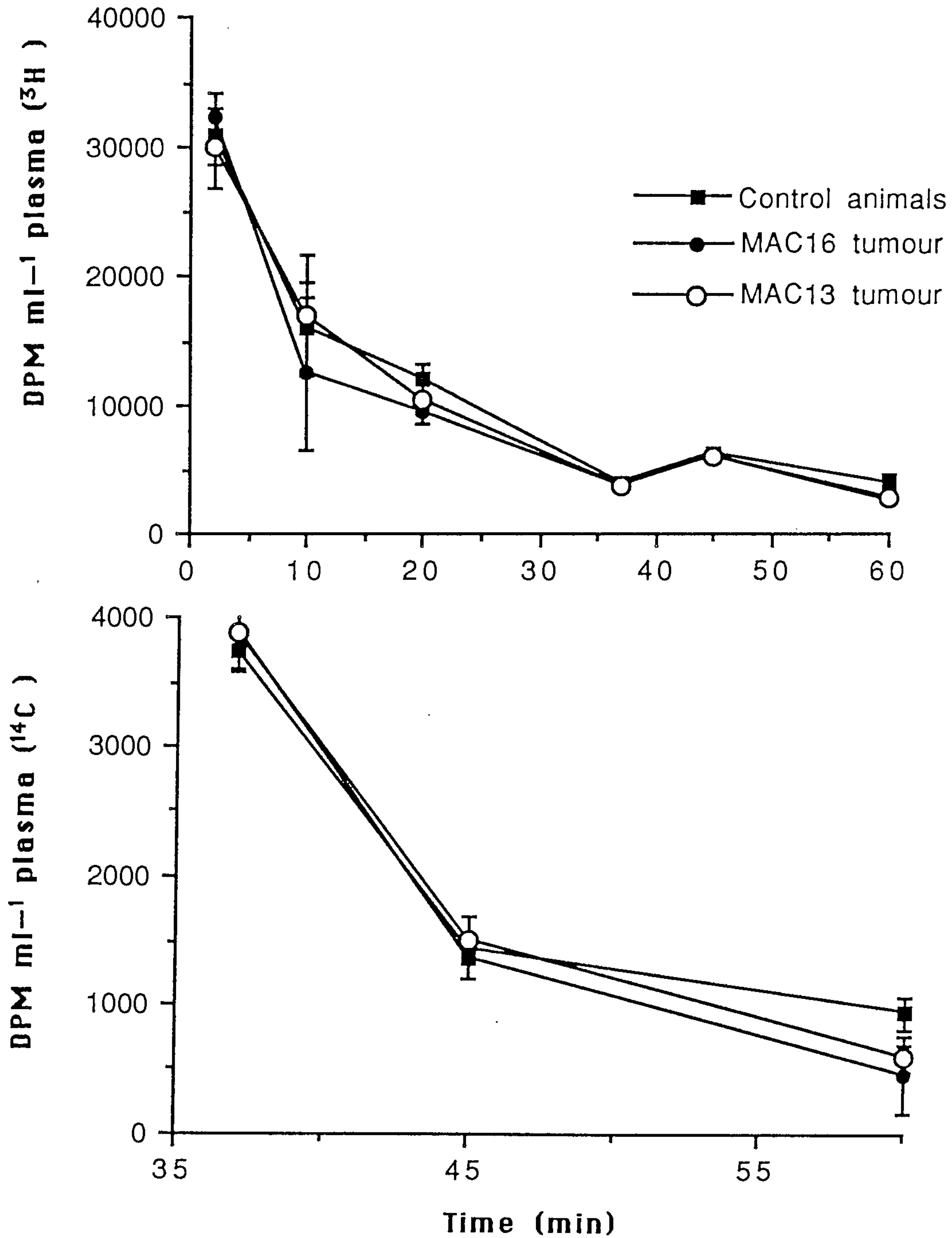


Results are expressed as mean ± S.E.M. of 3 - 4 animals per group per time point

a=p<0.005 from control values using the Students t-test

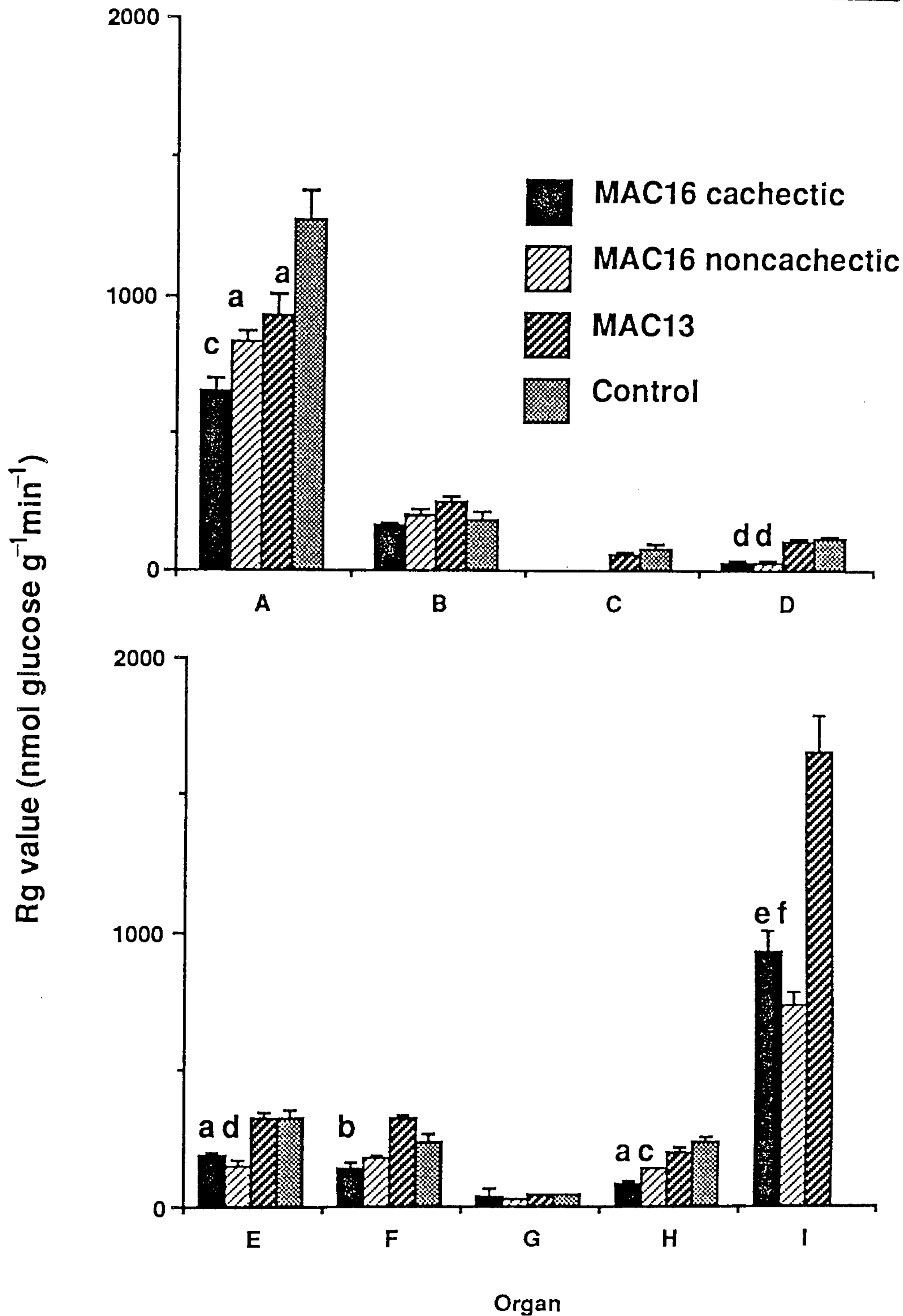
Figure 4.8

Measurement of the disappearance of [^{3}H]-2DG and [^{14}C]-2DG from the blood of tumour and non tumour-bearing animals



Results are expressed as mean ± S.E.M. of 3 - 4 animals per group

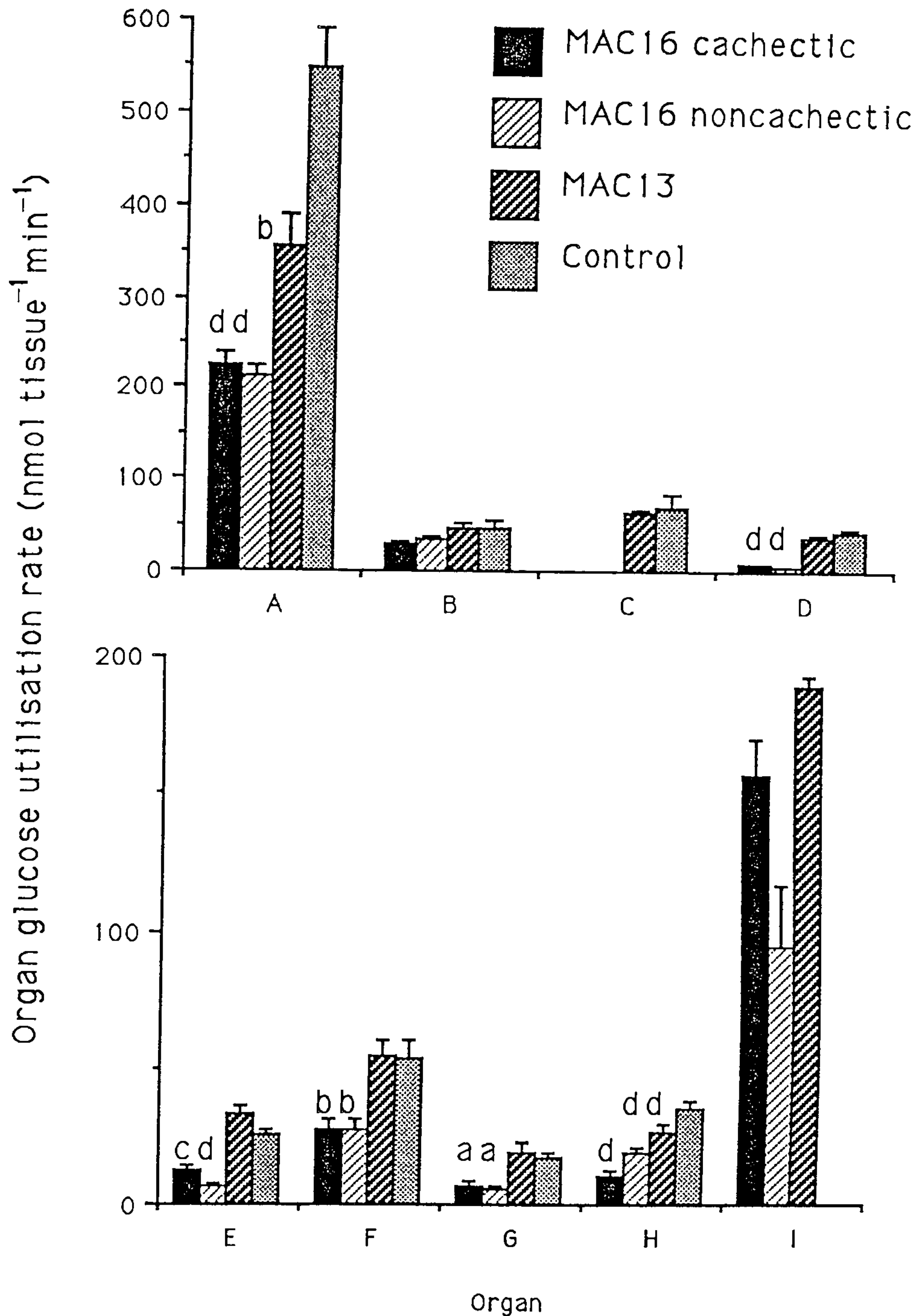
Figure 4.9
<u>The effect of tumour burden on tissue glucose metabolic rate (Rg) in tumour and host organs of male NMRI mice</u>



a=p<0.05, b=p<0.01, c=p<0.005, d=p<0.001 from controls, e=p<0.05, f=p<0.001 from MAC13 tumour values using the Students t-test. Results represent mean ± S.E.M. of 5 animals per group
A=brain, B=lungs, C=liver, D=kidneys, E=spleen, F=colon, G=epididymal fat pads, H=testes, I=tumour

Figure 4.10

The effect of tumour burden on organ glucose utilisation rate in male NMRI mice



A=brain, B=lungs, C=liver, D=kidneys, E=spleen, F=colon, G=epididymal fat pads, H=testes, I=tumour

a=p<0.05, b=p<0.01, c=p<0.005, d=p<0.001 from control values using the Students t-test. Results represent mean ± S.E.M. of 6 animals per group

compared with either group, irrespective of the development of cachexia. The rate of disappearance of the label from 2-deoxy-D-[2,6-^{3}H]-glucose ([^{3}H2DG]) (fig 4.8) or from 2-[1-^{14}C]-deoxy-D-glucose ([^{14}C2DG]) (fig 4.8) did not differ between the four groups.

Glucose utilisation by the MAC13 tumour was significantly ($p<0.05$) greater than the MAC16 tumour (fig 4.9), probably due to the higher rate of cell replication of the MAC13 tumour (2 - 3 days doubling time) when compared with the MAC16 tumour (3 - 4 days doubling time). There was no difference in glucose utilisation by the MAC16 tumour in the presence or absence of cachexia. The Rg values for testes, colon, spleen, kidney and particularly the brain ($p<0.005$) were significantly reduced in animals bearing the MAC16 tumour. The large reduction in the Rg value for brain was not specific for the cachectic state, since it was also seen in animals bearing the MAC16 tumour in the absence of cachexia and in MAC13 tumour bearing animals.

The contribution of various organs to overall glucose utilisation (fig 4.10) depends on both the Rg value and the weight of a particular organ. In all tumour-bearing states, the tumour was the second major consumer of glucose after the brain, and this was accompanied by a marked reduction in glucose utilisation by the brain. In MAC16 tumour-bearing animals, brain glucose consumption was

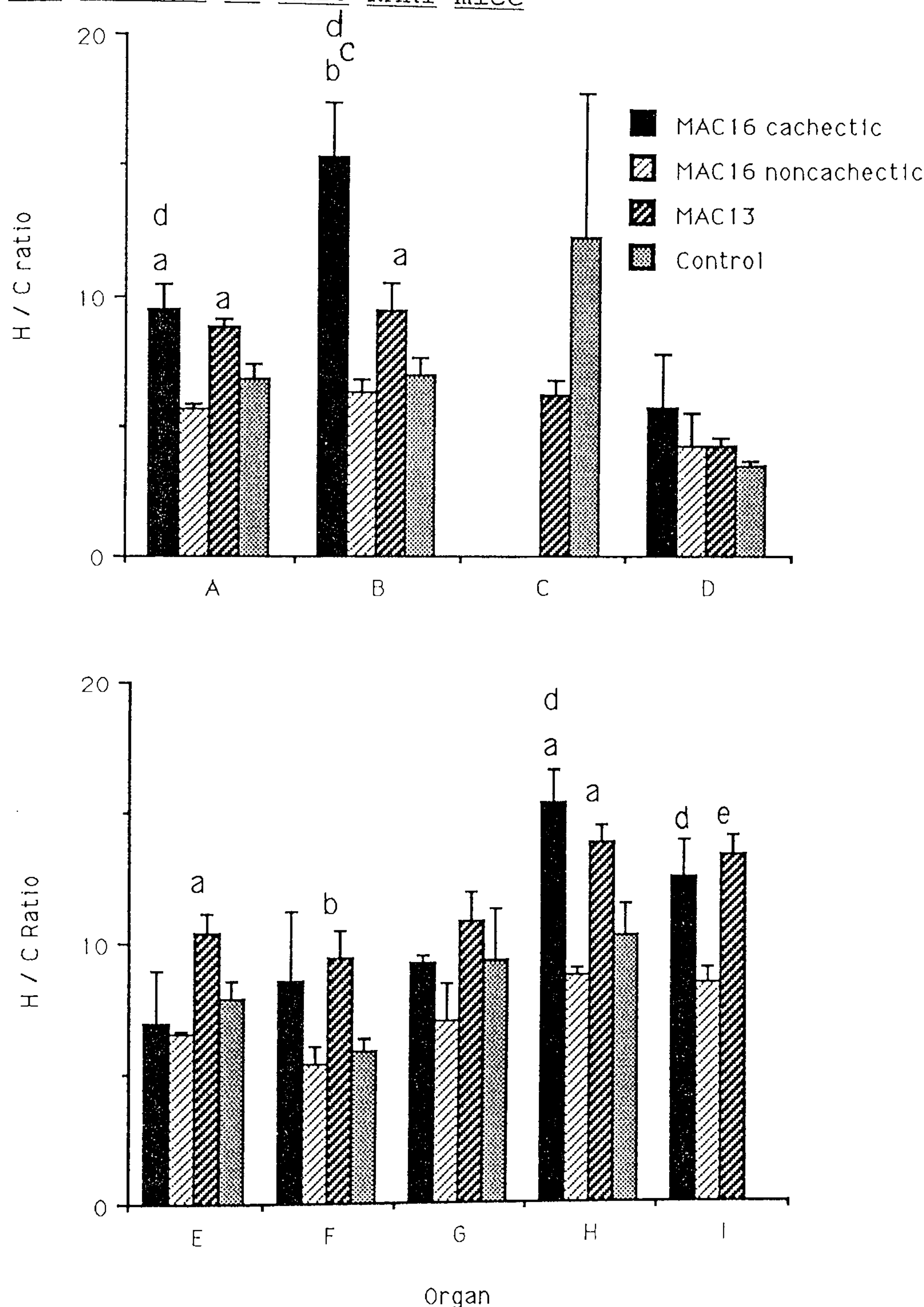
decreased to a greater extent than glucose consumption by the tumour, and glucose consumption was significantly reduced in epididymal fat pads, testes, colon, spleen and kidney, but was not related to the presence of cachexia.

The effect of cachexia on the retention of 2DGP in various organs was investigated using a sequential double-labelling technique followed by an analysis of the two labels in 2DGP. Since there was a marked initial decay of the precursor in the blood (fig 4.8), the bulk of the [^{3}H]2DGP was synthesised in the tissues during the initial 35 min of the labelling period and the ^{3}H/^{14}C ratio of 2DGP was measured at the end of the experiment. Loss of 2DGP from the tissue would therefore affect the ^{3}H component of the ratio more than the ^{14}C component. The ^{3}H/^{14}C ratio of 2DGP in the tissues was a measure of the retention of 2DGP, ie a low ratio indicated a high rate of loss. Since 10-times as much ^{3}H radioactivity was administered as ^{14}C, the ^{3}H/^{14}C ratio would be expected to be near 10. This was true for most control tissues except for lungs, colon, brain and kidney which were much lower (fig 4.11).

The lower ratio for brain has previously been reported (Meszaros, 1987). In cachectic MAC16 tumour-bearing animals, the ^{3}H/^{14}C ratio for testes, lung and brain were significantly higher when compared to control animals and non-cachectic MAC16 tumour-bearing animals. This suggests

Figure 4.11

The effect of tumour burden on the $^{3}H/^{14}C$ ratio of 2DGP in the tissues of male NMRI mice



A=brain, B=lungs, C=liver, D=kidneys, E=spleen, F=colon, G=epididymal fat pads, H=testes, I=tumour

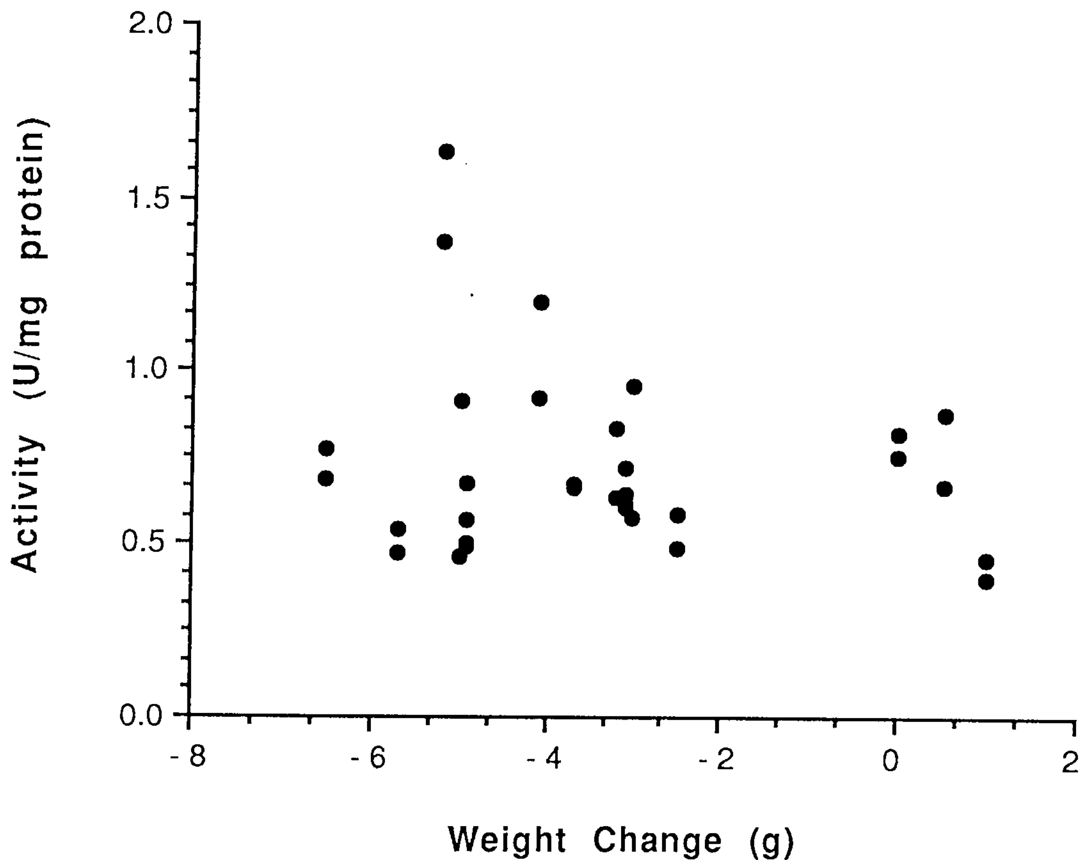
a=$p<0.05$, b=$p<0.01$ from controls, c=$p<0.01$ from MAC13 tumour values, d=$p<0.01$, e=$p<0.001$ from non-cachectic MAC16 tumour values using the Students t-test. Values represent mean $\pm$ S.E.M. of 5 animals per group.

that the tissues of cachectic animals adapt to use metabolic substrates other than glucose.

The enzyme glucose-6-phosphatase is abundant in certain tissues, especially the liver, and it plays a role in maintaining blood glucose levels by hydrolytically cleaving phosphorylated glucose, which cannot readily diffuse out of cells, to glucose which can. The results shown in figs 4.9, 4.10 and 4.11 show that the utilisation and retention of 2DGP by the livers of MAC16 tumour-bearing animals with and without cachexia were extremely low, and were in fact undetectable using this technique. We have studied the activity of glucose-6-phosphatase in the livers of these animals. Glucose-6-phosphatase activity was significantly elevated in the livers of MAC16 tumour-bearing animals compared with MAC13 tumour-bearing animals and non tumour-bearing controls, and the activity was elevated irrespective of weight loss (table 4.6 and fig 4.12). The MAC13 and MAC16 tumours induced profound changes in glucose utilisation by host tissues, particularly in the brain where glucose consumption was severely depressed. Previous studies (Nakamura, et al, 1986) demonstrated that sera and ascites fluid from patients with advanced primary lung and gastric cancers were able to suppress glucose consumption by murine peritoneal exudate macrophages, and that D-fructose-6-phosphate-1-phosphotransferase (the rate limiting enzyme in glycolysis) was the susceptible enzyme.

Figure 4.12

The lack of effect of weight loss on the activity of liver glucose-6-phosphatase



Each point represents the results from an individual animal

Table 4.6

Hepatic glucose-6-phosphatase activity in MAC16 and MAC13 tumour-bearing animals and non tumour-bearing controls

Group	Activity (U/mg protein)
MAC16 tumour bearing animals	0.693 ± 0.038 * **
MAC13 tumour bearing animals	0.541 ± 0.042
Non-tumour bearing animals	0.593 ± 0.041

* = $p<0.005$ from control values

**= $p<0.005$ from MAC13 tumour bearing values

using the Students t-test

Results represent mean ± S.E.M. of 8 - 15 animals per group

Table 4.7

Inhibition of phosphofructokinase activity by sera from MAC16 and MAC13 tumour-bearing animals and non tumour-bearing controls

Group	Percentage inhibition of activity
MAC16 serum	-12.0% ± 2.2
MAC13 serum	-7.7% ± 3.9
Control serum	-11.2% ± 1.7

Values represent the mean ± S.E.M. of 7 - 10 determinations per group

We investigated the effect of sera from MAC16 and MAC13 tumour-bearing animals and from non tumour-bearing animals on the activity of phosphofructokinase.

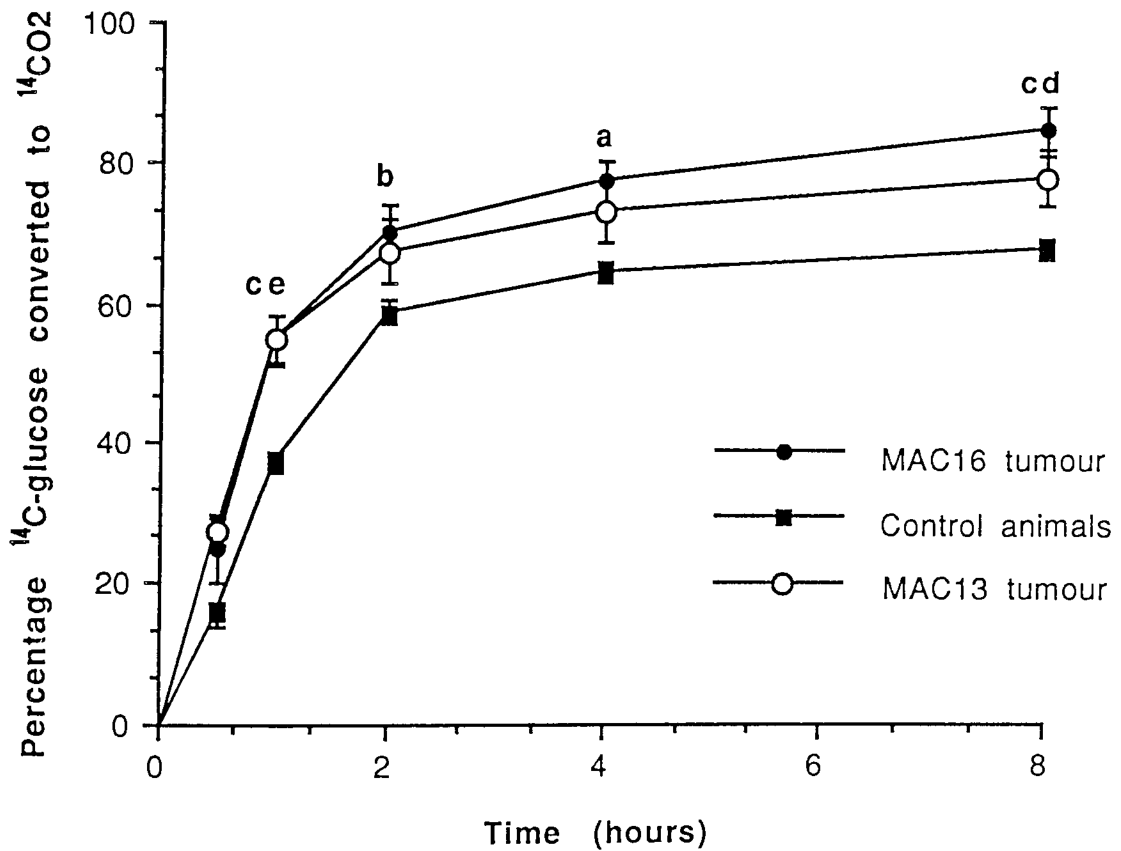
Sera from all 3 groups of animals inhibited the activity of the enzyme in this assay system (table 4.7) but there was no significant difference in the degree of inhibition between the three groups.

Conversion of D-[U-^{14}C]-glucose to $^{14}CO_2$ was higher in tumour-bearing (MAC13 and MAC16) than in non tumour-bearing animals at all time points examined with a higher total conversion over the 8 hour period (fig 4.13). There was no difference in glucose oxidation between cachectic and non-cachectic animals.

Although these results suggest that glucose oxidation is increased in the tumour-bearing state, it is important to remember that a bolus injection of U-[^{14}C]-glucose with measurement of $^{14}CO_2$ production does not strictly measure glucose oxidation. Re-cycling of the label, and its transfer to other substrates such as lipids must also be considered, since they may contribute to the amount of $^{14}CO_2$ produced.

Figure 4.13

Oxidation of U-[^{14}C]-glucose in vivo by MAC16 and MAC13 tumour-bearing animals and non tumour-bearing controls



a=p<0.05, b=p<0.01, c=P,0.005 for MAC16 values from controls, d=p<0.05, e=p<0.005 for MAC13 values from controls using Students t-test

Results are expressed as mean ± S.E.M. of 6 animals per group

4.3.3 Discussion

Glucose is an important metabolic substrate, especially for solid tumours (Nolop, et al, 1987) in which a poor vascularisation would prevent oxidative metabolism. In this study, respiration from glucose was elevated in tumour-bearing animals irrespective of the development of cachexia, probably due to glucose consumption by the tumour. This correlated well with the tissue glucose consumption rates, in which it was observed that glucose consumption by the colon adenocarcinomas of the MAC series was high (0.7 - 1.1 $\mu molg^{-1}min^{-1}$) and comparable with that of the brain (0.8$\mu molg^{-1}min^{-1}$). The rate of glucose utilisation by the MAC16 tumour was, however, lower than the MAC13 tumour and independent of the production of cachexia in the host. This suggests that the high glucose consumption by the tumour is not sufficient to explain host body weight loss. The tumour-bearing state induced changes in glucose utilisation by host organs, especially the brain, where glucose utilisation was severely depressed. Although other workers (Nakamura, et al, 1986) have shown that sera from cancer patients had an inhibitory action on D-fructose-6-phosphate-1-phosphotransferase, sera from MAC13 and MAC16 tumour-bearing animals did not significantly inhibit the activity of this enzyme.

Blood glucose levels were depressed in MAC16 tumour-bearing

animals regardless of the development of cachexia. This may indicate a failure of the mechanisms of glucose homeostasis to maintain normoglycaemia. The observed increase in liver glucose-6-phosphatase in MAC16 tumour-bearing animals may represent an attempt by the host to restore blood glucose levels.

The results from this investigation into glucose metabolism suggest that glucose is an important substrate for tumour metabolism, and that alterations in glucose metabolism in host tissues, especially the brain are induced in the tumour bearing host. There were, however, no indications of how glucose utilisation by the tumour could account for the development of cachexia in the host. In fact, since retention of 2DGP was high in cachectic MAC16 tumour-bearing animals, this suggests that glucose utilisation is actually reduced in this model of cachexia. This reduction in glucose utilisation must be accompanied by an alteration in the preference of the substrate for energy production, and this will be investigated in the following sections.

4.4 Lipid utilisation by tumour and non tumour-bearing animals in vivo

4.4.1 Introduction

Warburg in 1930 observed that slices of tumours arising from diverse tissues had one common property; they all utilised glucose readily and produced large quantities of lactate. According to Warburg, this persistence of glycolysis was an expression of impaired respiration and a compensatory mechanism for providing energy. This theory drew attention away from fatty acids as a metabolic fuel of cancer cells. The presence of mitochondria in tumours and the occurrence of the citric acid cycle awakened interest in the possibility that fatty acids may be a fuel for cancer cells (Weinhouse, 1951) and at least some tumour cells have been shown to possess the enzymatic ability to utilise fatty acids (Medes, and Weinhouse, 1958 and Weinhouse, et al, 1953).

Depletion of host adipose stores is a common finding in cancer cachexia. Anorexia alone is not sufficient to account for this fat depletion as shown by the results from pair-feeding experiments (Lundholm, et al, 1981). Hyperlipidaemia has been reported in some models of cachexia (Devereux, et al, 1982), but plasma levels of free fatty acids (FFA) and triglycerides are reduced in the

MAC16 model of cachexia (Mahony, et al, 1988), possibly due to an elevated level of lipoprotein lipase in skeletal muscle (Briddon, et al, 1991). This suggests that the fatty acids liberated during excessive lipid breakdown in this model are rapidly oxidised.

The FFA liberated during the cachectic process may also be required to maintain tumour growth. Mobilisaton of fat stores as a result of acute fasting (Sauer and Dauchy, 1987a) and streptozocin-induced diabetes (Sauer and Dauchy, 1987b) stimulate tumour growth and inhibition of host fat mobilisation in cancer cachexia is associated with inhibition of tumour growth (Tisdale and Beck, 1991). These observations suggest that tumour growth _in vivo_ may be limited by substances present in host fat stores, and provides further evidence for the theory that fatty acids are important fuels in some neoplastic processes.

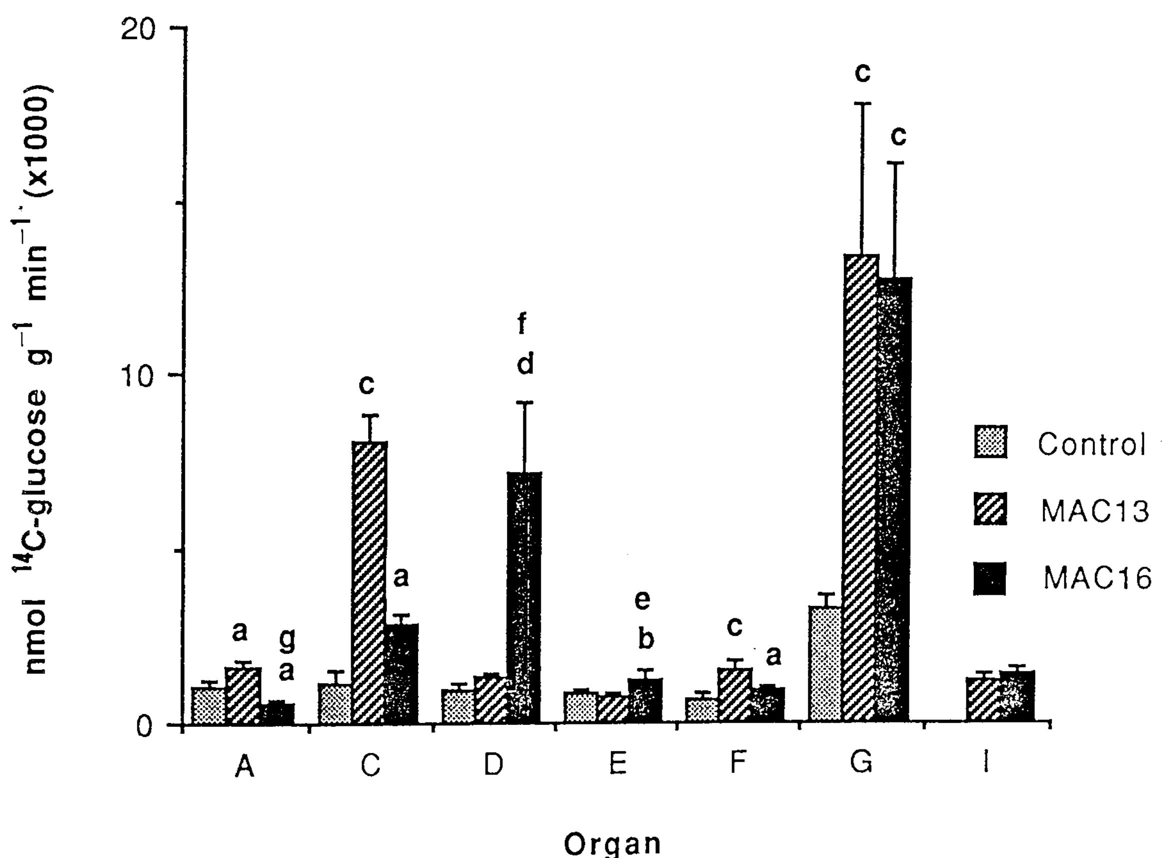
4.4.2 Results

4.4.2.1 Lipogenesis in tumour and host tissues in mice bearing colonic adenocarcinomas

In agreement with the _in vitro_ assay, (section 4.2), there was no difference between the rates of lipogenesis of the MAC16 and MAC13 tumours _in vivo_, using either [U-^{14}C]glucose (fig 4.14) or $^{3}H_2O$ (fig 4.15). The tumour-

Figure 4.14

Lipogenesis from U-[^{14}C]glucose in various organs in tumour and non tumour-bearing male NMRI mice

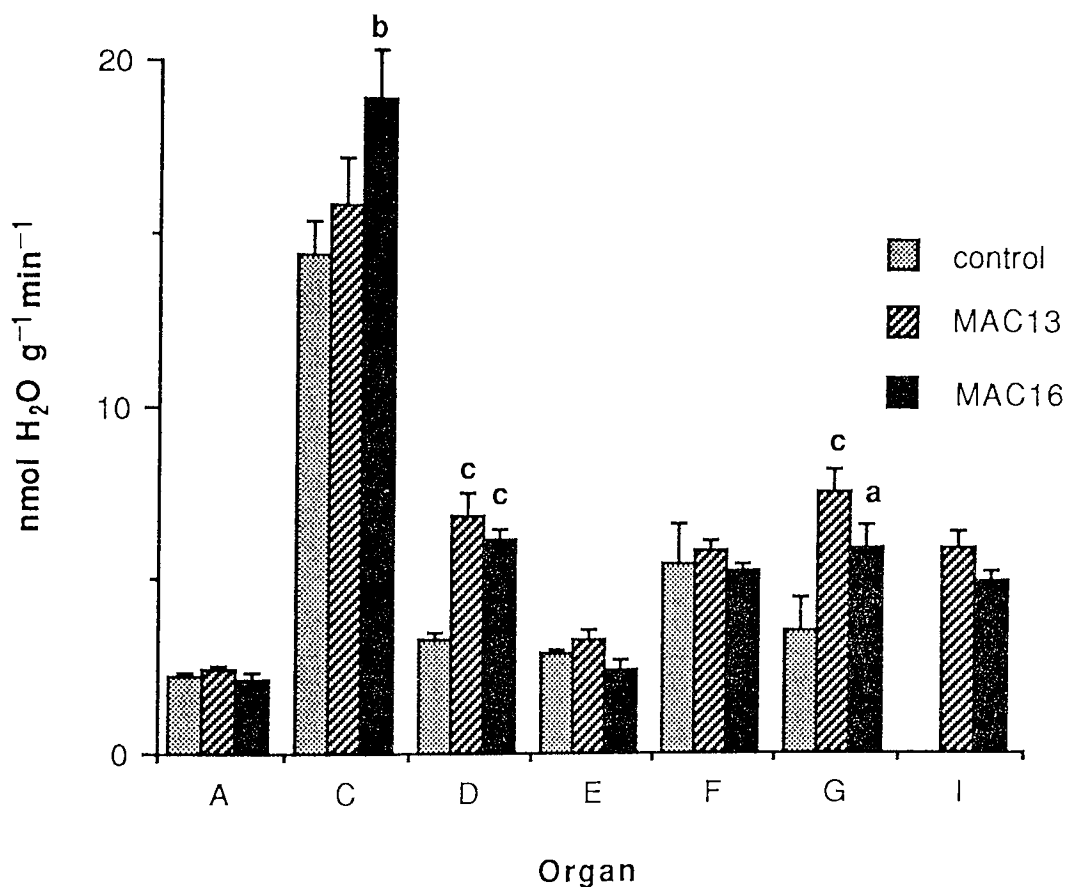


A=brain, C=liver, D=kidneys, E=spleen, F=colon, G=epididymal fat pads, I=tumour

a=$p<0.05$, b=$p<0.01$, c=$p<0.005$, d=$p<0.001$ from controls, e=$p<0.05$, f=$p<0.01$, g=$p<0.005$ from MAC13 tumour bearing values using Students t-test. Results are expressed as mean $\pm$ S.E.M. of 6 animals per group

Figure 4.15

Lipogenesis from ^3H_2O in various organs in tumour and non tumour-bearing male NMRI mice



A=brain, C=liver, D=kidneys, E=spleen, F=colon, G=epididymal fat pads, I=tumour

A=p<0.05, b=p<0.01, c=p<0.005 from control values using the Students t-test.

Results represent mean ± S.E.M. of 10 animals per group

bearing state produced profound alterations in the rate of lipogenesis in specific organs. In particular, the rates of lipogenesis were significantly elevated in the liver and epididymal fat pads in the tumour-bearing state using [U-^{14}C]glucose (fig 4.14) and in the kidney and epididymal fat pads using $^{3}H_2O$ (fig 4.15) and there was no significant difference in the extent of induction of lipogenesis in these organs between the cachexia-inducing MAC16 tumour and the MAC13 tumour. Using [U-^{14}C]glucose, lipogenesis was significantly increased in the kidneys of animals bearing the MAC16 tumour (fig 4.14) and using $^{3}H_2O$, lipogenesis was significantly elevated in the liver of animals bearing the MAC16 tumour (fig 4.15), while there was no significant elevation in animals bearing the MAC13 tumour. There appears to be a stimulation of the conversion of glucose to lipid in the tumour-bearing host, and for the fat pads this is irrespective of the development of cachexia.

The activity of acetyl-CoA carboxylase in the livers of control, non tumour-bearing animals and in animals bearing the MAC16 and MAC13 tumours after maximum stimulation by citrate is shown in table 4.8. In neither tumour type was the level of acetyl-CoA carboxylase increased above controls, nor was there any difference in the levels of citrate in the liver in the 3 groups (table 4.8).

Table 4.8

Hepatic citrate levels and the activity of acetyl CoA carboxylase in the livers of MAC16 and MAC13 tumour-bearing animals and non tumour-bearing controls

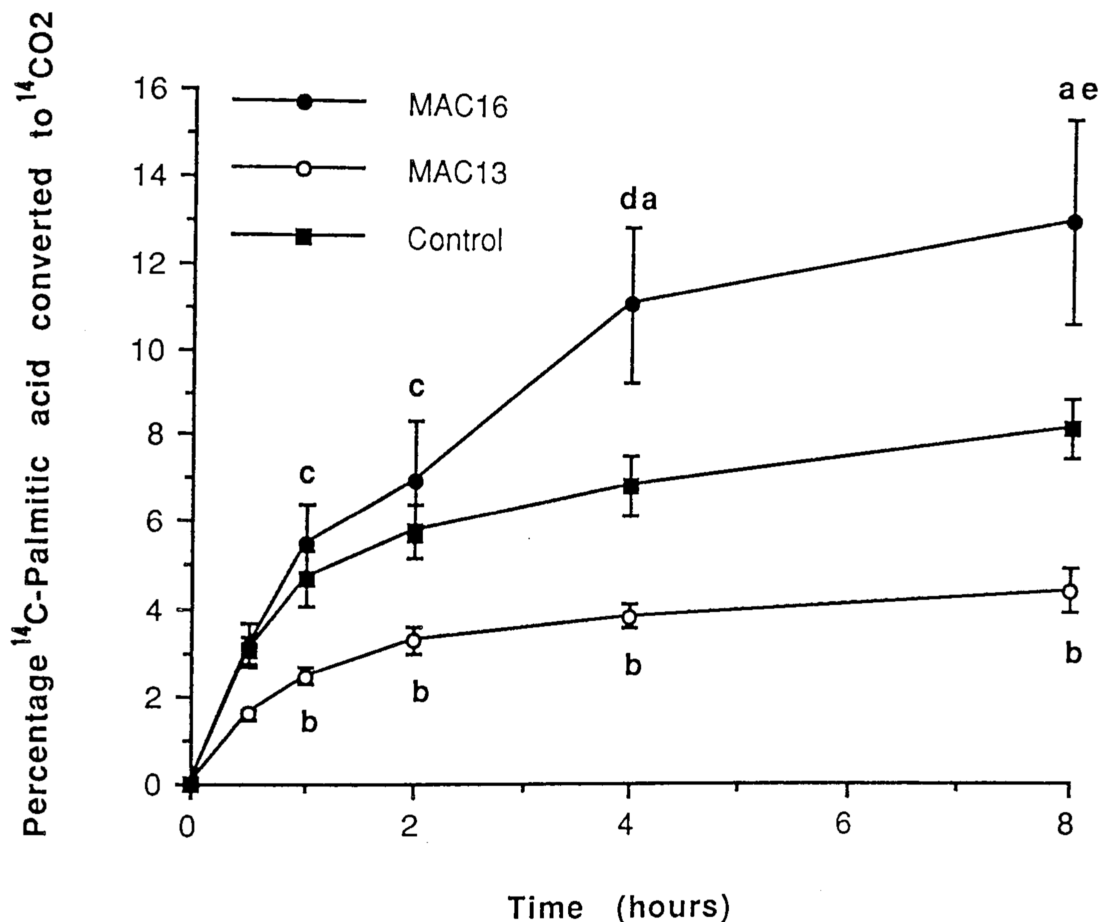
Group	Acetyl CoA carboxylase µmol malonyl CoA/min /mg protein	Citrate µmol/g
MAC16 tumour bearing animals	0.016 ± 0.001	0.210 ± 0.010
MAC13 tumour bearing animals	0.018 ± 0.002	0.216 ± 0.010
Non-tumour bearing animals	0.014 ± 0.001	0.234 ± 0.012

Results represent mean ± S.E.M. of 6 animals per group

* = $p<0.05$ from control values

Figure 4.16

Oxidation of U-[^{14}C]palmitic acid in vivo by tumour and non tumour-bearing male NMRI mice



a=$p<0.05$, b=$p<0.005$ from controls, c=$p<0.05$, d=$p<0.005$, e=$p<0.001$ MAC16 values from MAC13 values using the Students t-test.

Values represent mean $\pm$ S.E.M. of 6 animals per group

4.4.2.2 Production of $^{14}CO_2$ from [U-^{14}C]palmitic acid

Conversion of [U-^{14}C]palmitic acid to $^{14}CO_2$ in non tumour-bearing controls and in animals bearing the MAC16 and MAC13 tumours was significantly different between the 3 groups (fig 4.16). Animals bearing the MAC13 tumour, which does not induce weight loss, had a significantly ($p<0.005$) lower initial rate of conversion and a lower total conversion of [U-^{14}C]palmitic acid to $^{14}CO_2$ than non tumour-bearing animals. Animals bearing the MAC16 tumour (weight loss 2 - 4g) had a higher initial rate of conversion and a greater total conversion ($p<0.05$) of [U-^{14}C]palmitic acid to $^{14}CO_2$ than non tumour-bearing animals. Respiration from palmitate was significantly higher in animals bearing the MAC16 tumour than in animals bearing the MAC13 tumour at all time points examined. For all groups, conversion of palmitate to CO_2 was lower than the conversion of glucose to CO_2 over the 8h time period (section 4.3, fig 4.13).

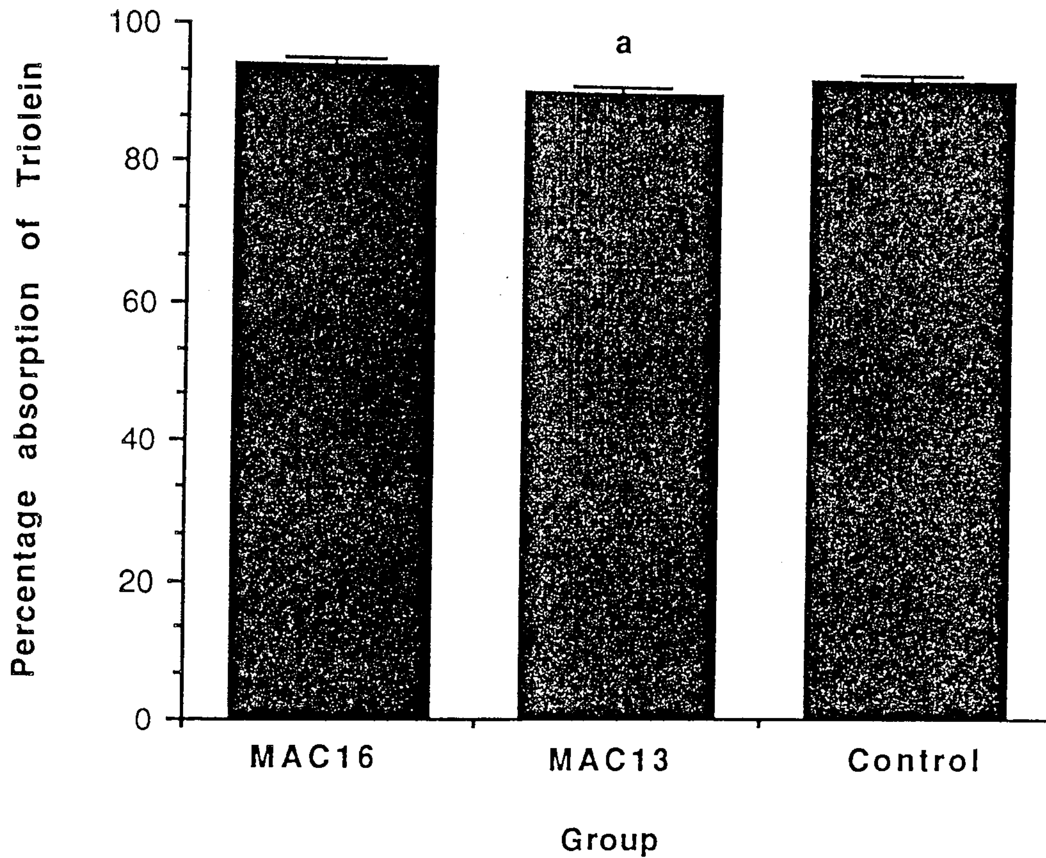
True values of respiration from palmitate would not be strictly measured by a bolus injection of U-[^{14}C]-palmitate. Infusion of $NaH^{14}CO_3$ and measurement of the percentage recovery of $^{14}CO_2$ would account for labelled CO_2 being re-synthesised into free fatty acids rather than being exhaled via the lungs, and this requires further investigation.

4.4.2.3 Lipid oxidation and tissue lipid accumulation

Absorption of an oral dose of [1-^{14}C]triolein was not significantly different in MAC16 tumour-bearing animals

Figure 4.17

Absorption of an oral dose of $[1\text{-}^{14}C]$-triolein

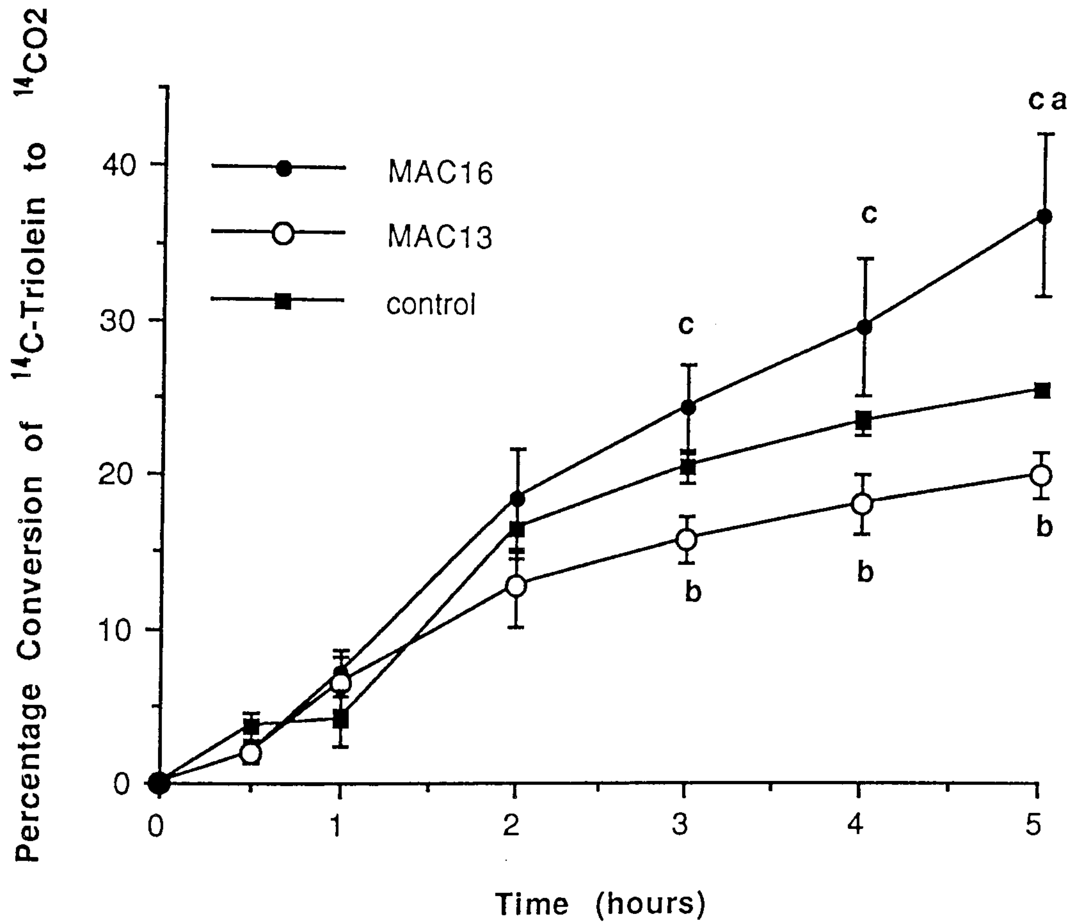


Results represent mean ± S.E.M. of 6 animals per group

a=$p<0.05$ from controls using the Students t-test

Figure 4.18

Oxidation of [1-^{14}C]triolein in vivo by tumour and non tumour-bearing male NMRI mice

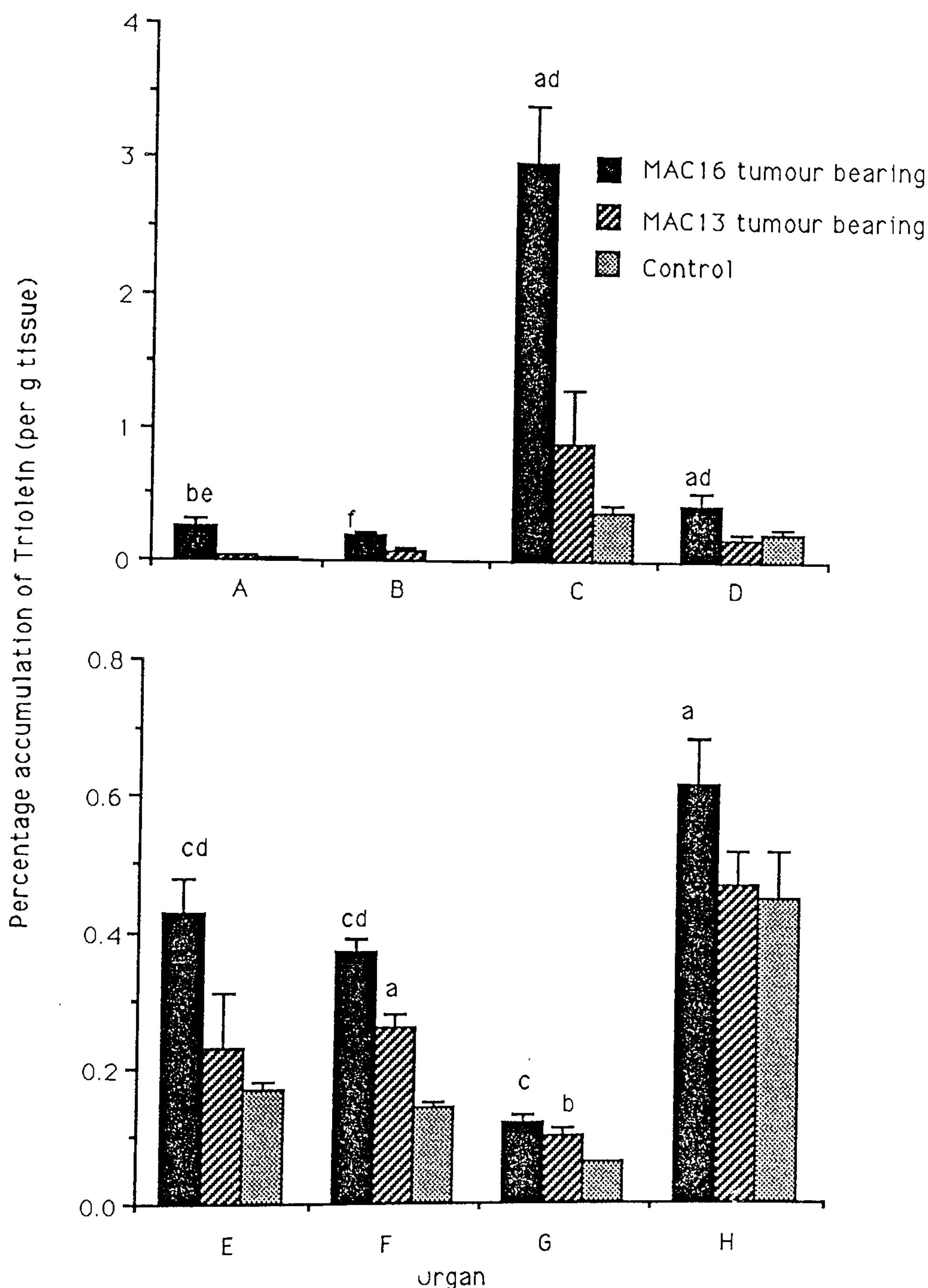


a=p<0.05 MAC16 values from control, b=p<0.05, MAC13 values from control, c=p<0.05 MAC16 values from MAC13 values

Results represent mean ± S.E.M. of 6 animals per group

Figure 4.19

Tissue lipid accumulation in tumour and non tumour-bearing male NMRI mice



A=plasma, B=tumour, C=liver, D=epididymal fat pads, E=thigh muscle, F=gastrocnemius muscle, G=brain, H=heart

a=p<0.05, c=p<0.005, c=p<0.001 from controls, d=p<0.05, e=p<0.005, f=p<0.001 MAC16 values from MAC13 values

Results represent mean ± S.E.M. of 6 animals per group

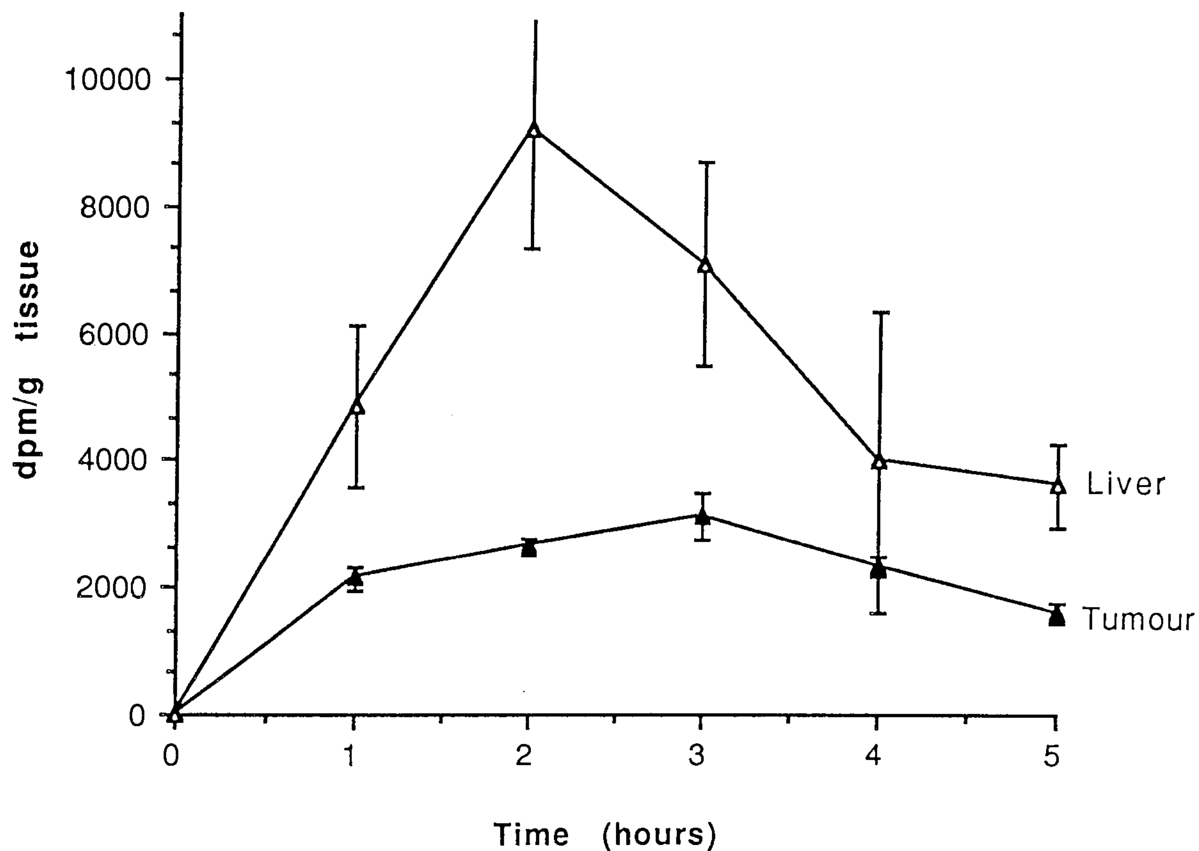
when compared with non tumour-bearing controls, while animals bearing the MAC13 tumour had a small but significant ($p<0.05$) reduced lipid absorption over the 5h period (fig 4.17).

The rate of oxidation of [1-^{14}C]triolein to $^{14}CO_2$ for the 3 groups was similar to that found for palmitic acid (fig 4.18). Animals bearing the MAC13 tumour had a lower excretion level of $^{14}CO_2$ than non tumour-bearing animals ($p<0.05$), while animals bearing the MAC16 tumour had a significantly ($p<0.05$) elevated rate of excretion (fig 4.18). The pattern of distribution of labelled lipid between tumour and host organs also differed between the 3 groups (fig 4.19). Heart, muscle, liver and adipose tissue labelled lipids were significantly elevated in animals bearing the MAC16 tumour when compared either with animals bearing the MAC13 tumour or with non tumour-bearing controls. The level of labelling of brain lipids was higher in both tumour-bearing states and there was no difference in urinary and faecal output in the 3 groups. Labelled tumour lipid levels were significantly ($p<0.001$) increased in the MAC16 tumour compared with the MAC13 tumour. The level of labelled lipids in plasma, liver, heart and adipose tissue in animals bearing the MAC13 tumour were not significantly different from that in non tumour-bearing controls.

The accumulation of triolein in the liver of MAC16 tumour-bearing animals reached a maximum 2h after administration of an oral dose of [1-^{14}C]triolein and then

Figure 4.20

Acummulation of [1-^{14}C]triolein in liver and tumour over time in MAC16 tumour-bearing animals



Results represent mean ± S.E.M. of 5 animals

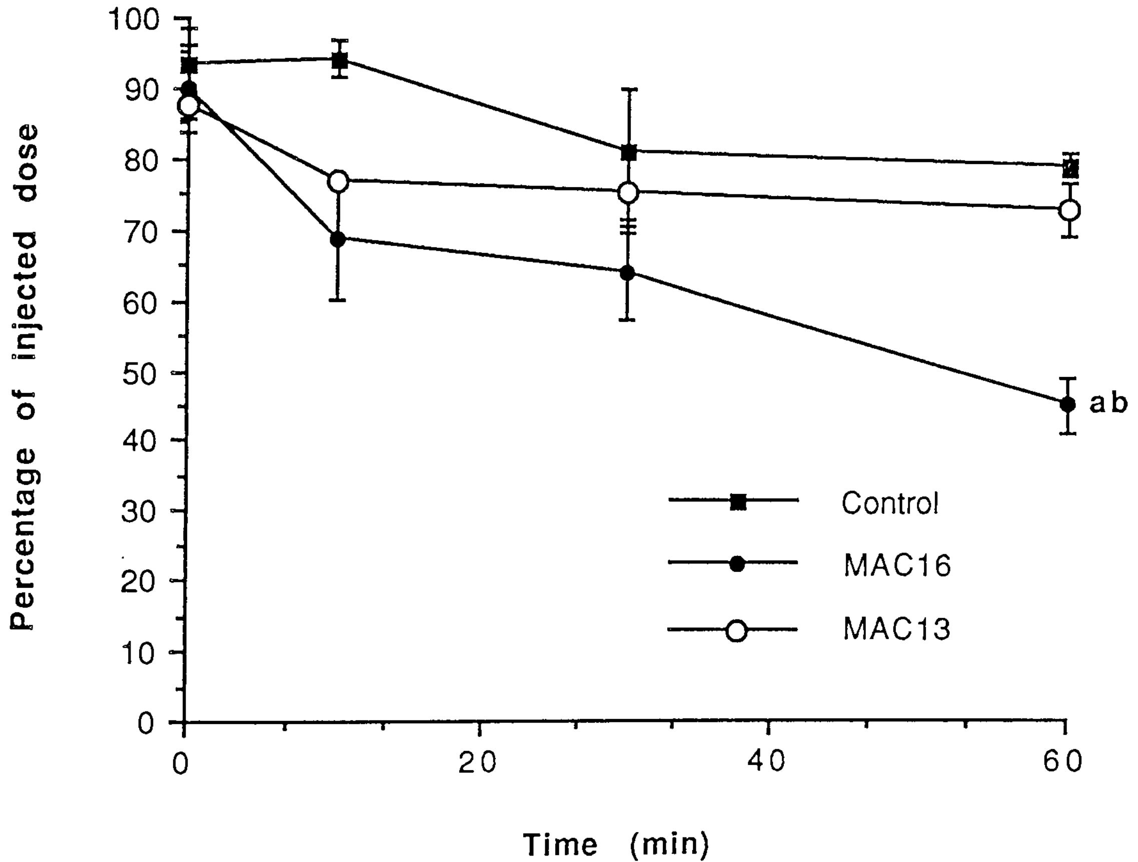
decreased over the rest of the 5h period (fig 4.20). Accumulation of radioactivity in the MAC16 tumour reached a maximum 3h after administration of the triolein and began to decrease over the next 2h (fig 4.20).

4.4.2.4 Lipid mobilisation from direct injection of tracer into epididymal fat pads

In order to understand further the path taken by lipids from host adipose tissue in the cachectic animal, [U-^{14}C]palmitic acid complexed to albumin was directly injected into the epididymal fat pads. The rate of disappearance of triacylglycerol fatty acid radioactivity was significantly greater in MAC16 tumour-bearing animals when compared with either MAC13 tumour-bearing animals or non tumour-bearing controls (fig 4.21). The amount of labelled lipid in the fat pads of control and MAC13 tumour-bearing animals did not change appreciably during the 60min period after injection, whereas there was a reduction of approximately 50% in animals bearing the MAC16 tumour. Incorporation of radioactivity into the liver at 60min was lower in both sets of tumour-bearing animals than in non tumour-bearing controls, and incorporation into the brain was higher (table 4.9). Incorporation of radioactivity into muscle was significantly greater in

Figure 4.21

The rate of loss of radioactivity from [U-^{14}C]palmitate labelled epididymal fat pads



a=$p<0.05$ from controls, b=$p<0.05$ from MAC13 values using Students t-test

Results represent mean ± S.E.M. of 3 animals per time point per group

Table 4.9

The distribution of U-[^{14}C]palmitate 60min after direct injection into the epididymal fat pads

	Organ Lipid Accumulation (% of injected dose)			
Group	Muscle	Liver	Brain	Tumour
Control	1.0±0.1	3.9±0.6	0.5±0.1	--
MAC16	1.7±0.1**+	2.1±0.4*	1.7±0.1***	1.7±0.1+
MAC13	0.8±0.1	1.7±0.6*	1.5±0.1**	0.1±0.1

Values represent ^{14}C-labelled lipid (percentage of injected dose) per organ 60min after direct injection of [U-^{14}C]palmitate into epididymal adipose tissue.
Results are expressed as mean ± S.E.M. of 6 animals per group
*=p<0.05, **=p<0.005 and ***-p<0.001 from controls
+=p<0.001 from MAC13 values using the Students t-test
Muscle value represents combined values for thigh plus gastrocnemius muscle from the left leg

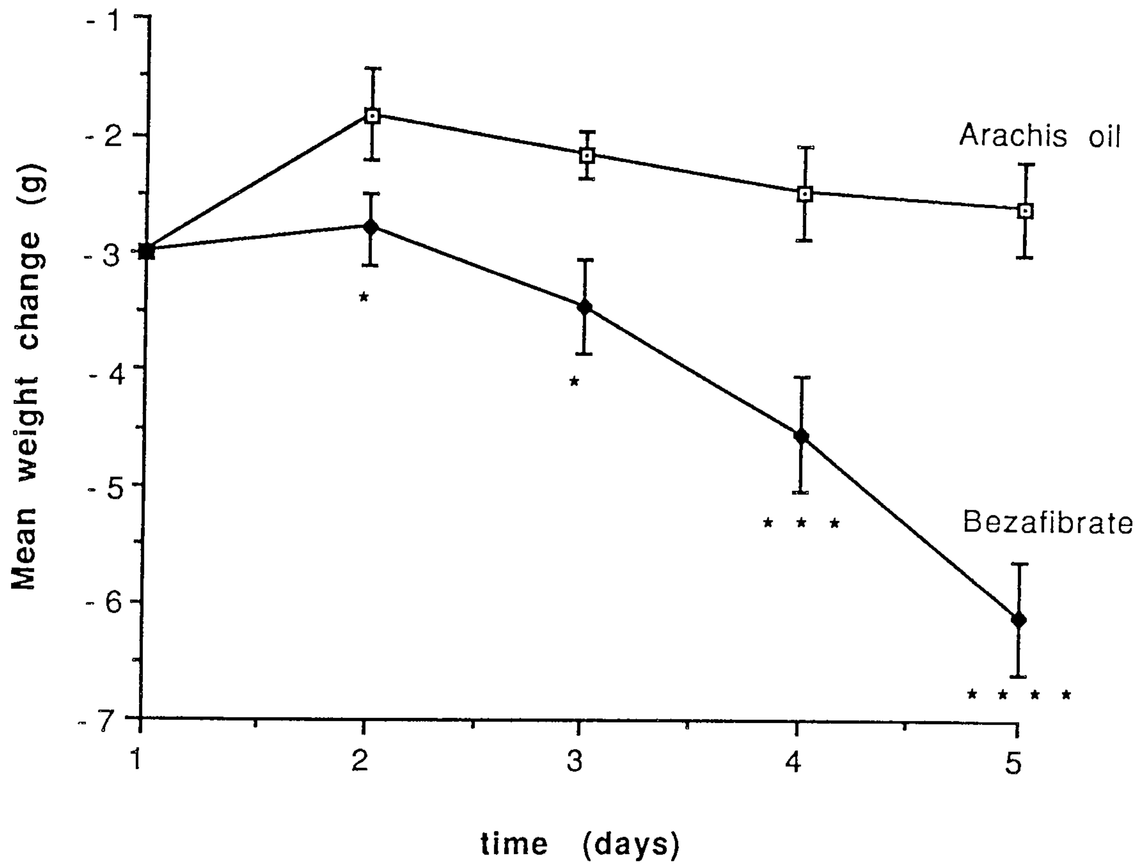
animals bearing the MAC16 tumour than in either control animals or those bearing the MAC13 tumour. The level of [^{14}C]lipid was significantly ($p<0.001$) higher in the MAC16 tumour than in the MAC13 tumour.

4.4.2.5 The effect of lipid lowering drugs on tumour growth and host weight loss

Administration of bezafibrate, a lipid lowering drug, resulted in a significant stimulation of tumour growth (fig 4.22) which was accompanied by a significant acceleration of weight loss in these animals when compared with solvent treated controls (fig 4.23). In contrast, bezafibrate had no effect on the growth of the MAC13 tumour *in vivo* (fig 4.24), nor did it cause any stimulation of the MAC16 cells *in vitro* (fig 4.25). Thus the stimulatory effect of bezafibrate was confined to the MAC16 tumour *in vivo* and may be related to lipid uptake by the tumour. To investigate this, the effect of bezafibrate on the accumulation of an oral dose of [^{14}C]triolein was examined. Accumulation of radioactivity was significantly increased in the heart, gastrocnemius muscle and tumour of MAC16 tumour-bearing animals after administration of bezafibrate when compared to a control group of MAC16 tumour-bearing animals (table 4.10).

Figure 4.22

The effect of bezafibrate on weight loss in MAC16 tumour-bearing animals

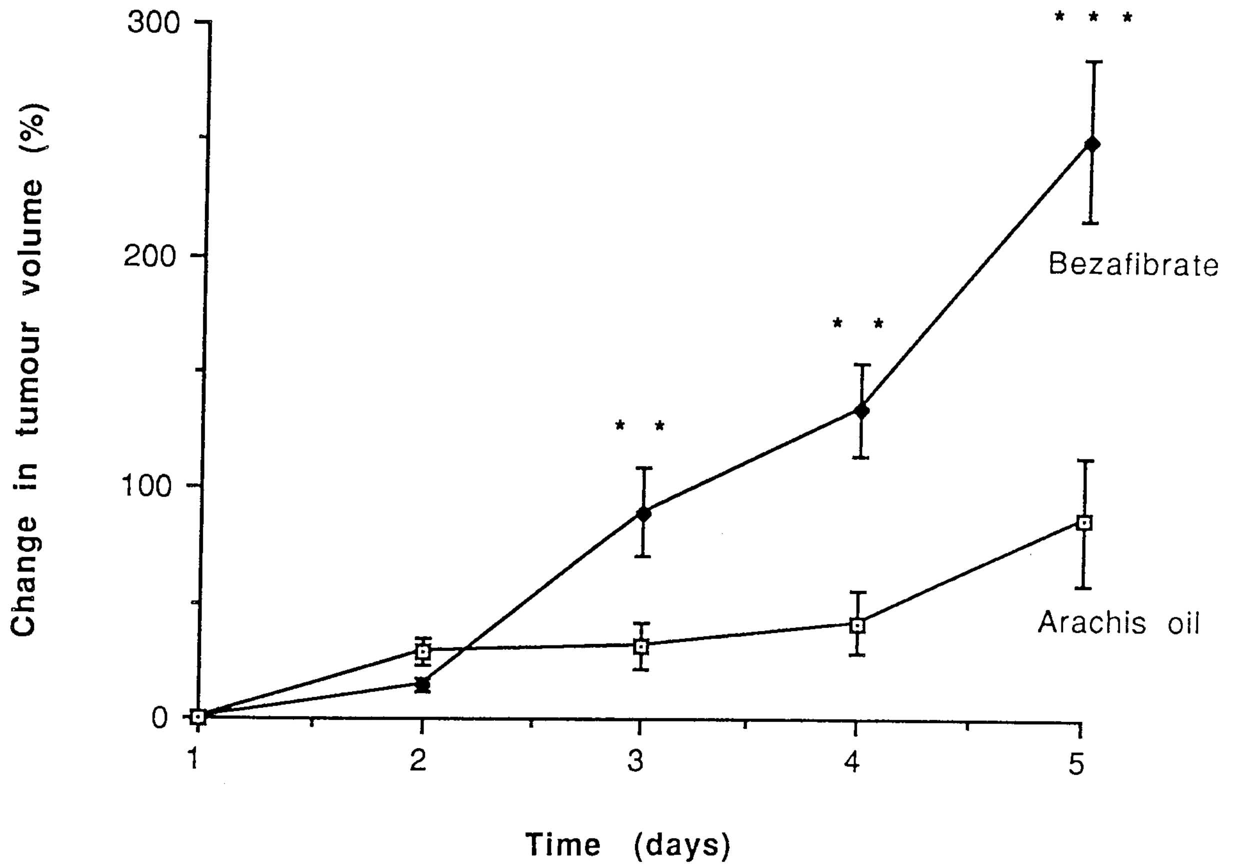


*=$p<0.05$, ***=$p<0.005$, ****=$p<0.001$ from control values using the Students t-test

Results represent mean ± S.E.M. of 5 male NMRI mice per group

Figure 4.23

The effect of bezafibrate on MAC16 tumour growth in vivo

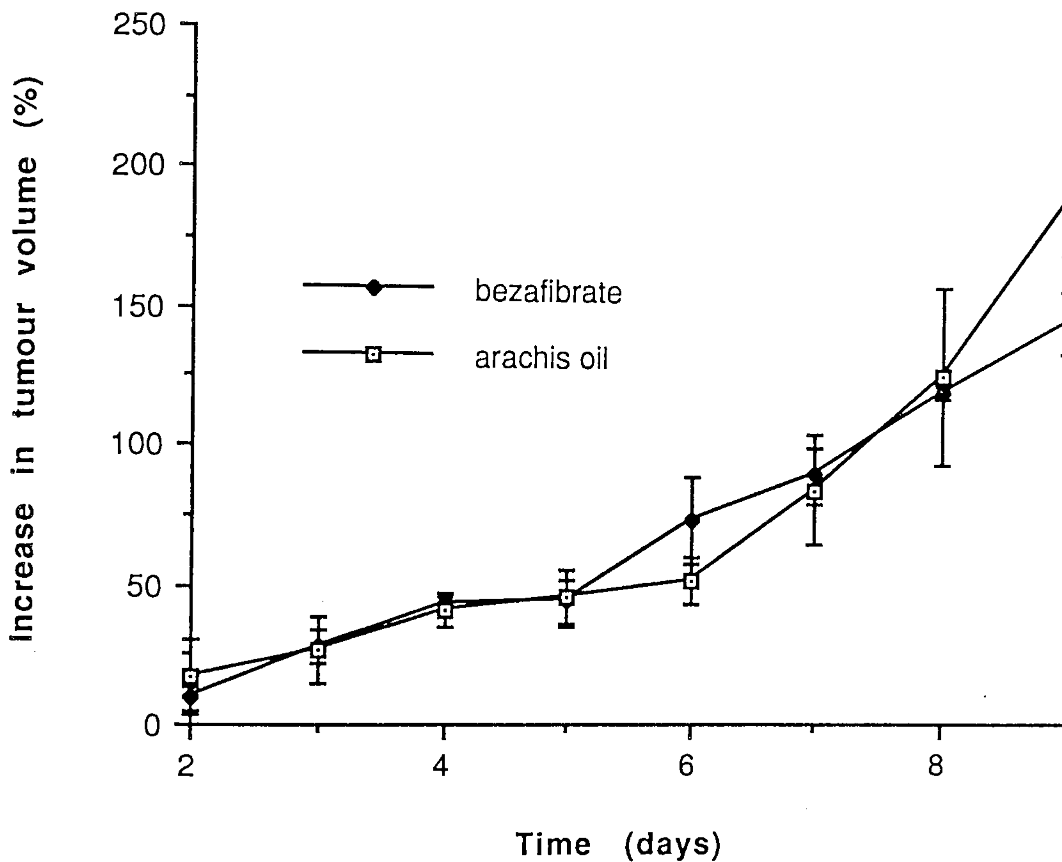


**=p<0.01, ***=p<0.005 from control group using the Students t-test.

Results represent mean ± S.E.M. of 5 male NMRI mice per group

Figure 4.24

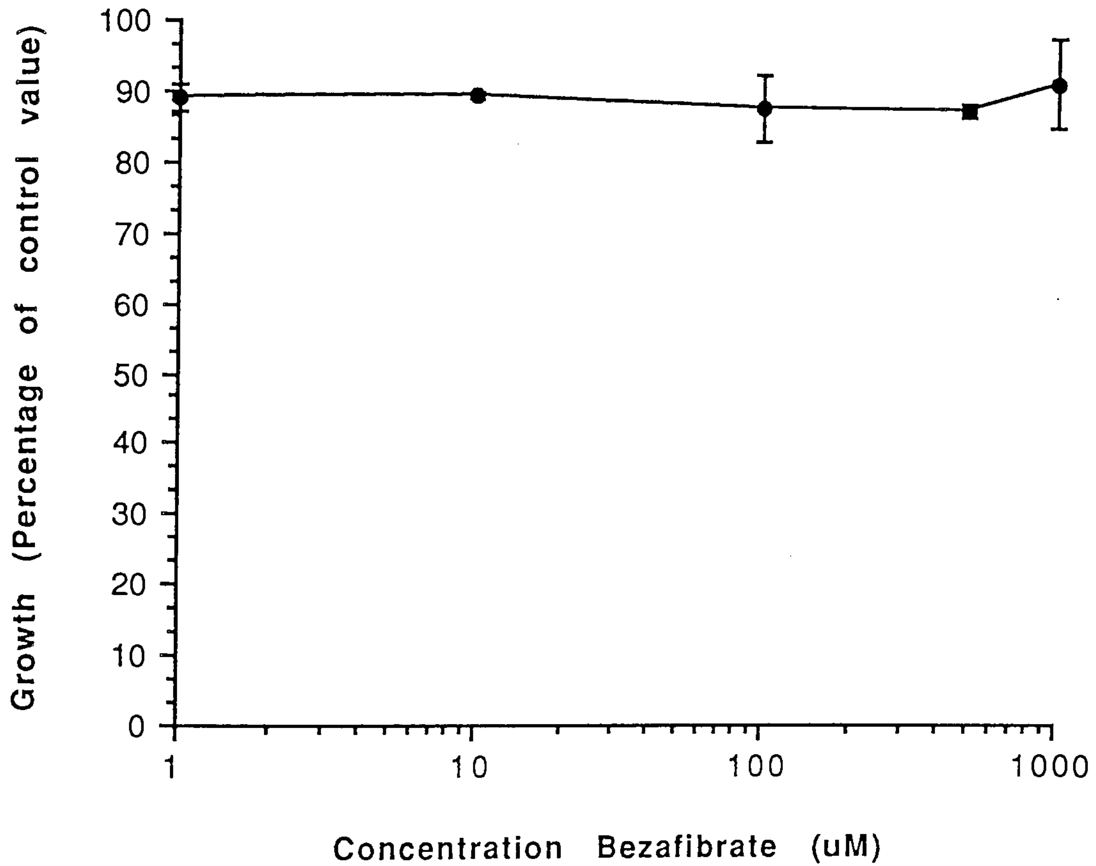
The effect of bezafibrate on MAC13 tumour growth in vivo



Results represent mean ± S.E.M. of 5 animals per group

Figure 4.25

The effect of bezafibrate on MAC16 cell growth in vitro



Results represent mean ± S.E.M. of 3 determinations per concentration of bezafibrate

Table 4.10

The effect of bezafibrate on the accumulation of an oral dose of [1-^{14}C]triolein in MAC16 tumour-bearing animals

Organ	% accumulation/g tissue of ^{14}C-lipid	
	Bezafibrate	Control
Brain	0.29 ± 0.02	0.27 ± 0.01
Heart	1.27 ± 0.09 *	0.96 ± 0.10
Liver	1.02 ± 0.16	0.70 ± 0.08
Fat	1.09 ± 0.41	0.81 ± 0.11
Thigh muscle	0.73 ± 0.07	0.68 ± 0.03
Gastrocnemius muscle	0.83 ± 0.05 *	0.70 ± 0.01
Tumour	0.26 ± 0.03 *	0.18 ± 0.02
Blood	0.15 ± 0.02	0.21 ± 0.04

*=$p<0.05$ from control values using the Students t-test

Results represent mean ± S.E.M. of 5 animals per group

4.4.3 Discussion

Alterations in the pattern of fuel utilisation with lipid sources predominating has been suggested to occur in patients with cancer (Young, 1977). Fat is a high energy fuel source, and it becomes important when the metabolic demands of an organism are high, as has been shown in septic patients with and without cancer (Levinson, et al, 1988). Resting metabolic rates have been shown to be elevated in cancer patients in some studies (Warnold, et al, 1976) and studies using the MAC16 tumour suggest an increase in metabolic rate, since there is an increase in oxygen consumption and an increased activity of brown adipose tissue during the period of weight loss (N. Rothwell, personal communication).

Unlike previous reports which have documented either no change (Thompson and Koons, 1981 and Lanza-Jacoby, et al, 1982) or a decrease (Lanza-Jacoby, et al, 1984) in the liver lipogenic capacity in the tumour-bearing state, we have found an increase in lipogenesis in host liver, kidney and epididymal adipose tissue in animals bearing either the cachexia-inducing MAC16 tumour, or the MAC13 tumour, using either D-[U-^{14}C]glucose or $^{3}H_2O$ as substrate. Whatever the original source of the precursor, the results were substantially the same.

Acetyl-CoA carboxylase catalyses the first committed and rate limiting step in fatty acid biosynthesis and is activated by citrate and inhibited by long chain fatty acetyl-CoA (Guynn, et al, 1972). Previous work has shown that liver fatty acetyl-CoA is reduced in animals bearing the MAC16 and MAC13 tumours (Tisdale and Leung, 1988). Although increased levels of citrate in the liver have been reported (McAllister, et al, 1982), we have been unable to detect differences in the levels of citrate in animals bearing either the MAC16 or MAC13 tumour from that found in control animals. There was also no difference in the activity of acetyl-CoA carboxylase in the livers of tumour-bearing mice and control mice after maximum stimulation with citrate. This suggests that both the increased activity of fatty acid synthase (Tisdale and Leung, 1988) and changes in the levels of regulatory metabolites may be important in maintaining a high rate of lipogenesis. The increased lipogenesis appears not to be specific to the cachectic state, but is more related to the presence of a tumour in an animal. There is also no difference in the rate of lipogenesis between the MAC16 and MAC13 tumours either _in vitro_ or _in vivo_, and although both tumours are capable of _de novo_ synthesis, both induce an increased rate of lipogenesis in host organs. This suggests that the lipid requirements of the tumour may exceed its biosynthetic capacity, or that the tumour-bearing state raises the requirement of host tissues

for lipids. An important consequence of the increase in lipogenesis is that if glucose or other carbohydrate sources are first converted into fat before being used to meet the energy requirements of the host, then there is a reduction in the amount of utilisable energy obtained from the intake of a given amount of carbohydrate.

Respiration from administered palmitate was specifically increased in cachectic tumour-bearing animals, suggesting that the tissues of such animals are more dependent on lipids as an energy source. We investigated alterations in the ability of the tumour-bearing host to deal with lipids by administering an oral load of triolein to cachectic and non-cachectic tumour-bearing animals. In MAC13 tumour-bearing animals, there was decreased lipid absorption and oxidation compared with non tumour-bearing animals. This resembles the effect of interleukin-1 and tumour necrosis factor alpha on lipid absorption and oxidation in rats (Evans and Williamson, 1988a, Argiles, et al, 1989 and Evans and Williamson, 1988b). In contrast, cachectic animals bearing the MAC16 tumour had normal lipid absorption but an increased oxidation of triolein compared with non tumour-bearing controls. Despite a massive mobilisation of body fat reserves, (Beck and Tisdale, 1987), no increase in serum triglycerides has been detected in MAC16 tumour-bearing animals and this may be attributed to an increase in the level of lipoprotein lipase in heart

and adipose tissue (Briddon, et al, 1991).

The increased requirement for lipid in cachexia was demonstrated after labelling of the adipose tissue in vivo with [U-^{14}C]palmitic acid complexed to albumin. By injecting volumes of labelled lipid less than 0.10ml per 100mg of fat pad, the pad was distended uniformly with very little lipid leakage (Baker, et al, 1984). Leakage of lipid after injection was also controlled by the use of Evans dye with which any leakage could be detected with the use of a magnifying glass. There was a 50% reduction in the radioactivity of fat pads in MAC16 tumour-bearing animals within 1h of injection, and increased accumulation of radioactive lipid in both the muscle and tumour of cachectic animals. The importance of lipids in cachexia was further demonstrated after the administration of bezafibrate, a lipid lowering drug which is thought to act by increasing peripheral uptake of lipids by an action on lipoprotein lipase (Shepherd and Packard, 1986). Bezafibrate stimulated MAC16 tumour growth and weight loss, but had no effect on the growth of the MAC13 tumour. This suggests again that lipids play a role in the growth of the MAC16 tumour and perhaps in the development of cachexia. Bezafibrate stimulated the uptake of ^{14}C-triolein into the tumour, but recent results have suggested that the activity of lipoprotein lipase is low in this tissue and not increased by bezafibrate, although activity in the heart

was doubled (M.J. Tisdale, personal communication). Since lipid uptake was stimulated by bezafibrate, some other mechanism must be occurring to account for this observation. Spector and Soboroff (1971) suggested that clofibrate, another lipid lowering drug was able to increase the uptake of free fatty acids by displacing them from their albumin binding sites. This would make the free fatty acids more available to the tumour cells. The peak of serum FFA in cachectic animals coincides with the maximum effect of bezafibrate in MAC16 tumour-bearing animals, i.e. at 2g weight loss (Briddon, et al, 1991), suggesting levels of free fatty acids must be elevated in the host before bezafibrate can exert an effect on tumour size and weight loss. This correlates well with the findings in this section that bezafibrate had no effect on MAC13 tumour growth nor did it have any effect on MAC16 tumour growth in animals that were not cachectic (M. J. Tisdale, personal communication).

The increased lipid oxidation in the cachectic state together with an elevated glucose oxidation (section 4.3), suggests an increased energy requirement which could be met by an increased energy intake. Although energy intake was increased in MAC13 tumour-bearing animals (section 4.1), it was not increased in MAC16 tumour-bearing animals when compared with non tumour-bearing controls, although the reason for this is not known. The increased metabolic

demand would therefore result in an energy deficiency leading to progressive weight loss.

4.5 Ketone body utilisation by tumour and non tumour-bearing animals

4.5.1 Introduction

Ketone bodies (acetoacetate and D-3-hydroxybutyrate) are metabolised by many mammalian tissues (Williamson, 1981). The enzymes involved in ketone body metabolism have been shown to be most active in the kidney, heart and interscapular brown adipose tissue of the adult rat, suggesting that ketone body utilisation is high in these tissues (Williamson, et al, 1971). Ketone bodies have been shown to replace glucose as the predominant fuel for brain metabolism during starvation (Owen, 1967), which correlates with their ability to act as substrates for oxidation by the brain (Robinson and Williamson, 1980). The liver contains only low activity of 3-oxoacid CoA transferase, whereas the activity of this enzyme has been shown to increase in hepatomas (Fenselau, et al, 1975). In contrast, the Walker 256 carcinosarcoma has been shown to be deficient in 3-oxoacid CoA transferase. The MAC16 tumour was shown to have only low levels of this enzyme when compared with non-involved colon (Tisdale and Brennan, 1986). This suggests that the MAC16 tumour may be unable

to metabolise ketone bodies as effectively as the tissue of origin. Plasma ketone bodies are not elevated in cachectic MAC16 tumour-bearing animals, despite a massive depletion of host lipid stores (Tisdale, et al, 1987). Attempts to reverse cachexia by feeding a high fat diet have produced conflicting views. A high fat ketogenic diet fed to MAC16 tumour-bearing animals was able to reduce host weight loss in proportion to the fat content of the diet and also reduce final tumour weights. The increase in carcass weight was attributed to an increase in fat and non-fat carcass mass (Tisdale, et al, 1987). Fearon et al (1988b) showed that ketogenic diets had no effect on host nitrogen balance or whole body protein synthesis, degradation or turnover rates in weight losing patients with cancer, although the patient's body weight was significantly increased. Systemic ketosis was unable to control cachexia and the growth rate of the Walker 256 carcinosarcoma in rats (Fearon, et al, 1985). Although some workers have shown that infusion of ketone bodies is able to spare lean body mass during starvation (Pawan and Semple, 1983), others have suggested that conservation of protein caused by infusion of 3-hydroxybutyrate may occur as a result of the generation of bicarbonate ions (Miles and Haymond, 1983). Studies of the effects of the infusion of sodium bicarbonate on urinary nitrogen excretion in fasting subjects confirms this theory (Hannaford, et al, 1982). Ketone body utilisation and the activities of 3-oxoacid CoA

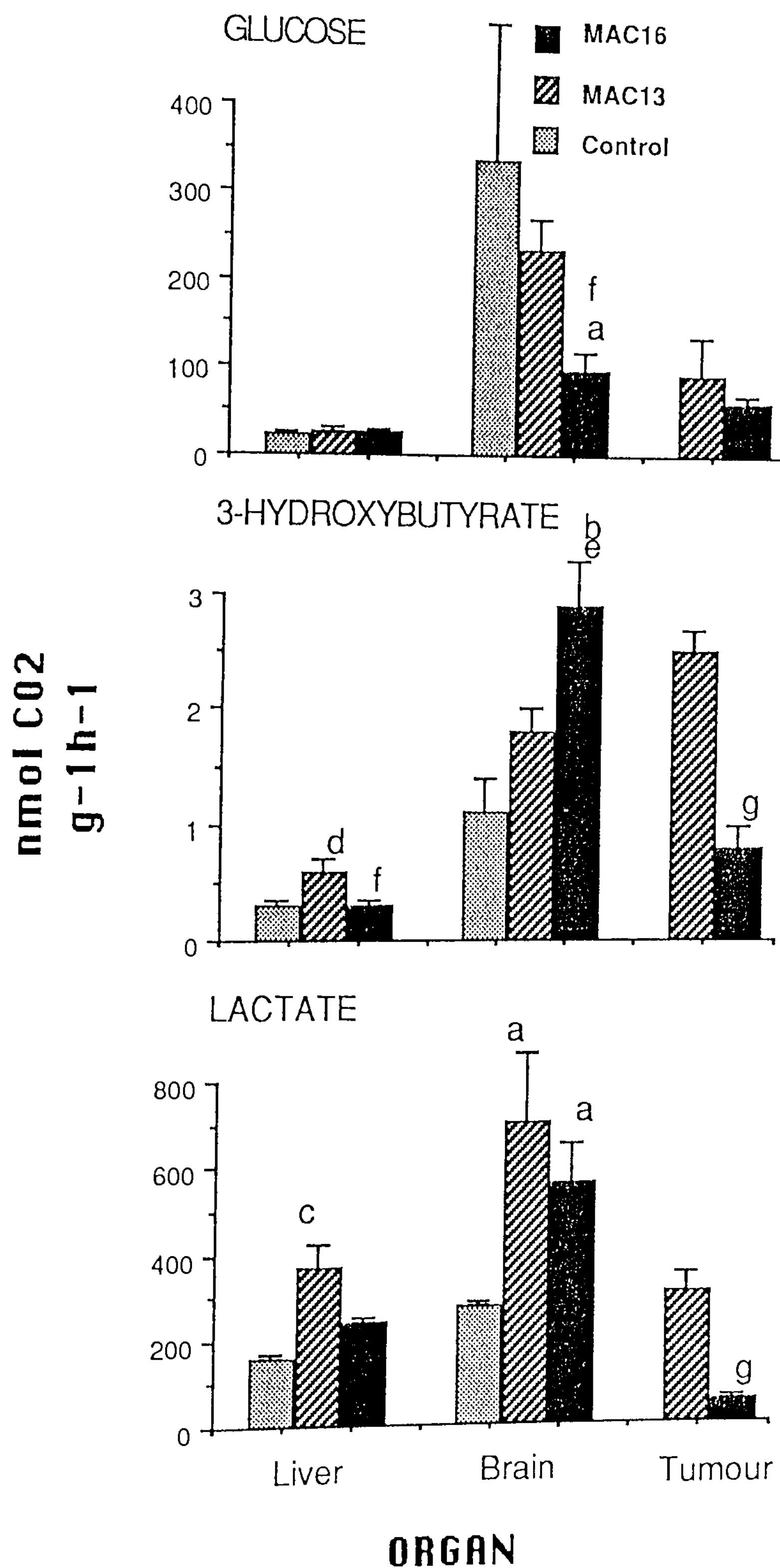
transferase have been investigated in cachectic and non-cachectic tumour-bearing animals and in non tumour-bearing controls and the results are discussed in the following section.

4.5.2. Results

The reduction in glucose utilisation by organs in tumour-bearing mice (section 4.3.2) must be accompanied by an alteration in the preference of the substrate for energy production. To determine the preferred metabolic substrate, the oxidation of glucose, lactate and 3-hydroxybutyrate was determined for slices of liver, brain and tumour of MAC16 and MAC13 tumour-bearing animals and non tumour-bearing controls (fig 4.26). At the concentrations employed, lactate was the most important oxidative substrate for the liver in control and tumour-bearing animals, with the rate of oxidation by liver slices from animals bearing the MAC13 tumour being significantly ($p<0.005$) elevated above control values (fig 4.26). Both glucose and 3-hydroxybutyrate oxidation by the liver were low and while the former did not change in the tumour-bearing state, oxidation of 3-hydroxybutyrate was significantly higher ($p<0.001$) in animals bearing the

Figure 4.26

In vitro oxidation of glucose, 3-hydroxybutyrate and lactate by liver, brain and tumour from MAC16 and MAC13 tumour-bearing animals and non tumour-bearing controls



a=p<0.05, b=p<0.01, c=p<0.005, d=p<0.001 from controls and e=p<0.05, f=p<0.005, g=p<0.001 from MAC13 tumour-bearing animals using the Students t-test. Results represent mean ± S.E.M. of 3 determinations per group

MAC13 tumour. Lactate was also the most important metabolic fuel for the tumour, and the rate of oxidation was significantly greater ($p<0.001$) in the MAC13 tumour. While the glucose oxidation rate was not significantly different for the two tumour types, the rate of oxidation of 3-hydroxybutyrate was much higher in the MAC13 tumour. For brain from non tumour-bearing animals glucose was the predominant metabolic fuel followed by lactate, while in the brain of animals bearing the MAC16 tumour with cachexia, the rate of glucose oxidation was reduced by two-thirds ($p<0.05$) and was also significantly less than that from brains of animals bearing the MAC13 tumour ($p<0.005$). This metabolic deficit produced by a decreased glucose oxidation was accompanied by a marked increase in both lactate ($p<0.05$) and 3-hydroxybutyrate ($p<0.01$) oxidation in the brains of animals bearing the MAC16 tumour when compared with non tumour-bearing controls.

Increased 3-hydroxybutyrate oxidation by the brains of cachectic animals must be due to increased 3-oxoacid CoA transferase activity or to an increased plasma level of 3-hydroxybutyrate or acetoacetate in the host. There was no significant difference between MAC16 tumour-bearing animals and non tumour-bearing controls with respect to plasma levels of 3-hydroxybutyrate, but levels were reduced in MAC13 tumour bearing animals (table 4.11). In contrast, levels of acetoacetate in MAC16 tumour-bearing animals were

elevated above control and MAC13 tumour-bearing animals at weight losses between 0 - 2g (fig 4.27), and this correlates well with the increased plasma level of free fatty acids at this weight loss (fig 4.27), (Briddon, et al, 1991). As weight loss progressed further, acetoacetate levels dropped dramatically (fig 4.27) (r = 0.91), as did FFA levels (Briddon, et al, 1991). Plasma 3-hydroxybutyrate levels in MAC16 tumour-bearing animals were not elevated above control values (table 4.11) nor did they alter with weight loss (fig 4.28). Brain 3-oxoacid CoA transferase activity was increased ($p<0.05$) in both tumour bearing states after maximum stimulation with acetoacetate (5mM) (Fig. 4.29). The activity of 3-oxoacid CoA transferase after maximum stimulation with acetoacetate, (5mM), was increased in the kidneys of MAC16 and MAC13 tumour-bearing animals when compared with non tumour-bearing controls (fig 4.29).

Production of $^{14}CO_2$ after i.v. administration of D-(-)-3-hydroxybutyrate (3-^{14}C) was rapid in all groups of animals and was significantly ($p<0.05$) greater in animals bearing both the MAC16 and the MAC13 tumours (fig 4.30).

Table 4.11

<u>Plasma ketone body levels</u> in tumour and non tumour-bearing animals

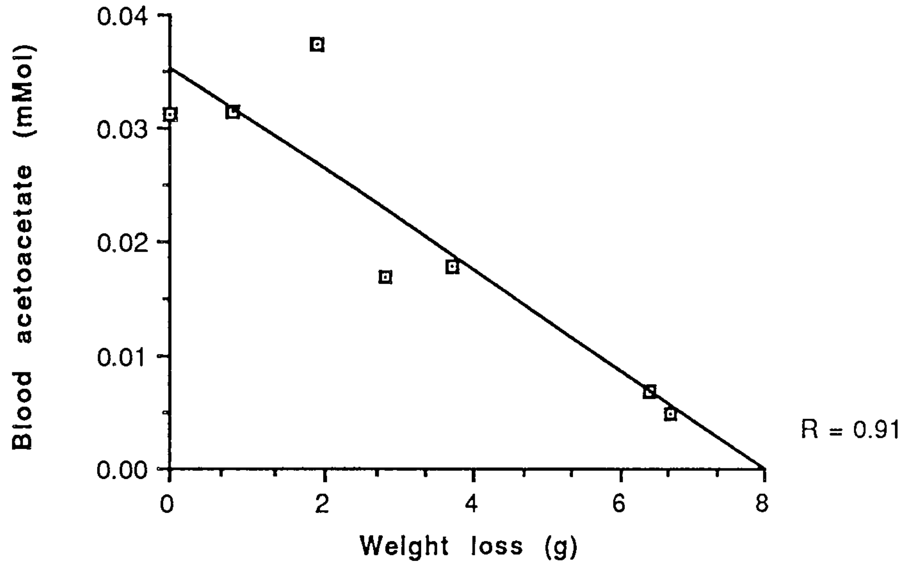
Group	Plasma metabolite Acetoacetate mM	3-Hydroxybutyrate mM
Non-tumour bearing	0.018 ± 0.003	0.0454 ± 0.004
MAC13 tumour bearing	0.019 ± 0.003	0.0236 ± 0.003 *
MAC16 tumour bearing	0.027 ± 0.004	0.0502 ± 0.004

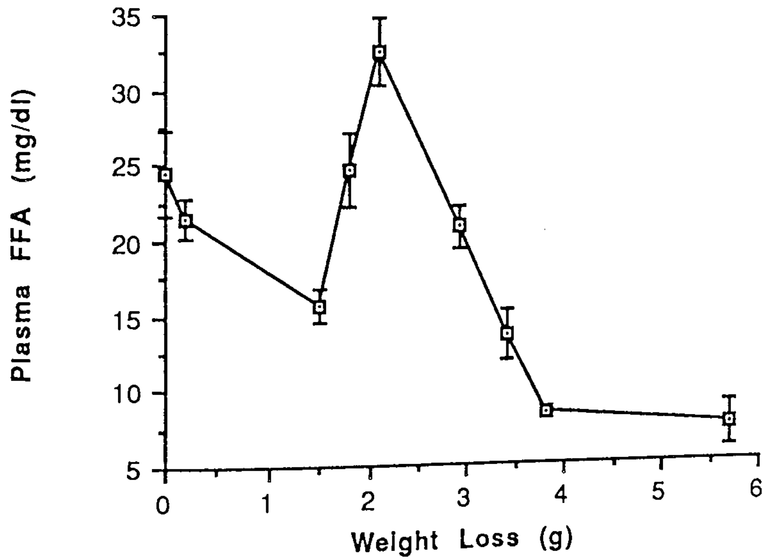
* = $p<0.005$ from control values using the Students t-test

Results represent the mean ± S.E.M. of 5 - 12 animals per group

Figure 4.27

Plasma acetoacetate and free fatty acids in MAC16 tumour-bearing animals and the relationship to weight loss



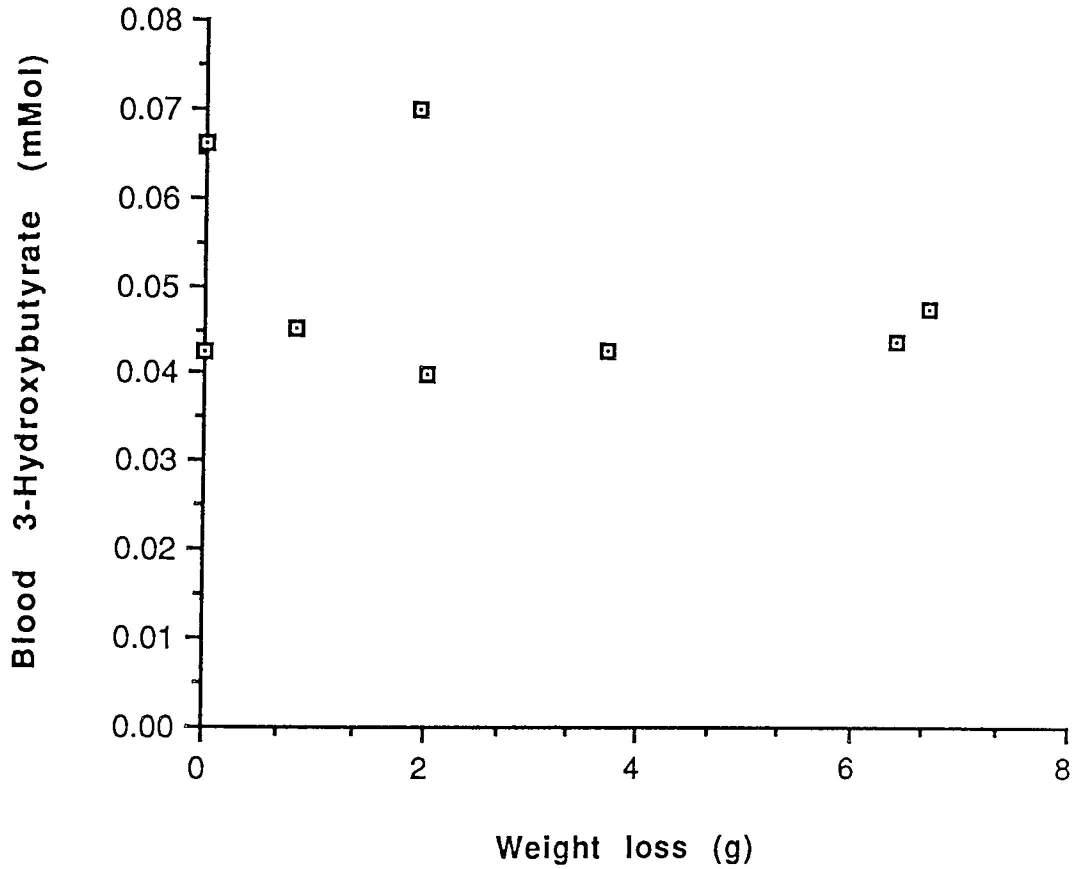


Each point represents the results from an individual animal

Plasma free fatty acids adapted from Briddon et al (1991)

Figure 4.28

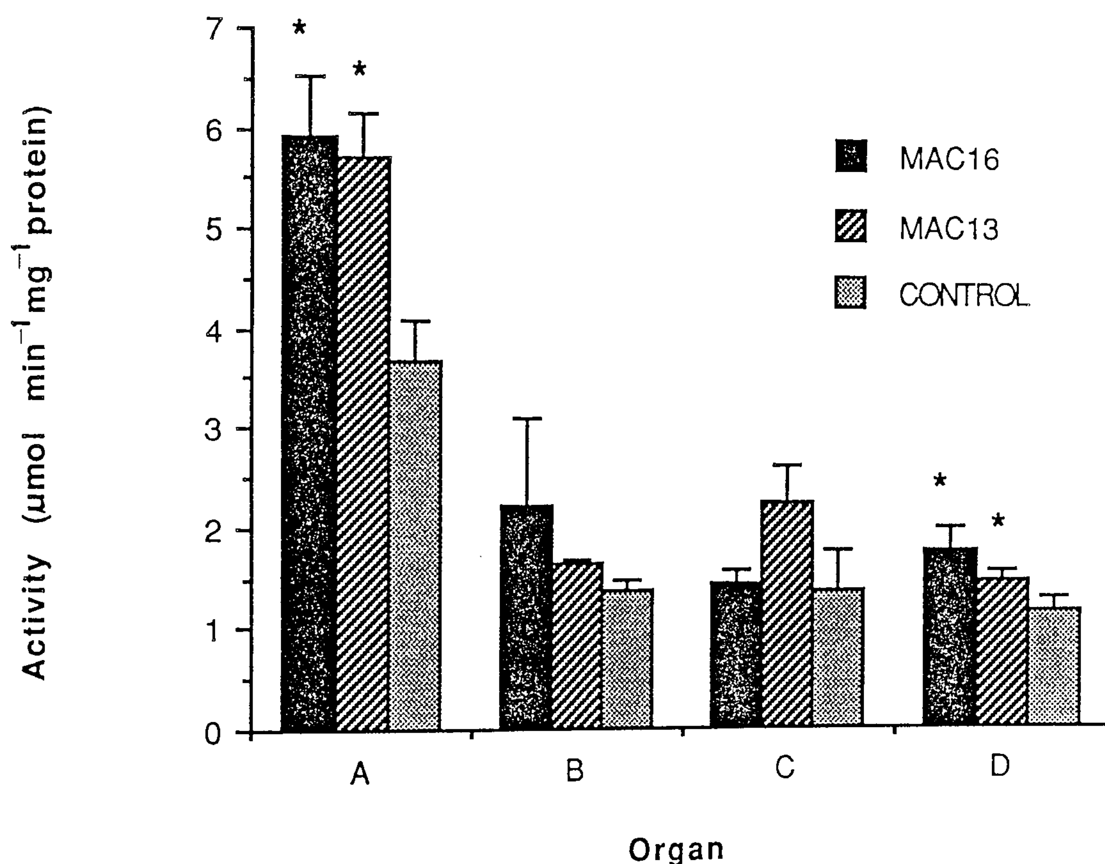
Plasma 3-hydroxybutyrate levels in MAC16 tumour-bearing animals and the relationship to weight loss



Each point represents the results from a single animal

Figure 4.29

The activity of 3-oxoacid CoA transferase in various organs in MAC16 and MAC13 tumour bearing animals and non tumour-bearing controls



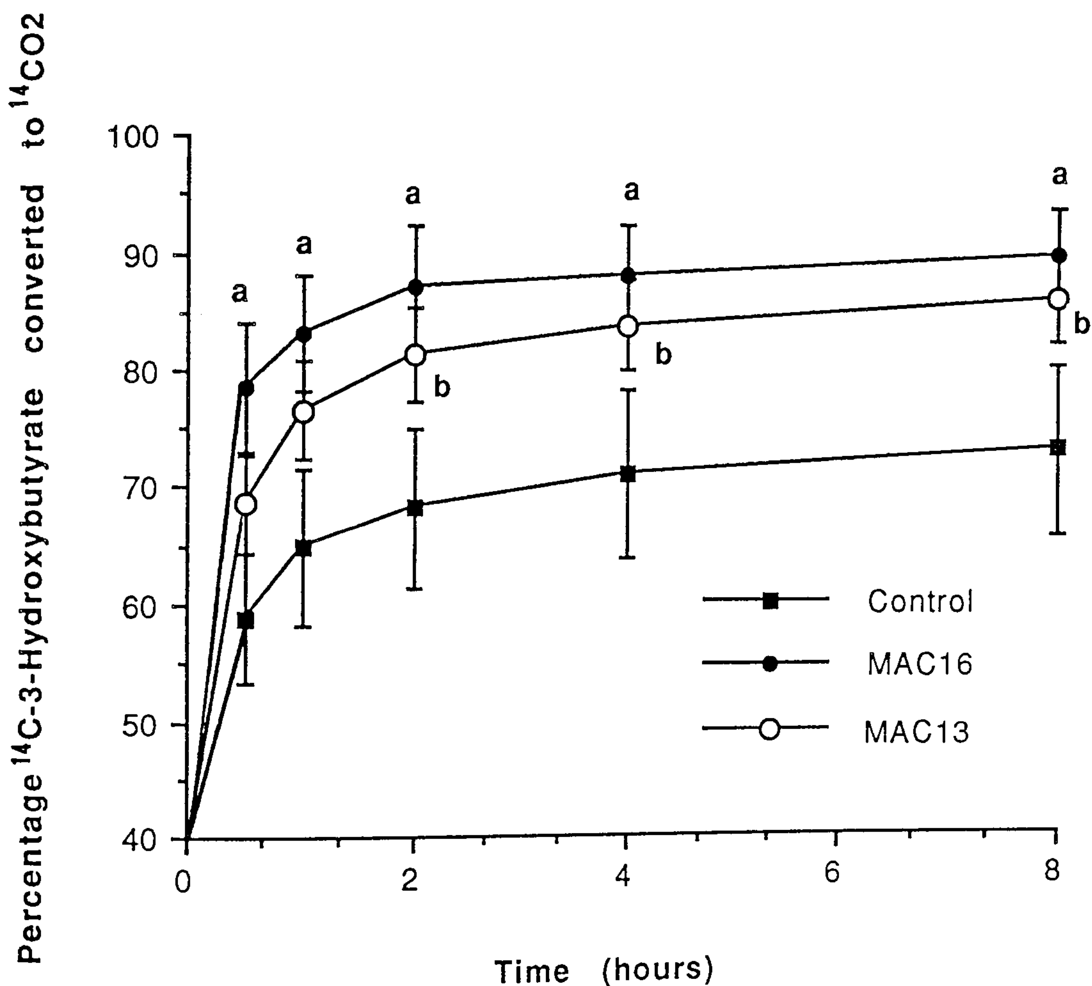
A=kidneys, B=gastrocnemius muscle, C=thigh muscle and D=brain

a=p<0.05 from control values using the Students t-test

Results represent mean ± S.E.M. of 4 - 5 determinations per group

Figure 4.30

Oxidation of [3-^{14}C]-hydroxybutyrate in MAC16 and MAC13 tumour-bearing animals and non tumour-bearing controls



Results represent mean ± S.E.M. of 6 animals per group

a=$p<0.05$ MAC16 values from controls, b=$p<0.05$ MAC13 values from controls using the Students t-test

4.5.3 Discussion

The results from the *in vitro* tissue slice assay system paralleled those from the *in vivo* study (section 4.3.2), in that while glucose was the predominant metabolic fuel for the brain in non tumour-bearing animals, its importance in oxidative metabolism was reduced in the tumour-bearing situation, especially when cachexia also accompanied tumour growth. In this situation, glucose was replaced by lactate and probably more importantly *in vivo* by 3-hydroxybutyrate. This resembles the situation seen in starvation (Owen, et al, 1967) although food intake is not decreased in these cachectic animals. Although the activity of 3-oxoacid CoA transferase was increased in the brains of MAC16 and MAC13 tumour-bearing animals, when compared with non tumour-bearing controls, recent results have failed to demonstrate any alteration in the Km value for this enzyme in the brains of tumour-bearing animals (R. Jones, personal communication). This correlates well with the reports quoted in the literature (Williamson, 1981), in which the activity of the enzyme 3-oxoacid CoA transferase was not increased in the brains of tumour-bearing animals. An increase in the Vmax value for this enzyme may explain the increase in its activity in the tumour-bearing state, but this requires further investigation. Although extensive mobilisation of adipose tissue is observed in animals bearing the MAC16 tumour throughout the course of

weight loss, the plasma levels of acetoacetate were only elevated at small tumour burdens when weight loss was between 0 and 2g, and 3-hydroxybutyrate was not elevated at any weight loss.

It has been shown previously that 3-oxoacid CoA transferase is also present in the MAC16 tumour, but at reduced levels when compared with normal colon (Tisdale and Brennan, 1986). This correlates well with the low utilisation of 3-hydroxybutyrate by the MAC16 tumour *in vitro*. For the MAC13 tumour, lactate was the most important metabolic fuel and lactate has also been shown to be utilised by a number of rat tumours *in vivo* (Sauer, et al, 1982).

Oxidation of 3-hydroxybutyrate *in vivo* was elevated in both tumour-bearing states. This suggests that in tumour-bearing animals, brain metabolism is supported by an increased utilisation of 3-hydroxybutyrate as occurs in starvation and this appears to be independent of the development of cachexia.

4.6 Monitoring lipid depletion using NMR spectroscopy

4.6.1 Introduction

Cancer therapy is at present hampered by the inability to evaluate the response of a tumour to treatment (Griffiths,

et al, 1987). Nuclear magnetic resonance (NMR) spectroscopy may provide a unique opportunity for solving this problem. It is possible to detect the presence of anticancer drugs themselves in tumours by NMR spectroscopy, particularly those that contain the ^{19}F atom (Stevens, et al, 1984). Levels of ATP and phosphocreatine and their breakdown product, inorganic phosphate and also the intracellular pH are all parameters likely to be affected by most non-surgical therapies. ^{31}P NMR spectroscopy is a method by which these parameters can be monitored, and alterations in tumour levels could be used as indices of therapeutic efficacy. ^{1}H NMR is also a useful tool as it is possible to use it to detect substances such as lactate in the tumour which may again be indicators of neoplastic growth. ^{1}H NMR spectroscopy is technically more demanding than ^{31}P, because the magnet must be adjusted to give a more uniform field and because signals from water and fat must be suppressed. This fact can in itself be a useful tool for investigating the effects of tumour induced cachexia on host tissues, since it is possible to detect levels of fat in the muscles of anaesthetised animals using this technique.

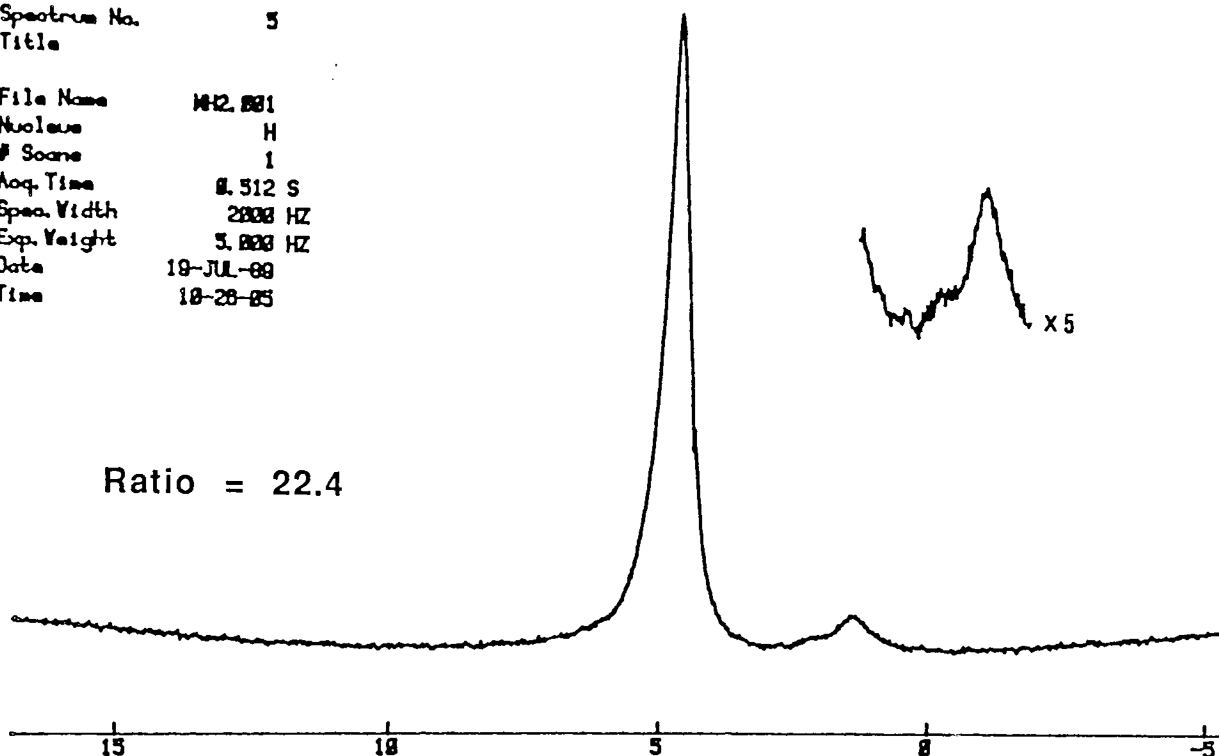
4.6.2 Results

Using ^{1}H NMR spectroscopy it was possible to obtain signals from the muscles of anaesthetised animals which correspond

to the water and fat content of the muscle (figs 4.31, 4.32 and 4.33). Peaks occurred between 10 and -5 ppm and were obtained using a 2cm coil and a spectrum width of 2000Hz. The first peak obtained corresponded to the water content of the muscle, and the second peak gave a measure of the fat content. The water to fat ratio of the tissue can therefore be used as a measure of the amount of fat present in the muscle. The water to fat ratio was significantly ($p<0.05$) increased in MAC16 tumour-bearing animals that had lost more than 4g in weight and it rose as weight loss became more severe (fig 4.34). The water to fat ratio in the hind leg muscle of MAC13 tumour-bearing animals and in MAC16 tumour-bearing animals that had lost 0 - 3g in weight was not significantly different from control values (fig 4.35).

Figure 4.31

^{1}H-NMR spectroscopy traces of leg muscle of non tumour-bearing animals



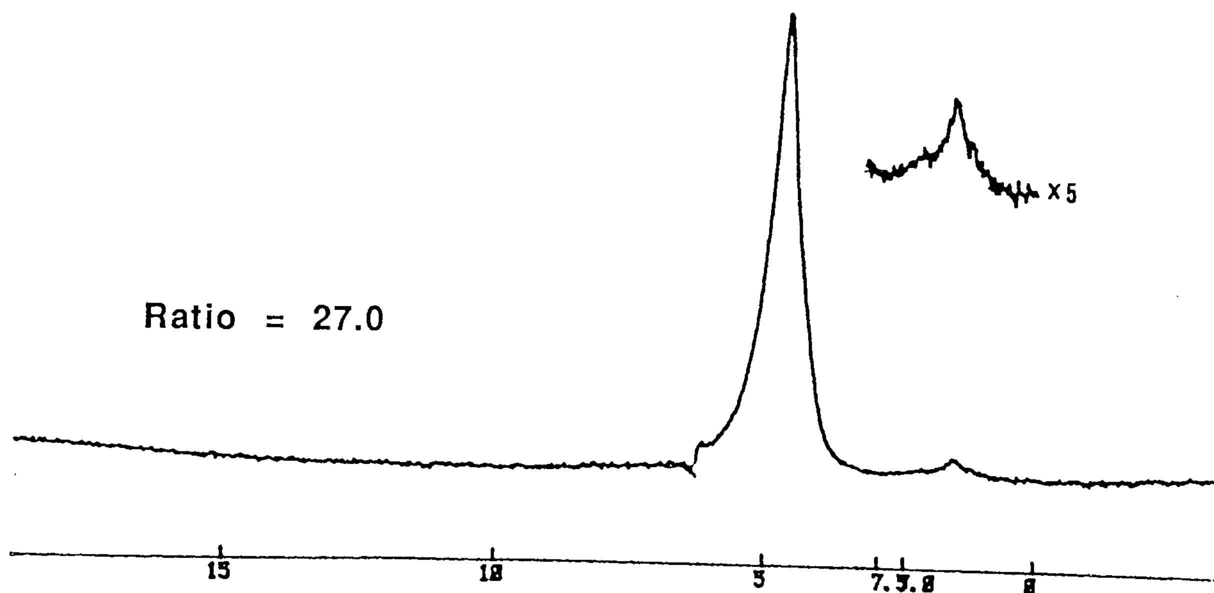
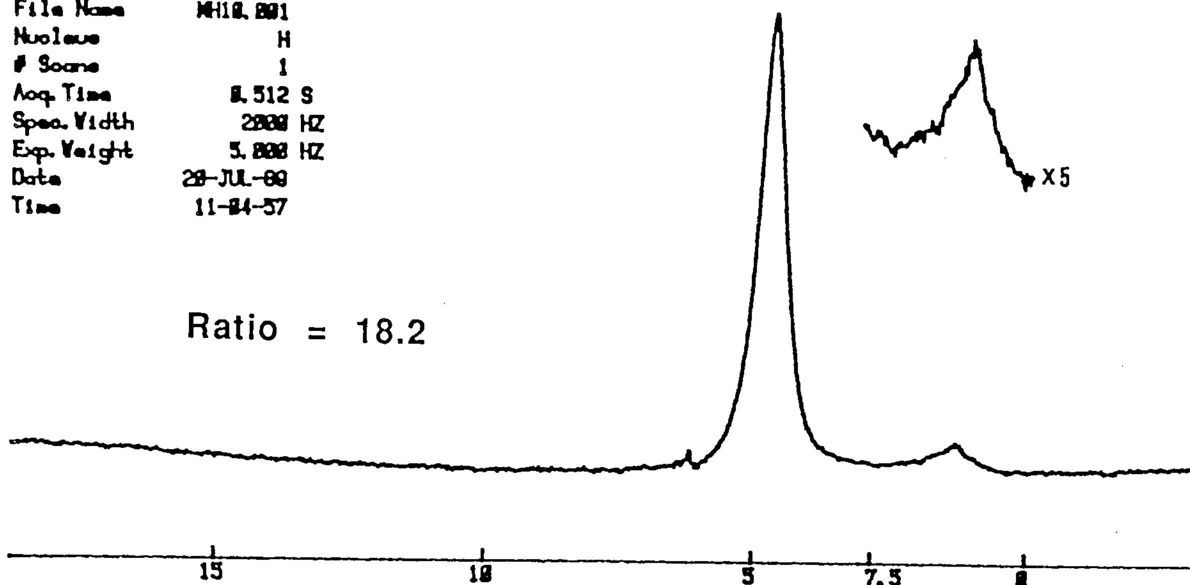


Figure 4.32

[1]<u>H-NMR spectroscopy traces of leg muscle of MAC13 tumour bearing animals</u>



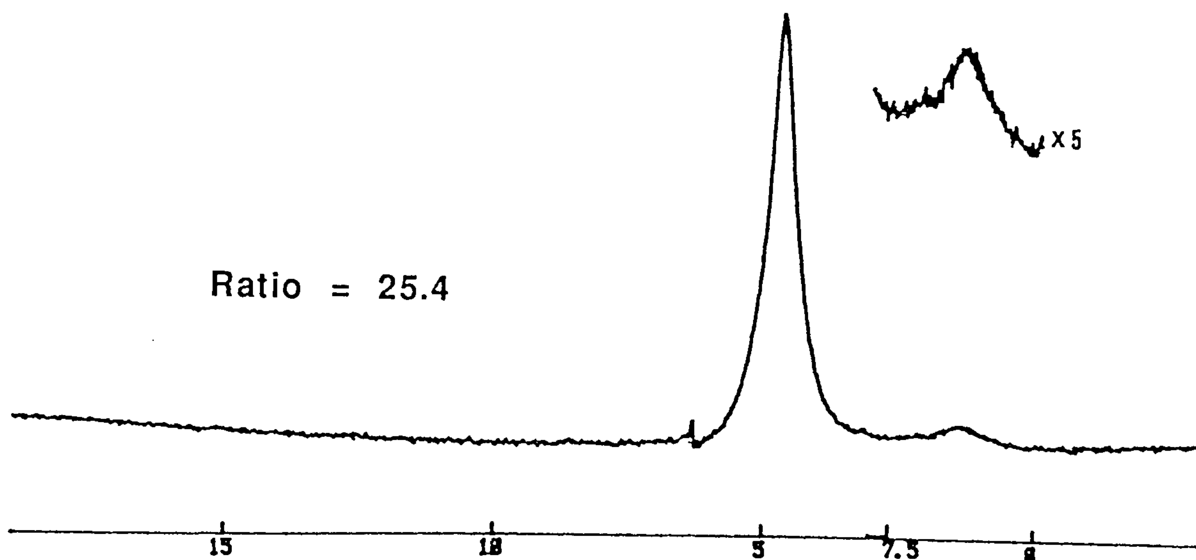
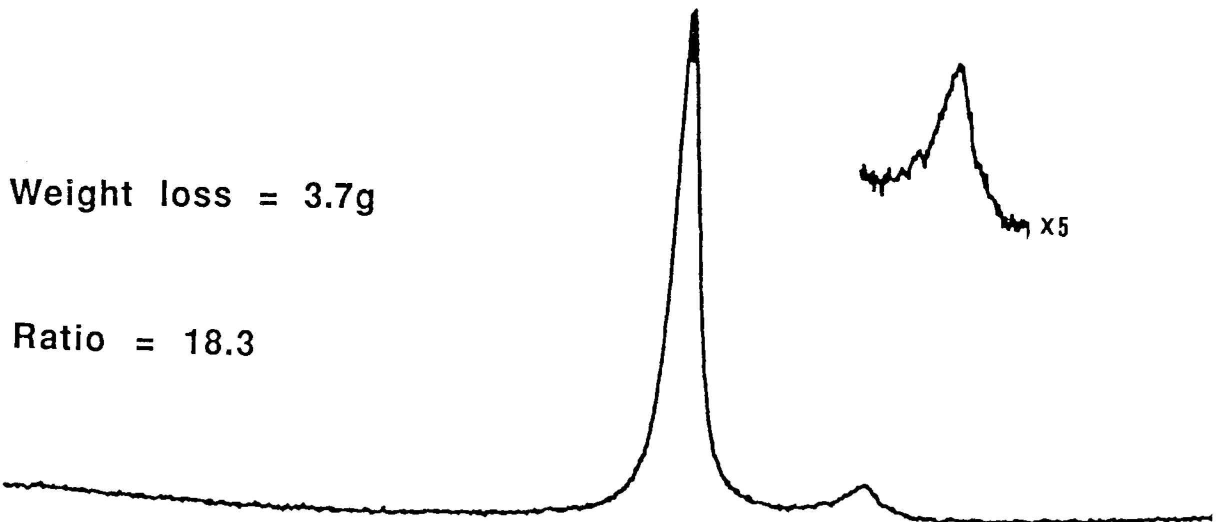


Figure 4.33

^{1}H-NMR spectroscopy traces of leg muscle of MAC16 tumour bearing animals

Spectrum No.	2
File Name	
Nucleus	H
# Scans	1
Acq. Time	0.512 S
Spec. Width	2000 HZ
Exp. Weight	5.000 H
Date	19-JUL-89
Time	09-30-53



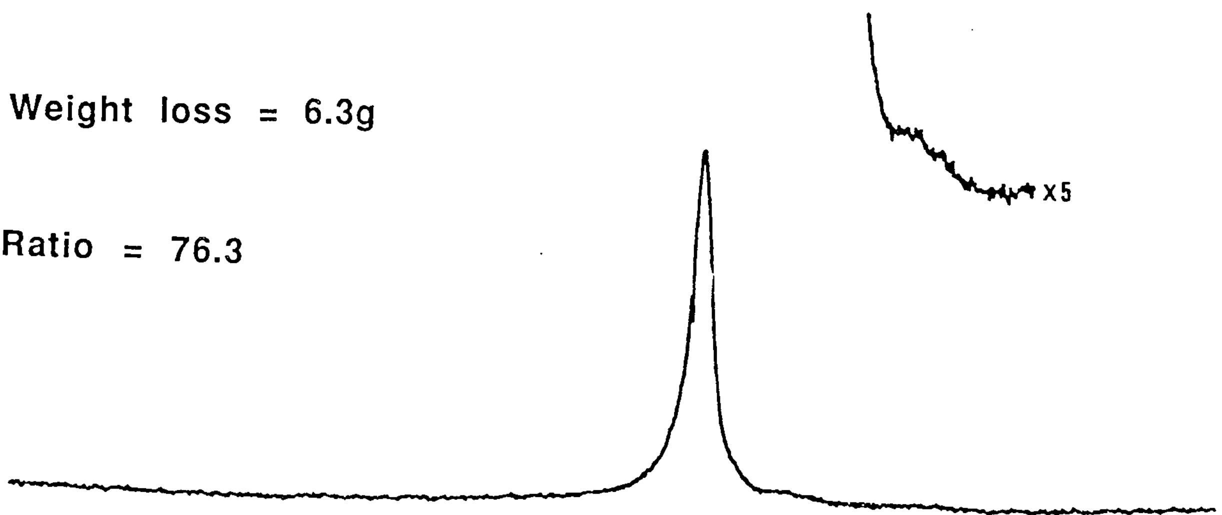
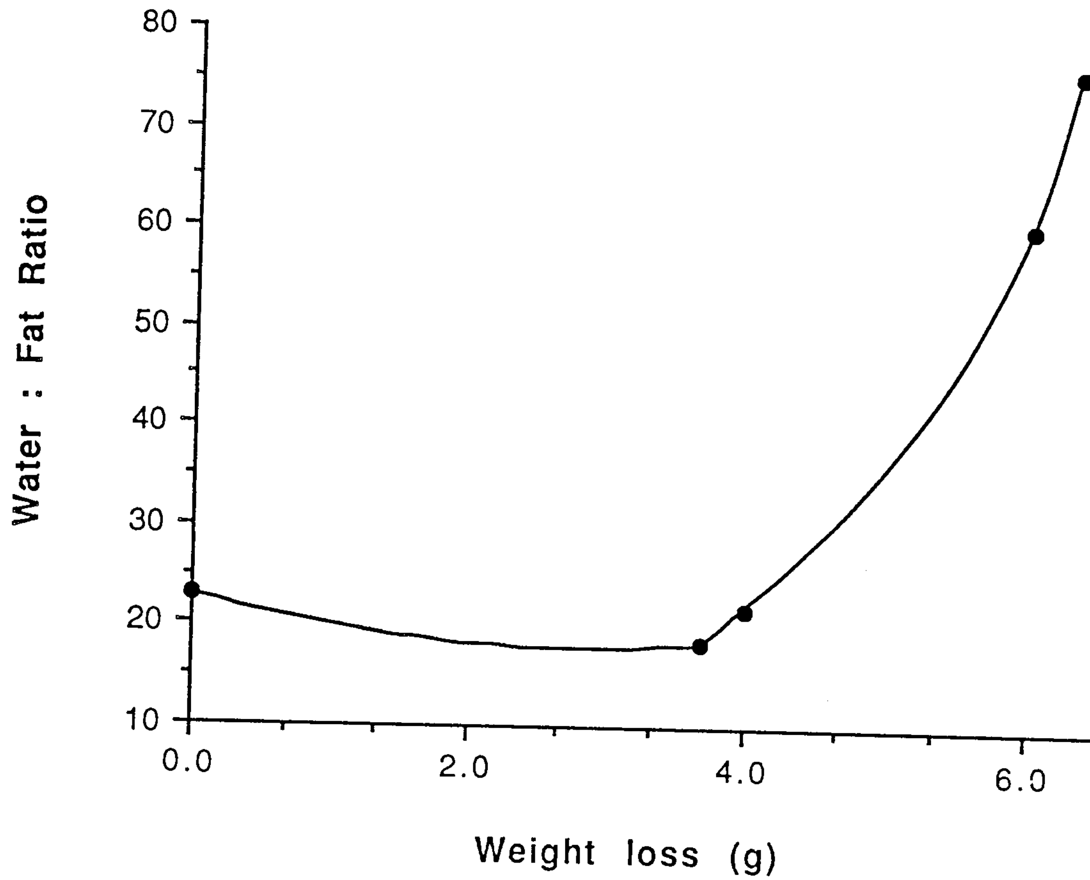


Figure 4.34

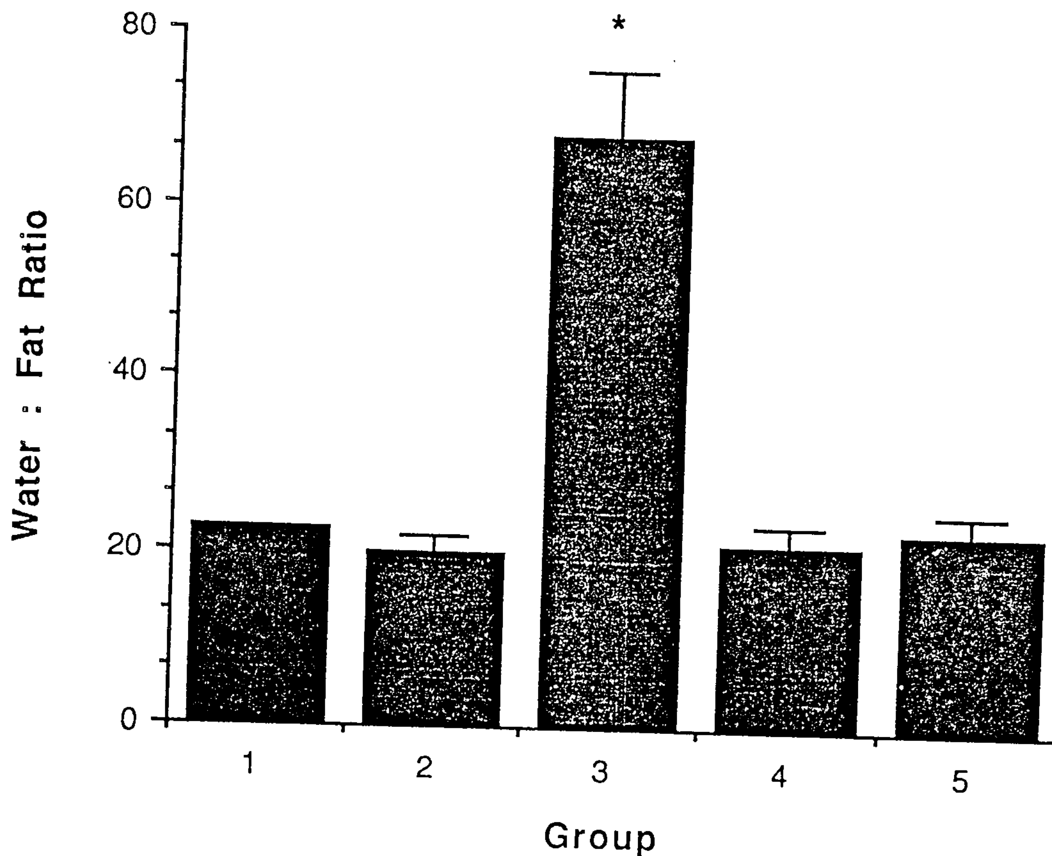
The effect of weight loss on the water:fat ratio of the leg muscle in MAC16 tumour-bearing animals



Each point represents the results from an individual animal

Figure 4.35

The water:fat ratio of leg muscles in tumour and non tumour-bearing animals



1=non-cachectic MAC16 tumour-bearing animals, 2=cachectic MAC16 tumour-bearing animals, weight loss 1 - 4g, 3=MAC16 tumour-bearing animals, weight loss >4g, 4=MAC13 tumour-bearing animals, 5=Control, non tumour-bearing animals

*= $p<0.05$ from controls using Students t-test. Results represent mean ± S.E.M. of 3 animals per group

4.6.3 Discussion

NMR spectroscopy is a useful tool for measuring parameters of tumour growth and the effect of the tumour on the host. In this investigation, ^{1}H NMR spectroscopy was used to determine the effect of tumour growth and tumour-induced cachexia on the lipid content of muscle tissues. At weight losses greater than 4g, there was a significant decrease in the lipid content of the muscle. MAC13 tumour growth and MAC16 tumours that did not cause cachexia in the host had no effect on muscle lipid content when compared with controls. In agreement with the results presented in previous sections, these results are an indication of the massive depletion of host lipid stores that occurs in cancer-induced cachexia and suggests once again the importance of lipids to the cachectic host.

4.7 The effect of a MAC16 tumour derived lipolytic factor in vivo

4.7.1 Introduction

Attention has recently been directed towards the isolation and identification of the factors responsible for the complex metabolic changes associated with cancer cachexia. Tumour necrosis factor alpha (TNF) or cachectin has been associated with significant weight loss in animals (Oliff,

et al, 1987, Mahony, et al, 1988). Clinical trials using recombinant TNF (Sherman, et al, 1988) have however failed to demonstrate any evidence of accelerated cachexia, although in some patients, anorexia was present during administration. These results suggest that other tumour-derived factors may be the mediators of cachexia in cancer patients, since TNF appears to be involved in anorexia rather than cachexia.

More recently, a lipolytic factor distinct from TNF (Mahony, et al, 1988) has been identified in a cachexia-inducing murine tumour and enhanced lipolytic activity has been detected in a number of other tumour models and in the body fluids of human cancer patients (Beck, et al, 1991). The MAC16 tumour-associated lipolytic factor has been characterised by DEAE cellulose chromatography of MAC16 tumour extracts. Active material was retained on the column and could be eluted as four successive peaks, under the influence of a linear salt gradient (0.08 - 0.2M NaCl) (Beck, et al, 1991). Each peak of lipolytic activity from the DEAE cellulose column when subjected to Sephadex G50 exclusion chromatography produces 3 fractions of apparent molecular weights corresponding to 3, 1.5 and 0.8kDa (Beck, et al, 1991), although recent calculations suggest higher molecular weights of 5, 10,and 20kDa (M. J. Tisdale, personal communication). Serum from cancer patients with clinical cancer cachexia also show an

activity distribution pattern of a form similar to that of the MAC16 tumour when subjected to DEAE cellulose chromatography and eluted with a salt gradient. A control sample of normal human serum contained no corresponding fractions of retained lipolytic activity. Exclusion chromatography again produced 3 fractions of similar molecular weights (Beck, et al, 1991). Structural elucidation of the cachexia-related lipolytic factor awaits its further purification, although it is known to be stable to heat (90^{0}C for 15 min), acid (pH<1.0), RNAase, DNAase, periodate, trypsin and chymotrypsin. Although the structure of this MAC16 tumour- associated lipolytic factor is not yet known, it is possible to partially purify the factor (section 3.7), and its effects *in vivo* are described in the remainder of this section.

4.7.2 Results

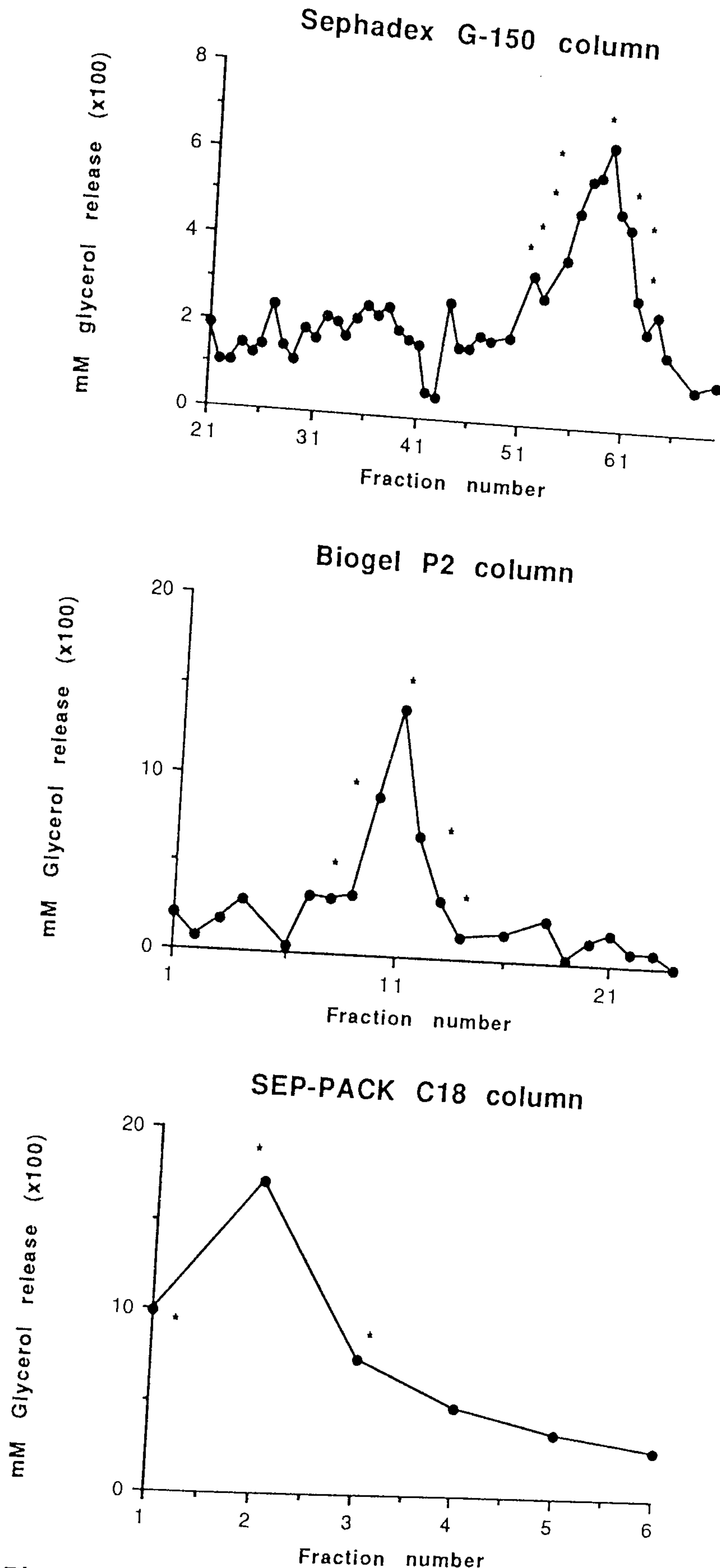
The partially purified MAC16 tumour-associated factor was prepared from tissue culture medium in which the MAC16 cells had been growing by a series of exclusion chromatography procedures (fig 4.36). The active fractions (*) from each column were pooled after each procedure (section 3.7.2). Subcutaneous administration of the partially purified MAC16 tumour cell-derived factor had no effect on weight loss in non tumour-bearing mice (table 4.12). Intraperitoneal administration of the factor

resulted in daily weight loss, but weight was regained overnight (fig 4.37). There was no effect on food and water consumption in either group (table 4.13), except on day 2 when the main weight loss occurred. There was a significant ($p<0.005$) reduction in blood glucose levels and a slight, but not significant increase in the plasma lactate levels in the factor injected group. There was no difference between the 2 groups with regard to the plasma concentrations of acetoacetate (table 4.14). Injection of partially purified factor produced a dramatic loss of body weight in non-cachectic MAC16 tumour-bearing animals when compared with saline injected controls (fig 4.38). This very rapid weight loss did, however, cause distress to the animals and resulted in food intake being reduced to half that of control values (table 4.15). There was a significant ($p<0.05$) reduction in total carcass fat in the factor injected mice and a significant ($p<0.05$) reduction in the content of the gastrocnemius muscle (table 4.16). Injection of the lipolytic factor did not affect the percentage water composition of the carcass (table 4.16). There was no difference between the 2 groups with regard to weight loss when the partially purified lipolytic factor or normal saline were administered by intragastric intubation (fig 4.39), to MAC13 tumour-bearing animals. Both the partially purified K562 tissue culture medium (fig 4.40) and the L1210 tissue culture medium (fig 4.41) failed to produce weight loss in MAC13 tumour-bearing animals when

compared with the partially purified MAC16 tissue culture medium. The K562 and L1210 tissue culture mediums were purified by the same procedures as the MAC16 medium, but they were lacking in lipolytic activity. A steady weight loss was achieved and maintained by injecting a smaller dose of the factor (50µl) (fig 4.41). Food and water intakes were not reduced when compared with the control group (table 4.17) and percentage water composition remained the same in both groups (table 4.18). There was a slight but not significant decrease in carcass fat content in the MAC16 tumour-associated lipolytic factor injected group, and there was no difference between the two groups with respect to the muscle content of the thigh and gastrocnemius muscles (table 4.18). The non-fat carcass mass was significantly reduced in the MAC16 lipolytic factor injected group when compared with the control L1210 injected group (table 4.18). Since 70% of the weight loss that occurred in the factor injected group was probably due to water loss, it was very difficult to detect any decrease in body fat since it would be too small to measure accurately, i.e. loss of adipose and lean body tissues would only account for 0.3g weight loss in this experiment.

Figure 4.36

Purification of the MAC16 tumour-associated lipolytic factor



The most active fractions (*) were pooled after each procedure. 250 - 300ml of tissue culture medium was purified.

Table 4.12

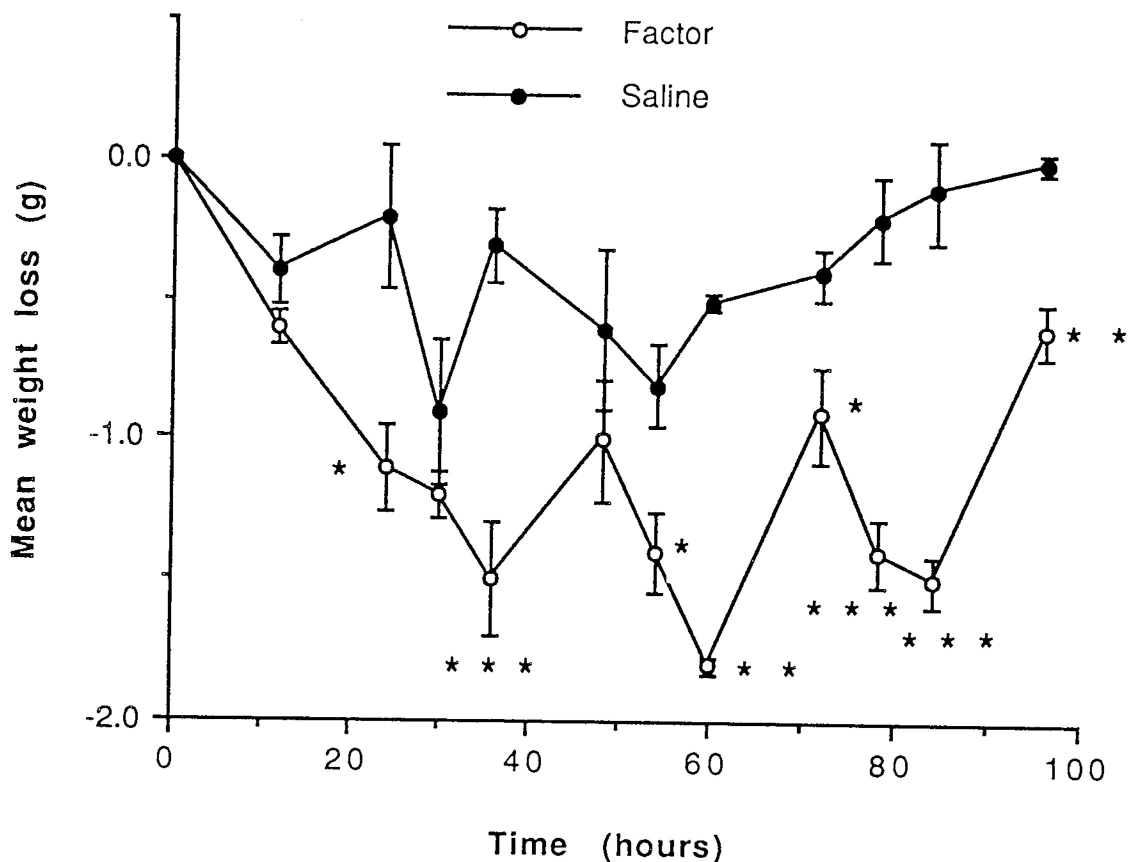
The effect of subcutaneous administration of lipolytic factor on body weight in non tumour-bearing animals

Time (days)	Average weight change (g)	
	Control	Lipolytic factor
2	+0.20 ± 0.21	-0.03 ± 0.64
3	+0.50 ± 0.31	+0.23 ± 0.47
4	+0.07 ± 0.29	+0.30 ± 0.31
5	+0.43 ± 0.33	+1.03 ± 0.41

Mice were injected with 200µl of partially purified factor. The activity of the factor was such that 100µl resulted in the production of 0.1mM glycerol release when incubated with adipocytes. Each result represents mean ± S.E.M. of 4 animals per group

Figure 4.37

The effect of intraperitoneal administration of lipolytic factor on weight loss in non tumour-bearing mice



*=p,0.05, **=p<0.01, ***=p<0.005 from saline controls using the Students t-test

Mice were injected three times a day with 100µl of partially purified lipolytic factor. Activity of factor was as described for table 4.13. Results represent mean ± S.E.M. of 3 animals per group

Table 4.13

The effect of intraperitoneal administration of lipolytic factor on food and water intake in non tumour-bearing mice

Time (days)	Lipolytic factor Food (g)	Lipolytic factor Water (ml)	Control Food (g)	Control Water (ml)
2	3.33	2.7	5.13	4.7
3	3.17	4.0	3.53	4.0
4	3.27	3.3	3.50	3.3
5	4.40	4.0	3.73	4.0
Mean	3.54	3.5	3.97	4.0
± S.E.M.	0.30	0.3	0.39	0.3

Table 4.14

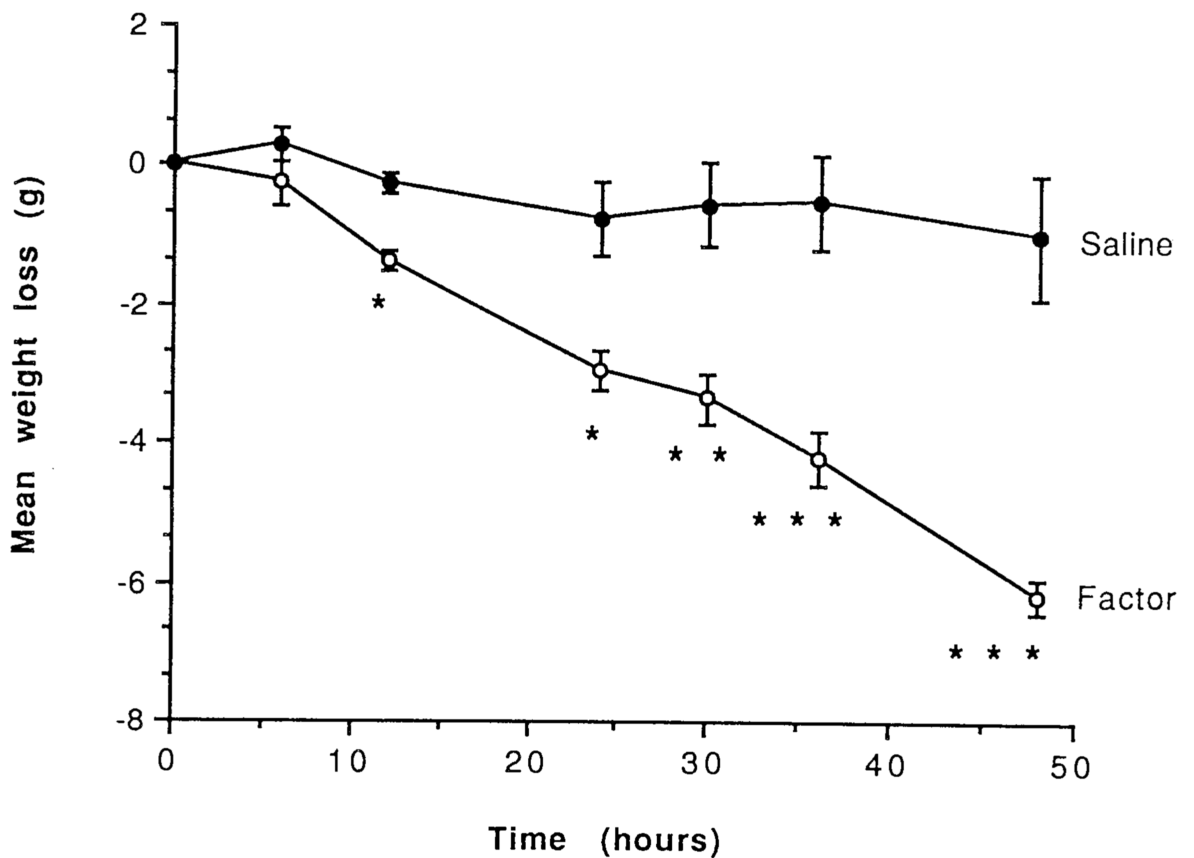
<u>The effect of intraperitoneal administration of lipolytic factor on levels of plasma metabolites in non tumour-bearing animals</u>

	Plasma concentration	
	Lipolytic factor	Control
Glucose (mg/100ml)	80.85 ± 7.19 *	128.39 ± 5.09
Acetoacetate (µmol)	0.102 ± 0.012	0.114 ± 0.004
Lactate (µmol/ml)	4.696 ± 0.704	2.945 ± 1.622

*=$p<0.005$ from control values using the Students t-test

Figure 4.38

The effect of intraperitoneal administration of lipolytic factor on weight loss in non-cachectic MAC16 tumour-bearing animals



*=p<0.05, **=p<0.01, ***=p<0.005 from control values using the Students t-test

Results represent mean ± S.E.M. of 3 animals per group

Mice were injected three times a day with 100µl of partially purified lipolytic factor. Activity of factor was as described in table 4.13

Table 4.15

Effect of intraperitoneal administration of lipolytic factor on food and water intakes in non-cachectic MAC16 tumour-bearing animals

Group	Intake per mouse	
	Food (g)	Water (ml)
MAC16 associated lipolytic factor	4.6 ±0.4	3.1 ±0.1
Saline controls	9.5 ±0.4	3.3 ±0.2

Table 4.16

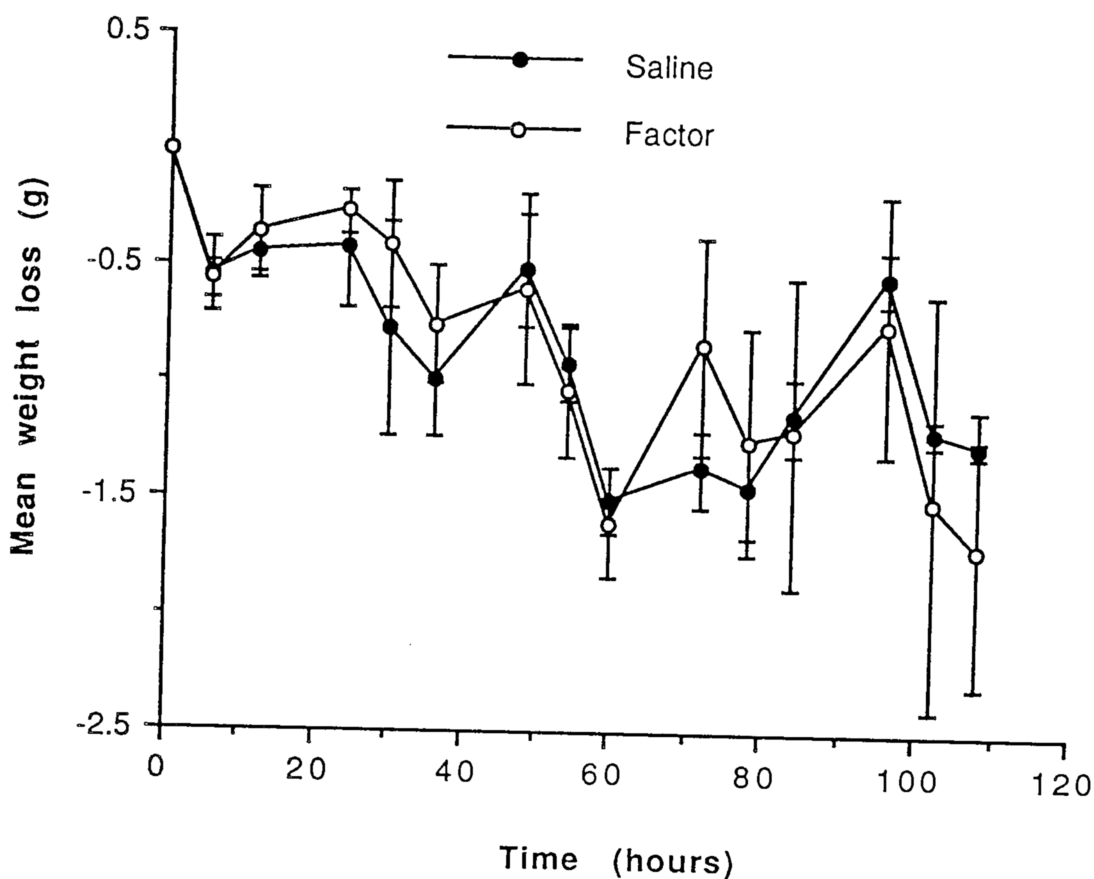
The effect of intraperitoneal administration of lipolytic factor on body composition of non-cachectic MAC16 tumour-bearing animals

	Group	
Body composition	MAC16 associated factor	Saline controls
Water (%)	65.9 ± 0.9	65.3 ± 0.3
Fat (g)	1.42 ± 0.21 *	1.95 ± 0.10
Gastrocnemius muscle (g)	0.033 ± 0.002 *	0.046 ± 0.004
Thigh muscle (g)	0.036 ± 0.001	0.041 ± 0.003

* = $p<0.05$ from controls using the Students t-test

Figure 4.39

The effect of an oral dose of lipolytic factor on weight loss in MAC13 tumour-bearing animals

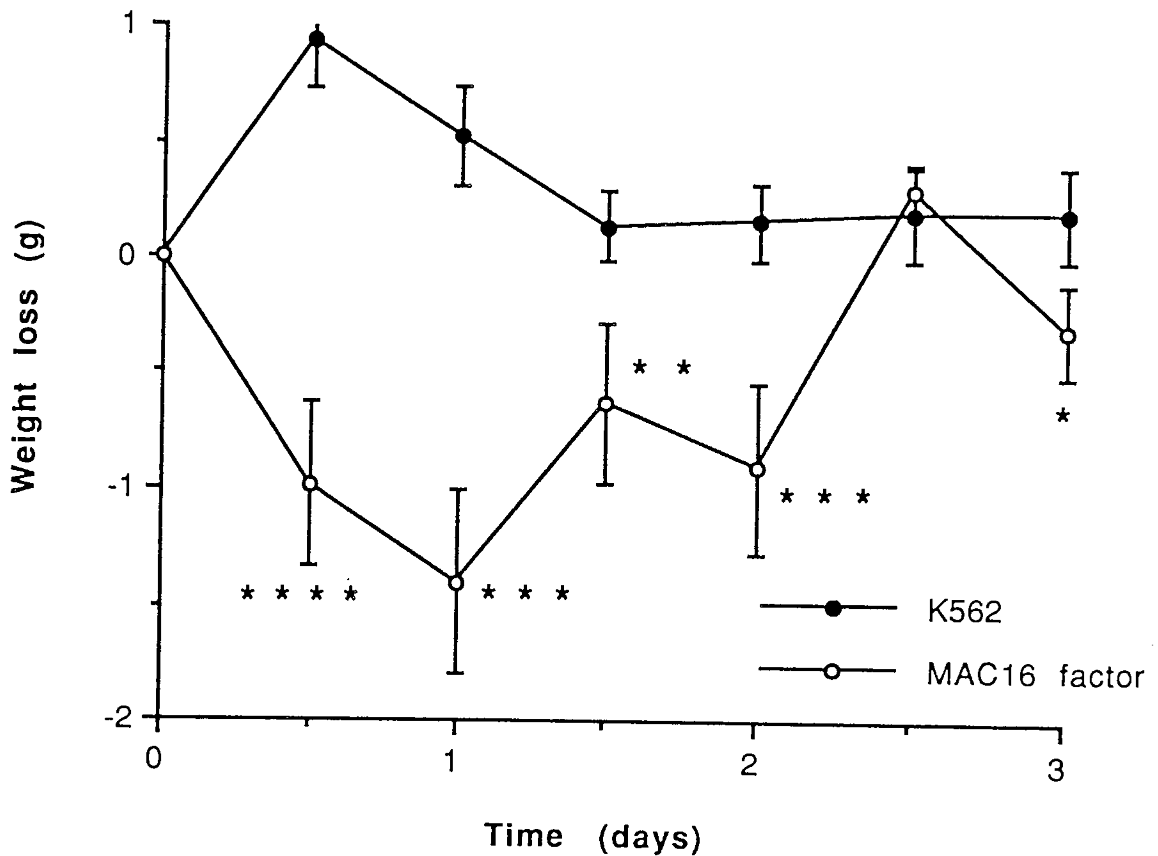


Each point represents the mean ± S.E.M. of 5 animals per group

Mice were given 200µl of partially purified lipolytic factor by intragastric intubation three times a day. Activity of factor was such that 100µl produced 0.1mM glycerol release when incubated with adipocytes.

Figure 4.40

The effect of MAC16 tumour-associated lipolytic factor and purified K562 tissue culture medium on weight loss in MAC13 tumour-bearing animals

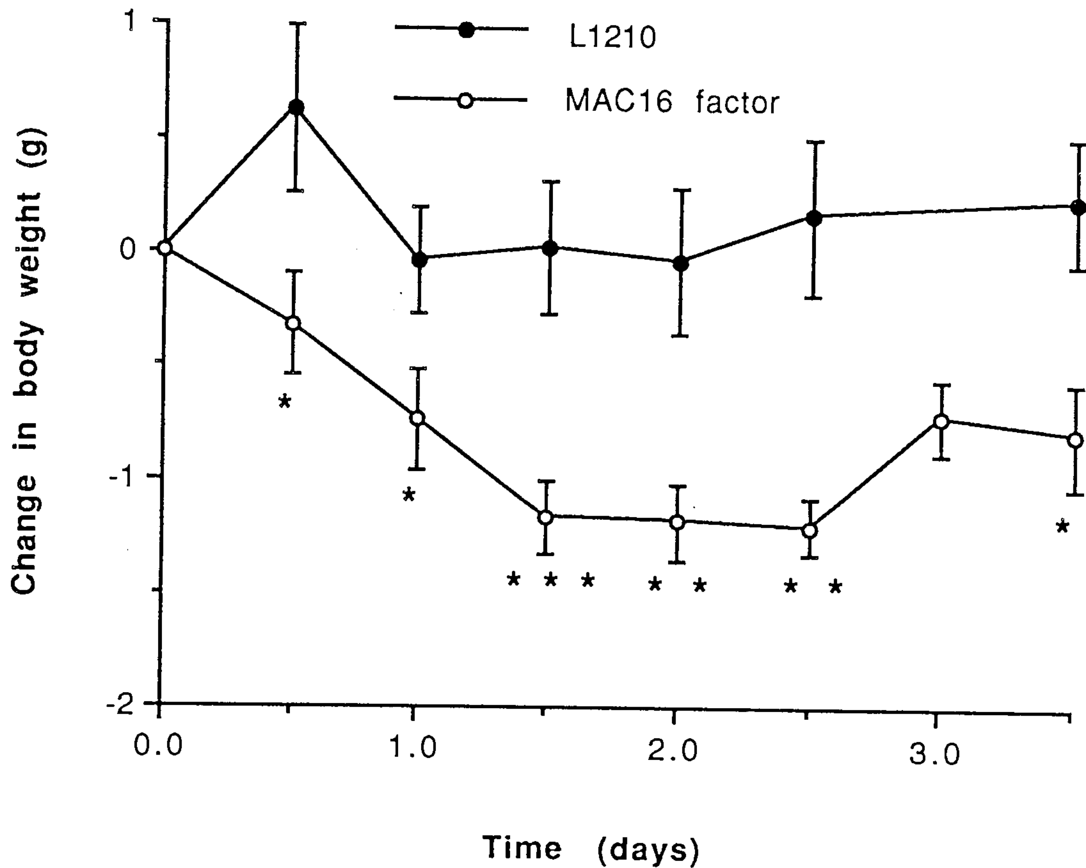


*=p<0.05, **=p<0.01, ***=p<0.005, ****=p<0.001 from K562 values using the Student t-test

Mice were injected twice a day with either 100µl of partially purified MAC16 culture medium or K562 medium. Activity of MAC16 culture medium was such that 100µl produced 0.1mM glycerol release when incubated with adipocytes. Results are expressed as mean ± S.E.M. of 5 animals per group

Figure 4.41

The effect of MAC16 tumour-associated lipolytic factor and purified L1210 tissue culture medium on weight loss in MAC13 tumour-bearing animals



*=p<0.05, **=p,0.01, ***=p<0.005 from L1210 values using the Students t-test

Mice were injected twice a day with either 50µl of partially purified MAC16 culture medium or L1210 culture medium. Activity of MAC16 culture medium was such that 100µl produced 0.1mM glycerol release when incubated with adipocytes. Results are expressed as mean ± S.E.M. of 4 - 5 animals per group

Table 4.17

The effect of MAC16 tumour-associated lipolytic factor and purified L1210 culture medium on food and water intakes in MAC13 tumour-bearing animals

Group	Intake per mouse/24 hours	
	Food (g)	Water (ml)
MAC16 associated lipolytic factor	2.56 ±0.23	3.5 ±0.3
L1210 medium	2.84 ±0.32	4.3 ±0.7

Food and water intakes were determined every 24 hours and the mean intakes ± S.E.M. were calculated for the duration of the experiment.

Table 4.18

The effect of MAC16 tumour-associated lipolytic factor and purified L1210 culture medium on body composition of MAC13 tumour-bearing mice

Body composition	Group: MAC16 associated factor	L1210 medium
Water (%)	68.9 ± 0.4	67.7 ± 0.5
Fat (g)	0.98 ± 0.11	1.19 ± 0.16
Gastrocnemius muscle (g)	0.027 ± 0.004	0.028 ± 0.004
Thigh muscle (g)	0.018 ± 0.001	0.017 ± 0.002
Non-fat	4.60 ± 0.20*	5.20 ± 0.20

*= $p<0.05$ from L1210 controls using the Students t-test

4.7.3 Discussion

The MAC16 tumour *in vivo* and the corresponding MAC16 cell line *in vitro* produce a lipolytic factor. Although this same factor is also detectable in the MAC13 tumour (which does not produce cachexia), (Beck, 1989), it is only produced in very small quantities when compared with the MAC16 tumour. The factor may therefore be required for tumour growth, with an over-expression of the factor leading to the production of cachexia in the host. The preliminary results reported in this section suggest that the lipolytic factor is only capable of producing cachexia in the presence of a tumour. Unlike TNF, weight loss induced by the lipolytic factor was maintained over a prolonged period of time when injected into tumour-bearing animals, whereas tachyphylaxis rapidly develops in response to repeated injections of TNF (Mahony, et al, 1988). Since there is extensive loss of host lipid stores in cachectic animals and a preference for lipids in the cachectic state, the overproduction of a lipolytic factor by the MAC16 tumour and the increased lipolytic activity in the body fluids of cachectic cancer patients may be the key to understanding the causes of cachexia in some cancer patients. Further investigations into the mechanism of action and a structural elicidation of this factor may lead to the ability to reverse cachexia and perhaps even inhibit tumour growth.

SECTION 5: CONCLUSION

5.0 Conclusion

The results presented in section 4 confirm that the MAC16 adenocarcinoma is a good model with which to study alterations in host metabolism in tumour-induced cachexia, since it induces weight loss in the host at only small tumour burdens and without any accompanying anorexia.

Although glucose consumption by the colon adenocarcinomas of the MAC series was high and comparable with that of the brain *in vivo*, the rate of utilisation by the MAC16 tumour was lower than the MAC13 tumour and independent of the production of cachexia in the host. This suggests that high glucose consumption by the tumour is not sufficient to explain the loss of host body compartments that occurs in cachexia. The tumour-bearing state induced profound changes in glucose utilisation by host organs, particularly the brain, where glucose utilisation *in vivo* was severely depressed. Evidence from an *in vitro* assay system presented in section 4 confirms the importance of glucose as a fuel for oxidative metabolism in the brain of non tumour-bearing animals. Brain glucose metabolism was, however, reduced in the tumour-bearing state, especially in animals bearing the MAC16 tumour, where its use was replaced by lactate and 3-hydroxybutyrate. Ketone bodies have been shown to replace glucose as the predominant fuel for brain metabolism during starvation (Owen, et al, 1967).

This suggests that the brains of cachectic animals bearing the MAC16 tumour resemble that during starvation, although food intake is not decreased in these animals. Oxidation of 3-hydroxybutyrate in vivo was elevated in both tumour-bearing states, suggesting organ metabolism in tumour-bearing animals is supported by an increased utilisation of ketone bodies.

The decreased glucose utilisation by peripheral tissues in the tumour-bearing state suggests an increased dependence on fat as an energy source. In view of its high calorific value, fat is an important fuel source when the metabolic demands of an organism are high. Studies involving MAC16 tumour-bearing animals indicate that metabolic rates are elevated in these animals, since there is an increase in oxygen consumption and an increased activity of brown adipose tissue during the period of weight loss (N. Rothwell, personal communication). While respiration from glucose was elevated in tumour-bearing animals, irrespective of cachexia, (probably due to glucose consumption by the tumour), respiration from administered palmitate and triolein was specifically increased in cachectic MAC16 tumour-bearing animals, indicating that the tissues of such animals are more dependent on lipids as an energy source. This increased requirement for lipid is shown not only by catabolism of adipose tissue, but also by an increased conversion of glucose to lipids. This would

result in a reduction in the amount of utilisable energy obtained from the intake of a given amount of carbohydrate. The increased lipid and glucose oxidation in the cachectic state suggests that energy requirements are increased in cachexia. Although energy intake was increased in MAC13 tumour-bearing animals, it was not increased in MAC16 tumour-bearing animals when compared with non tumour-bearing controls. The increased metabolic demand therefore results in an energy deficit leading to progressive weight loss in the host.

The MAC16 adenocarcinoma has been shown to produce circulatory catabolic factors that have lipolytic and proteolytic activities in the host (Beck, et al, 1990). Lipolytic factors of similar molecular weights have also been isolated in small amounts from other non-cachectic tumour models and in the body fluids of tumour-bearing animals and cancer patients (Beck, 1989). Injection of this partially purified lipolytic factor into non tumour-bearing animals produced a significant but transient weight loss. A greater, sustained weight loss could be achieved in non-cachectic MAC16 and MAC13 tumour-bearing animals. Injection of smaller doses of lipolytic factor was able to to produce weight loss in recipient tumour-bearing animals without any reduction in food and water intakes. Since weight loss was only maintained in tumour-bearing animals, the tumour may be acting as a sink

for the free fatty acids produced as a result of the mobilisation of triglycerides from adipose stores.

The importance of this tumour-associated lipolytic factor in the clinic depends on its abundance in human tumours. If it is present in a large number tumours it may be playing an essential role in tumour growth, with an overexpression of the factor resulting in cachexia and the accompanying defects in lipid and carbohydrate metabolism discussed in this section. Future experiments must aim to further investigate the metabolic effects of tumour growth and the effects of tumour-produced or associated catabolic factors on host metabolism. Comprehensive studies on the actions of these tumour-associated catabolic factors in vivo depend on their complete purification and subsequent structural elucidation. Isolation, sequencing and synthesis of these factors are important, since they may have a number of potential uses, such as tumour markers for early diagnosis of cancer, or the production of monoclonal antibodies for treating and suppressing the symptoms of cachexia. Finally, if these factors are essential for tumour growth, but not involved in the growth of normal non-malignant cells in the body, they may provide a novel and selective therapeutic action for the treatment of cancer.

REFERENCES

AISENBERG, A. C. and MORNS, H. P. (1961) Energy pathways of hepatoma No. 5123. Nature, 191, 1314 - 1315

ANDERSON, H. L., BENEVENGA, N. J. and HARPUR, A. E. (1968) Associations among food and protein intake, serine dehydratase and plasma amino acids. Am. J. Physiol., 214, 1008 - 1013

ARGILES, J. M. and AZCON-BIETO, J. (1988) The metabolic environment of cancer. Molec. and Cell. Biochem., 81, 3 - 17

ARGILES, J. M., LOPEZ-SORIANO, F. J., EVANS, R. D. and WILLIAMSON, D. H. (1989) Interleukin-1 and lipid metabolism in the rat. Biochem. J., 259, 673 - 678

BAGINSKI, E. S., FOA, P. P. and ZAK, B. (1974) Glucose - 6 - phosphatase. Methods of enzymatic analysis, Volume 2, pp. 876 - 880, Ed. Bergmeyer, H. U.

BAILLE, C. A., ZINN, W. M. and MAYER, J. (1970) Effects of lactate and other metabolites on food intake of monkeys. Am. J. Physiol., 219, 1606 - 1613.

BAKER, N., SANDBORG, C., MORNS, D. and OOKHTENS, M. (1977) Competition for host essential and non-essential fatty acids in Ehrlich ascites carcinoma in mice. Cancer Res., 37, 2218 - 2225

BAKER, N., HILL, V., JACOBSON, M. and BRUCKDORFER, K. R. (1984) Fatty acid metabolism in adipose tissue of ageing mice after direct tracer injection into fat pads. Mechanisms of Ageing and Development, 27, 295 - 313

BALDUCCI, L. and HARDY, C. (1985) Cancer and malnutrition : a critical interaction. Am. J. hematol., 18, 91 - 103

BECK, S. A. (1989) Catabolic factors in tumour-induced cachexia. PhD Thesis, Aston University, Birmingham

BECK, S. A. and TISDALE, M. J. (1987) Production of lipolytic and proteolytic factors by a murine tumour-producing cachexia in the host. Cancer Res., 47, 5919 - 5923

BECK, S. A. and TISDALE, M. J. (1989a) Effect of insulin and ketone bodies on tumour growth and host body weight in a cachexia model. (Abstract) Joint meeting of the 30th British Association for Cancer Research and the 4th meeting of the Association of Cancer Physicians, Glasgow, UK.

BECK, S. A. and TISDALE, M. J. (1989b) Nitrogen excretion in cancer cachexia and its modification by a high fat diet. Cancer Res., 49, 3800 - 3804

BECK, S. A., MULLIGAN, H. M. and TISDALE M. J. (1990) Lipolytic factors associated with murine and human cancer cachexia. J. Natl. Cancer Inst., 82, 1922 - 1926

BENSON, R. C., RIGGS, B. L., PICKARD, B. M. and ARNAUD, C, D. (1974) Radioimmunoassay of parathyroid hormone in hypercalcaemic patients with malignant disease. Am. J. Med., 56, 821 - 826

BERGMEYER, H. A. (1974) Fructose-6-phosphate kinase. In: Methods of Enzymatic Analysis, volume 1, pp. 451.

BERNSTEIN, I. L. (1986) Etiology of anorexia in cachexia. Cancer, 58, 1881 - 1886

BEUTLER, B., MAHONEY, J., LETRANG, N., PEKALA, P. and CERAMI, A. (1985) Purification of cachectin, a lipoprotein lipase - suppressing hormone secreted by endotoxin-induced RAW 264.7 cells. J. Exp. Med., 161, 984 - 995

BEUTLER, B. and CERAMI, A. (1986) Cachectin and Tumour necrosis factor as two sides of the same biological coin. Nature, 320, 584 - 588

BHARGAVA, P. M. (1977) Regulation of cell division and malignant transformation. A new model for control by uptake of nutrients. J. Theoret. Biol., 68, 101 - 137

BJORNTORP, F. (1966) Effect of ketone bodies on lipolysis in adipose tissue *in vitro*. J. Lipid Res., 7, 621 - 626

BLACKWELL, J. B. (1961) Malabsortion in the presence of primary carcinoma of the small bowel. Gut, 2, 337 - 347.

BOWMAN, W. C. and RAND, M. J. (1980) Subcellular organisation and cellular metabolism. In: Textbook of pharmacology, 2nd edition. Blackwell Scientific press. pp 213 - 234

BOXER, G. E. and DEVLIN, T. M. (1961) Pathways of intracellular hyhdrogen transport. Science, 134, 1495 - 1501

BRADFORD, M. M. (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilising the principal of protein-dye binding. Anal. Biochem., 72, 248 - 254

BRENNAN, M. F. (1977) Uncomplicated starvation versus cancer cachexia. Cancer Res., 37, 2359 - 2364.

BRIDDON, S., BECK, S. A. and TISDALE, M. J. (1991) Cancer Lett. In press

BUSE, M. G., BIGGERS, J. F., FRIDERICI, K. H. and BUSE, J. F. (1972) Oxidation of branched chain amino acids by isolated hearts and diaphragms of the rat. The effects of fatty acids, glucose and pyruvate respiration. J. Biol. Chem., 247,, 8085 - 8096

BUZBY, G. P., MULLEN, J. L., STEIN, P., MILLER, E. E., HOBBS, C. L. and ROSATO, E. F. (1980) Host - tumour interaction and nutrient supply. Cancer, 45, 2940 - 2948

BYGRAVE, F. L. (1976) Mitochondrial calcium transport and the regulation of metabolism by calcium in tumour cells. In: Control mechanisms in cancer. New York Raven press, Eds. Criss, Onon and Sabine, pp. 411 - 423

CARRASCOSA, J. M., MARTINEZ, P.and NUNEZ DE CASTRO, I. (1984) Nitrogen movement between host and tumour in mice inoculated with Ehrlich ascitic tumour cells. Cancer Res., 44, 3831 - 3835

CARSON, J. A. S. and GORMICAN, A. (1977). Taste acuity and food attitudes of selected patients with cancer. J. Am. Diet. Assoc., 70, 361 - 365

CEDERBAUM, A. I., BECKER, F. F. AND RUBIN, E. (1976) Ethanol metabolism by a transplantable hepatocellular carcinoma; role of microsomes and mitochondria. J. Biol. Chem., 251, 5366 - 5374

CERAMI, A., IKEDA, Y., LETRANG, N., HOTEZ, P. J. and BEUTLER, B. (1985) Weight loss associated with an endotoxin-induced mediator from peritoneal macrophages: the role of cachectin (tumour necrosis factor). Immunology letters, 11, 173 - 177.

CHANCE, W. T., VAN LAMMEREN, F. M., CHEN, M. H., MURPHY, R. F., JOFFE, S.N. and FISCHER, J. E. (1983) Alteration in plasma levels of insulin and glucagon associated with cancer anorexia. Surg. Forum, 34, 441 - 443

CHLEBOWSKI, R. T., HEBER, D. and RICHARDSON, B. (1984) Influence of hydrazine sulfate on abnormal carbohydrate metabolism in cancer patients with weight loss. Cancer Res., 44, 857 - 861

CLARK, C. M. and GOODLAD, G. A. J. (1971) Depletion of proteins of phasic and tonic muscles in tumour-bearing rats. Europ. J. Cancer, 7, 3 - 9

COHN, S. H., VARTSKY, D. and VASWANI, A. N. (1982) Changes in body composition of cancer patients following combined nutritional support. Nutrition and cancer, 4, 107 - 119

CONYERS, R. A. J., NEED, A. G., DURBRIDGE, T. (1979a) Cancer ketosis and parenteral nutrition. Med. J. Australia, 1, 398 - 399

CONYERS, R. A. J., NEED, A. G., ROFE, A. M., POTEZNEY, N. and KIMBER, R. J. (1979b) Nutrition and cancer. British Medical Journal, 1, 1146

COPELAND, E. M., RODMAN, C. A. and DURICK, S. J. (1979) Nutritional concepts of neoplastic disease. In : Nutrition and cancer. Eds. Van Eys, J., Seelig, M. S. and Nichols, B. L. Spectrum. New York pp. 133 - 156

COSTA, G. (1963) Cachexia, the metabolic component of neoplastic disease. Prog. Exp. Tumour Res., 3, 321 - 369

COX, R. P. and GHOSH, N. K. (1978) Current concepts in the ectopic production of foetal proteins and hormones by neoplastic cells. Am. J. Med. Sci., 275, 232 - 248

DAGLEY, S. (1974) Citrate. UV Spectrophotometric determination. In : Methods of enzymatic analysis, volume 3, pp. 1562 - 1565 Ed. Bergmeyer, H. U.

DEMETRAKOPOULOS, G. E. and BRENNAN, M. F. (1982) Tumoricidal potential of nutritional manipulations. Cancer Res., 42, (Supplement) 756s - 765s

DEVEREUX, D. F., ROBY, C., DECKERS, P. J., PIETRAFITTA, J. and MOZDEN, P. (1982) Effect of tumour bearing and removal on lipid metabolism in the rat. Surg. Forum, 33, 406 - 408

DE WYS, W. D. and HERBST, S. H. (1977) Oral feeding in the nutritional management of the cachexia patient. Cancer Res., 37, 2429 - 2431)

DE WYS, W. D. (1978) Changes in taste sensation and feeding behaviour in cancer patients: a review. J. Hum. Nutr., 32, 447 - 453

DE WYS, W. D. (1979) Anorexia as a general effect of cancer. Cancer, 43, 2013 - 2019

DE WYS, W. D. (1985) Management of cancer cachexia. Seminars in Oncology, 12, 452

DICKERSON, J. W. T. (1984) Nutrition in the cancer patient : a review. J. Royal Soc. Med., 77, 309 - 315

DONALDSON, S. S. (1977) Nutritional consequences of radiotherapy. Cancer Res., 37, 2407 - 2413

DONALDSON, S. S. and LENON, R. A. (1979) Alterations of nutritional status. Impact of chemotherapy and radiation therapy. Cancer, 43, 2036 - 2052

DOUBLE, J. A., BALL, C. R. and COWEN, P. N. (1975) Brief communication: Transplantation of adenocarcinoma of the colon in mice. J. Natl. Cancer Inst., 54, 271 - 275

EDEN, E., EDSTROM, S., BENNEGARD, K., SCHERSTEIN, T. and LUNDHOLM, K. (1984) Glucose flux in relation to energy expenditure in malnourished patients with and without cancer during periods of fasting and feeding. Cancer Res., 44, 1718 - 1724

ELLISON and NEVILLE (1973) Neoplasia and ectopic hormone production. In : Modern trends in oncology Vol 1 R.W. Ravern (Ed) (London : Butterworth), 163 - 181

EMERY, P. W., LOVELL, L. and RENNIE, M. J. (1984) Protein synthesis measured in vivo in muscle and liver of cachectic tumour-bearing mice. Cancer Res., 44, 2279 - 2284

EVANS, R. D. and WILLIAMSON, D. H. (1988a) Tissue-specific effects of rapid tumour growth on lipid metabolism in the rat during lactation and on litter removal. Biochem. J., 252, 65 - 72

EVANS, R. D. and WILLIAMSON, D. H. (1988b) Tumour necrosis factor-alpha (cachectin) mimics some of the effects of tumour growth on the disposal of a [^{14}C]lipid load in virgin, lactating and litter-removed rats. Biochem. J., 256, 1055 - 1058

FEARON, K. C. H., TISDALE, M. J., PRESTON, T., PLUMB, J. A. and CALMAN, K. C. (1985) Failure of systemic ketosis to control cachexia and the growth rate of the Walker 256 carcinosarcoma in rats. Br. J. Cancer, 52, 87 - 92

FEARON, K. C. H., and CARTER, D. C. (1988a) Cancer cachexia. Ann. Surg., 208, 1 - 5

FEARON, K. C. H., BORLAND, W., PRESTON, T., TISDALE, M. J., SHENKIN, A. and CALMAN, K. C. (1988b) Cancer cachexia : influence of systemic ketosis on substrate levels and nitrogen metabolism. Am. J. Clin. Nutr., 47, 42 - 48

FEARON, K. C. H., McMILLAN, D. C., PRESTON, T., WINSTANLEY, P., CRUICKSHANK, A. M. and SHENKIN, A. (1988c) Elevated circulating interleukin-6 is associated with an acute-phase response, but reduced fixed hepatic protein synthesis in patients with cancer. Ann. Surg., 213, 26 - 31

FELDMAN, J. M. and PLONK, J. W. (1976) Effect of intravenous glucose and insulin on plasma tryptophan and tyrosine concentrations in normal subjects and patients with carcinoid tumours. Metabolism, 25, 97 - 103

FENSELAU, A., WALLIS, K. and MORRIS, H. P. (1975) Acetoacetate coenzyme A transferase activity in rat hepatomas. Cancer Res., 35, 2315 - 2320

FERRE, P., LETURQUE, A., BURNOL, A., PENICAUD, L. and GIRARD, J. (1985) A method to quantify glucose utilisation in vivo in skeletal muscle and white adipose tissue of the anaesthetised rat. Biochem. J., 228, 103 - 110

FIELDS, A. L. A., WOLMAN, S. A., CHEEMA-DHADLI, S., MORRIS, H. P. and HALPERIN, M. L. (1981) Regulation of energy metabolism in Morris hepatoma 7777 and 7800. Cancer Res., 41, 2762 - 2766

FIELDS, A. L. A., CHEEMA-DHADLI, S., WOLMAN, S. L. and HALPERIN, M. L. (1982) Theoretical aspects of weight loss in patients with cancer. Cancer, 50, 2183 - 2188

FLETCHER, M. J. (1968) A colorimetric method of estimating serum triglycerides. Clin. Chim. Acta, 22 393 - 396

FREDERICK, G. L. and BEGG, R. W. (1956) A study of hyperlipidaemia in the tumour bearing rat. Cancer Res., 16, 548 - 558

FREUND, H., HOOVER, H. C. Jr., ATAMIAN, S. and FISCHER, J. E. (1979) Infusion of the branched chain amino acids in postoperative patients. Anticatabolic properties. Annals of Surgery, 190, 18 - 23

FURST, P., BERGSTROM, J., HELLSTROM, B., VINNARS, E., HERFARTH, O., KLIPPEL, C., MERKEL, N., SCHULTIS, K., ELWYN, D., HARDY, M. and KINNEY, J. (1981) Amino acid metabolism in cancer. In : Nutrition and metabolism in cancer. George Thieme Verlag. Stuttgart pp. 75 - 89

GLICKSMAN, A. S. and RAWSON, (1956) Diabetes and altered carbohydrate metabolism in patients with cancer. Cancer, 9, 1127 - 1134

GOLD, J. (1968) Proposed treatment of cancer by inhibition of gluconeogenesis. Oncology, 22, 185 - 207

GOLD, J. (1978) Potentiation by clofibrate of in vivo tumour inhibition by hydrazine sulphate and cytotoxic agents in Walker 256 carcinosarcoma. Cancer Biochemistry Biophysics, 3, 41 - 45

GOLD, J. and SYRACUSE, M. D. (1987) Tumour glycolysis - host gluconeogenesis in cancer cachexia. Arch. Surg., 122, 850

GOODLAD, G. A. J. and CLARK, C. M. (1972) Activity of gastrocnemius and soleus polyribosomes in rats bearing the Walker 256 carcinoma. Europ. J. Cancer, 8, 647 - 651

GOODLAD, G. A. J., MITCHELL, A. J. H., McPHAIL, L. and CLARK, C. M. (1975) Serum insulin and somatomedin levels in the tumour bearing rat. Eur. J. Cancer, 11, 733 - 737

GORSHEIN, D. (1977) Posthypophysectomy taste abnormalities. Their relationship to remote effects of cancer. Cancer, 39, 1700 - 1703

GRIFFITHS, J. R. (1987) Monitoring cancer therapy by nuclear magnetic resonance spectroscopy. In: Physiological nuclear magnetic resonance spectroscopy: From isolated cells to man. Annals. N.Y. Acad. Sci., 508, 183 - 199

GRUBBS, B., ROGERS, W. and CAMERON, I. (1979) Total parenteral nutrition and inhibition of gluconeogenesis on tumour - host responses. Oncology, 36, 216 - 223

GUTMANN, I. and WAHLEFIELD, A. W. (1974) L-(+)-Lactate. Determination with lactate dehydrogenase and NAD. Methods of enzymatic analysis, volume 3, pp. 1464 - 1468. Ed. Bergmeyer, H. U.

GUYNN, R. W., VELOSO, D. and VEECH, R. L. (1972) The concentration of malonyl-coenzyme A and the control of fatty acid synthesis in vivo. J. Biol. Chem., 247, 7325 - 7331

HANNAFORD, M. C., LEITER, L. A., JOSSE, M. B., GOLDSTEIN, E. B. and HALPERIN, M. L. (1982) Protein wasting due to acidosis of prolonged fasting. Am. J. Physiol., 243, E251 - E256

HARDY, C., WALLACE, C., KHANSUR, T., VANCE, R.B., THIGPEN, J. T. and BALDUCCI, L. (1986) Nutrition, cancer and ageing : an annotated review. J.A.G.S., 34, 219 - 228

HARNET, W. L. (1952) A survey of cancer in London. British Empire Cancer Campaign, London. pp. 26

HARPER, H. A., RODWELL, V. W. and MAYES, P. A. (1979) Metabolism of lipids: II Role of the tissues. In: Review of Physiological chemistry, 17th Edition. Lange Medical publications, Chapt. 23, 343

HATANAKA, M. (1974) Transport of sugars in tumour cell membranes. Biochem. Biophys. Acta, 355, 77 - 104

HAWKINS, R. A., ALBERTI, K. G. M. M., HOUGHTON, C. R. S., WILLIAMSON, D. H. and KREBS, H. A. (1971) The effect of acetoacetate on plasma insulin concentration. Biochem. J., 125, 541 - 544

HEBER, D., CHLEBOWSKI, R.T., ISHIBASHI, D.E., HERROLD, J. N. and BLOCK, J. B. (1982) Abnormalities in glucose and protein metabolism in non-cachectic lung cancer patients. Cancer Res., 42, 4815 - 4819

HOLROYDE, C. P., GABUZDA, T. G., PUTNAM, R. C., PAUL, P. and REICHARD, G. A. (1975) Altered glucose metabolism in metastatic carcinoma. Cancer Res., 35, 3710 - 3714

HOLROYDE, C. P. and REICHARD, G. A. (1981) Carbohydrate metabolism in cancer cachexia. Cancer Treat. Report., 65, 55 - 58

HOLROYDE, C. P., SKUTCHES, C. L., BODEN, G. and REICHARD, G. A. (1984) Glucose metabolism in cachectic patients with colorectal cancer. Cancer Res., 44, 5910 - 5913

HUME, D. A. and WEIDERMAN, M. J. (1979) Role and Regulation of glucose metabolism in proliferating cells. J. Natl. Cancer Inst., 62, 3 - 8

HYVARINEN, A. and NIKKILA, E. A. (1962) Specific determination of blood glucose with o-toluidine. Clin. Chim. Acta, 7, 140 - 143

INAGAKI, J., RODRIGUEZ, V., and BODEY, G. P. (1974) Causes of death in cancer patients. Cancer, 33, 568 - 573

INOUE, H. and LOWENSTEIN, J. M. (1975) Acetyl Coenzyme A carboxylase from rat liver. Methods in enzymology, Volume 35, Lipids part B. Ed. Lowenstein, J. M. pp. 3 - 11

JAIN, R. K., SHAH, S. A. and FINNEY, P. L. (1984) Continuous non-invasive monitoring of pH and temperature in rat Walker 256 carcinoma during normoglycaemia and hyperglycaemia. J. N. C. I., 73, 429 - 436

JENKINS, A. B., FURLER, S. M. and KRAEGEN, E. W. (1986) 2-Deoxy-D-glucose metabolism in individual tissues of the rat in vivo. Int. J. Biochem., 18, 311 318

KELLEY, M. L., TROUP, S. B., LOGAN, V. W. and TERRY, R. (1961) Carcinoma of Cecum with primary malabsorption syndrome. Arc. Int. Med., 108, 284 - 291

KESSLER, G. and LEDERER, H. (1966) Fluorometric measurement of triglycerides. In : Automation in analytic chemistry (Ed. Skeggs, L.T.) Mediad Inc. New York pp. 341 - 344

KIEN, C. L. and CAMITTA, B. M. (1983) Increased whole-body protein turnover in sick children with newly diagnosed leukaemia or lymphoma. Cancer Res., 43, 5586 - 5592

KISNER, D., HAMOSH, M., BLECHER, M., HALLER, D., JACOBS, E., PETERSON, B. and SCHEIN, P. (1978) Malignant cachexia: insulin resistance and insulin receptors. Proc. Am. Assoc. Cancer Res., 19, 199 (abstract)

KITADA, S., HAYS, E. F. and MEAD, J. F. (1980) A lipid mobilising factor in the serum of tumour-bearing mice. Lipids, 15, 168 - 174

KNOX, L. S. (1983) Nutrition and cancer. Nursing clinics of North America, 18, 97 - 109

LACKNER, R. A., CHALLISS, R. A. J., WEST, D. and NEWSHOLME, A. A. (1984) A problem in the radiochemical assay of glucose-6-phosphatase in muscle. Biochem. J., 218, 649 - 651

LANZA-JACOBYS, S., MILLER, E. E. and ROSATO, F. E. (1982) Changes in the activities of lipoprotein lipase and the lipogenic enzymes in tumour-bearing rats. Lipids, 17, 944 - 949

LANZA-JACOBYS, S., LANSEY, S. C., MILLER, E. E. and CLEARY, M. P. (1984) Sequential changes in the activities of lipoprotein lipase and lipogenic enzymes during tumour growth in rats. Cancer Res., 44, 5062 - 5967

LAWSON, D. H., RICHMOND, A., NIXON, D. W. and RUDMAN, D. (1982) Metabolic approaches to cancer cachexia. Ann. Rev. Nutr., 2, 227 - 301

LASZLO, J. Ed. (1986) In: Physicians guide to cancer care complications : Prevention and management. pp. 48 - 52, Marcel Dekker

LAZO, P. A. (1981a) Amino acids and glucose utilisation by different metabolic pathways in ascites tumour cell. Eur. J. Biochem., 117, 19 - 25

LAZO, P. A. (1981b) Tumour induction of host leucine starvation. FEBS Letters, 135, 229 - 231

LAZO, P. A. and SOLS, A. (1980) Energetics of tumour cells : Enzymic basis of aerobic glycolysis. Biochemical Society Transactions, 8, (5), 579

LEVINSON, M. R., GROEGER, J. S., JEEVANANDAM, M. and BRENNAN, M. F. (1988) Free fatty acid turnover and lipolysis in septic mechanically ventilated cancer-bearing humans. Metabolism, 37, 618 - 625

LINDSEY, A. M. (1986) Cancer cachexia: Effects of the disease and its treatment. Seminars in Oncology nursing, 2, 19 - 29

LONNROTH, C., MOLDAWER, L. L., GELIN, J., KINDBLOM, L., SHERRY, B. and LUNDHOLM, K. (1990) Tumour necrosis factor-alpha and Interleukin-1 production in cachectic tumour-bearing mice. Int. J. Cancer, 46, 889 - 896

LUNDHOLM, K., EDSTROM, S., KARLBERG, I., EKMAN, L. and SCHERSTEN, T. (1980) Relationship of food intake, body composition and tumour growth to host metabolism in non-growing mice with sarcoma. Cancer Res., 40, 2516 - 2522

LUNDHOLM, K., EDSTROM, S., EKMAN, L., KARLBERG, I. and SCHERSTEIN, T. (1981) Metabolism in peripheral tissues in cancer patients. Cancer Treatment Reports, 65, (suppl. 5) 79 - 83

LUNDHOLM, K., EDSTROM, S., KARLBERRY, I., EKMAN, L. and SCHERSTEN, T. (1982) Glucose turnover, gluconeogenesis from glycerol and estimation of net glucose cycling in cancer patients. Cancer, 50, 1142 - 1150

MAGEE, B. A., POTEZNEY, N., ROFE, A.M. and CONYERS, R. A. J. (1979) The inhibition of malignant cell growth by ketone bodies. Australian J. Exp. Biol. and Med. Sci., 57, 529 - 539

MAHONY, S. M., BECK, S. A. and TISDALE, M. J. (1988) Comparison of weight loss induced by recombinant tumour necrosis factor with that produced by a cachexia-inducing tumour. Br. J. Cancer, 57, 385 - 389

MAHONY, S. M., and TISDALE, M. J. (1988) Induction of weight loss and metabolic alterations by human recombinant tumour necrosis factor. Br. J. Cancer, 58, 345 - 349

MALLINSON, C. N., BLOOM, S. R., WARIN, A. P., SALMON, P. R. and COX, B. (1974) A glucagonoma syndrome. Lancet, 2, 1 - 5

MANNEL, D. N., MOORE, R. N., and MERGENHAGEN, S. E. (1980) Macrophages as a source of tumoricidal activity (tumour necrotizing factor). Infec. Immun., 30, 523 - 530

MASUNO, H., YAMASAKI, N. and OKUDA, H. (1981) Purification and characterisation of a lipolytic factor (Toxohormone-L) from cell-free fluid of Ascites sarcoma 180. Cancer Res., 41, 284 - 288

MASUNO, H., YOSHIMURA, H., OGAWAS, N. and OKUDA, H. (1984) Isolation of a lipolytic factor (Toxohormone-L) from ascites fluid of patients with hepatoma and its effects on feeding behaviour. J. Cancer Clin. Oncol., 20, 1177 - 1185

MATTHEWS, N. and WATKINS, J. F. (1978) Tumour necrosis factor from the rabbit. I. Mode of action, specificity and physicochemical properties. Br. J. Cancer, 38, 302 - 315

MCALLISTER, R. A., CAMPBELL, E. H. G. and CALMAN, K. C. (1982) Metabolic changes in liver of tumour bearing mice. J. Surg. Res., 33, 500 - 509

McINTOSH, J. K., JABLONS, D. M., MULE, J. J., NORDAN, R. P., RUDIKOFF, S., LOTZE, M. T. and ROSENBERG, S. A. (1989) In vivo induction of IL-6 by administration of exogenous cytokines and detection of de novo serum levels of IL-6 in tumour-bearing mice. J. Immunol., 143, 162 - 167

MEDES, G. and WEINHOUSE, S. (1958) Metabolism of neoplastic tissue XIII. Substrate competition in fatty acid oxidation in Ascites tumor cells. Cancer Res., 18, 352 - 359

MELLANBY, J. and WILLIAMSON, D. H. (1974) Acetoacetate. In: Methods of Enzymatic Analysis, Volume 4. pp. 1840 - 1843. Ed. Bergmeyer, H. U.

MESZAROS, K., BAGBY, G. J. LANG, C. H. and SPITZER, J. J. (1987a) Increased uptake and phosphorylation of 2-deoxyglucose by skeletal muscles in endotoxin-treated rats. Am. J. Physiol., 253, E33 - E39

MESZAROS, K., LANG, C. H., BAGBY, G. J. and SPITZER, J. J. (1987b) Contribution of different organs to increased glucose consumption after endotoxin administration. J. Biol. Chem., 262, 10965 - 10970

MIDER, G. B. (1951) Some aspects of nitrogen and energy metabolism in cancerous subjects. Cancer Res., 11, 821 - 829

MILES, J. M. and HAYMOND, M. W. (1983) 3-hydroxybutyrate to conserve protein during therapeutic starvation. The Lancet, Feb. 19, pp. 409

MORRISON, S. D. (1973) Control of food intake during growth of a Walker 256 carcinosarcoma. Cancer Res., 33, 526 - 528

MUELLER, P. S. and WATKIN, D. (1961) Plasma unesterified fatty acid concentrations in neoplastic disease. J. Lab. Clin. Med., 57, 95 - 108

NAKAMURA, K., NAKAJIMA, Y. and NAKAMURA, Y. (1986) Suppression of glucose utilisation of murine peritoneal exudate macrophages by body fluids from cancer patients and identification of the susceptible enzyme. J. N. C. I., 77, 1035 - 1038

NATHANSON, L. and HALL, T. C. (1974) A spectrum of tumours that produce paraneoplastic syndromes. Lung tumours : how they produce their syndromes. Ann. N. Y. Acad. Sci., 230, 367 - 377

NIXON, D. W., HEYMSFIELD, S. B. and COHEN, A. E. (1980) Protein calorie undernutrition in hospitalised cancer patients. Am. J. Med., 68, 683 - 690

NIXON, D. W., LAWSON, D. H., KUTNER, M., ANSLEY, J. and SCHWARZ, M. (1981) Hyperalimentation of the cancer patient with protein - calorie undernutrition. Cancer Res., 41, 2038 - 2045

NOLOP, K. B., RHODES, C. R., BRUDIN, L. H., BEANEY, R. P., KRAUSZ, T., JONES, T. and HUGHES, J. M. B. (1987) Glucose utilisation in vivo by humanpulmonary neoplasms. Cancer 60, 2682 - 2689

NORTON, J. A., MOLEY, J. F., GREEN, M. V., CARSON, R. E. and MORRISON, S. D. (1985) Parabiotic transfer of cancer anorexia/cachexia in male rats. Cancer Res., 45, 5547 - 5552

ODELL, W. D. and WOLFSEN, A. R. (1980) Hormones from tumours : Are they ubiquitous? Am. J. Med., 68, 317 - 318

OHE, K., MORRIS, H. P. and WEINHOUSE, S. (1967) ß-hydroxybutyrate dehydrogenase activity in liver and liver tumours. Cancer Res., 27, 1360 - 1371

OHNUMA, T. and HOLLAND, J. F. (1977) Nutritional consequences of cancer chemotherapy and immunotherapy. Cancer Res., 37, 2395 - 2406

OLIFF, A., DEFEO-JONES, D., BOYER, M., MARTINEZ, D., KLEFER,D., VUOCOLO, G., WOLFE, A. and SOCHER, S. H. (1987) Tumors secreting human TNF/cachectin induce cachexia in mice. Cell, 50, 555 - 563

OLLER do NASCIMENTO, C. M. and WILLIAMSON, D. H. (1986) Evidence for conservation of dietary lipid in the rat during lactation and the immediate period after removal of the litter : Decreased oxidation of oral (1-^{14}C)-triolein. Biochem. J., 239, 233 - 236

OOKHTENS, M. and BAKER, N. (1979) Fatty acid oxidation to H_2O by Ehrlich ascites carcinoma in mice. Cancer Res., 39, 973 - 980

OOKHTENS, M., KANNAN, R., LYON, I. and BAKER, N. (1984) Liver and adipose tissue contributions to newly formed fatty acids in an ascites tumour. Am J. Physiol., 247, R146 - R153

OWEN, O.E., MORGAN, A. P., KEMP, H. G., SULLIVAN, J. M., HERRARA, M. G. and CAHILL, G. F. (1967) Brain metabolism during fasting. J. Clin. Invest., 46, 1589 - 1595

PAVELIC, K. and SLIJEPCEVIC, M. (1978) Growth of a thymoma in diabetic mice treated with insulin. Eur. J. Cancer, 14, 675 - 679

PAWAN, G. L. S. and SEMPLE, S. J. G. (1983) Effect of 3-hydroxybutyrate in obese subjects on very-low-energy diets and during therapeutic starvation. The Lancet, Jan. 1/8, pp. 15 - 17

PEDERSON, P. L. (1978) Tumour mitochondria and the bioenergetics of cancer cells. In : Progress in Experimental Tumour Research Vol. 22. Karger, Basel. pp. 190 - 274

REDGRAVE, T. G., DEVEREUX, D. F. and DECKERS, P. J. (1984) Hyperlipidaemia in tumour-bearing rats. Biochim. et Biophys. Acta., 795, 286 - 292

RICH, A. J. (1987) Nutritional status in cancer. Anticancer Res., 7, 271 - 280

ROBINSON, A. M. and WILLIAMSON, D. H. (1980) Physiological roles of ketone bodies as substrates and signals in mammalian tissues. Physiol. Rev., 60, 143 - 187

ROH, M. S., EKMAN, L., JEEVANANDAM, M. and BRENNAN, M. F. (1984) Gluconeogenesis in tumour-influenced hepatocytes. Surgery, August 1984, 427 - 434

ROUZER, C. A. and CERAMI, A. (1980) Hypertriglyceridaemia associated with Trypanosoma brucei brucei infection in rabbits : Role of defective triglyceride removal. Mol. Biochem. Parasitol., 2, 31 -38

RUFF, M. R. and GIFFORD, G. E. (1981) Tumour necrosis factor in : Lymphokine Reports, 2, 235 - 272. Pick (Ed). Academic Press : New York

SAUER, L. A. and DAUCHY, R. T. (1983) Ketone body, glucose, lactic acid and amino acid utilisation by tumours in vivo in fasted rats. Cancer Res., 43, 3497 - 3503

SAUER, L. A. and DAUCHY, R. T. (1986) In vivo lactate production and utilisation by Jensen sarcoma and Morris hepatoma 7288CTC. Cancer Res., 46, 689 - 693

SAUER, L. A. and DAUCHY, R. T. (1987a) Blood nutrient concentrations and tumour growth in vivo in rats: Relationships during the onset of an acute fast. Cancer Res., 47, 1065 - 1068

SAUER, L. A. and DAUCHY, R. T. (1987b) Stimulation of tumour growth in adult rats in vivo during acute Streptozotocin-induced diabetes. Cancer Res., 47, 1756 - 1761

SCHEIN, P. S., MACDONALD, J. S., WATERS, C. and HAIDAK, D. (1975) Nutritional complications of cancer and its treatment. Sem. Oncology, 2, 337 - 347

SCHEIN, P. S., KISNER, D., HALLER,D., BLECHER, M. and HAMOSH, M. (1979) Cachexia of malignancy. Potential role of insulin in nutritional management. Cancer, 43, 2070 - 2076

SEMB, H., PETERSON, J., TAVERNIER, J. and OLIVECRONER, T. (1987) Multiple effects of tumour necrosis factor on lipoprotein lipase in vivo. J. Biol. Chem., 262, 8390 - 8394

SHAMBAUGH, G. E. (1985) Ketone body metabolism in the mother and fetus. Federation Proc., 44, 2347 - 2351

SHAMBERGER, R. J. (1984) Cancer cachexia. In : Nutrition and cancer. Plenum press. pp. 353 - 366

SHAPOT, V. S. (1972) Some biochemical aspects of the relationship between the tumour and the host. Adv. Cancer Res., 15, 253 - 285

SHAPOT, V. S. and BLINOV, V. A. (1974) Blood glucose levels and gluconeogenesis in animals bearing transplantable tumours. Cancer Res., 34, 1827 - 1832

SHEPHERD, J. and PACKARD, C. J. (1986) An overview of the effects of p-chlorophenoxyisobutyric acid derivatives on lipoprotein metabolism. In: Pharmacological control of hyperlipidaemia. Proceedings of an international telesymposium on hyperlipidaemia. Ed. Fears, R. Pub. Prous Science, S.A.

SHERRY, B. A., GELIN, J., FONG, Y., MARANO, M., WEI, H., CERAMI, A., LOWRY, S. F., LUNDHOLM, K. G. and MOLDAWER, L. L. (1989) Anticachectin/Tumor necrosis factor-alpha antibodies attenuate development of cachexia in tumor models. F.A.S.E.B. J., 3, 1956 - 1962

SHERMAN, M. L., SPRIGGS, D. R., ARTHUR, K. A., IMUMURA, K., FREI III, E. and KAFE, D. N. (1988) J. Clin. Oncol., 6, 344 - 350

SHERWIN, R. S., HENDLER, R. G. and FELIG, P. (1975) Effect of ketone infusions on amino acid and nitrogen metabolism in man. J. Clin. Invest., 55, 1382 - 1390

SOCHER, S. H., MARTINEZ, D., CRAIG, J. B., KUHN, G. and OLIFF, A. (1988) Tumour necrosis factor not detectable in patients with clinical cancer cachexia. J. Natl. Cancer Inst., 80, 595 - 598

SOKOLOFF, L., REIVICH, M., KENNEDY, C., DES ROSIERS, M. H., PATLAK, C. S., PETTIGREW, K. D., SAKURADA, O. and SHINOHARA, M. (1977) The (^{14}C)-Deoxyglucose method for the measurement of local cerebral glucose utilisation : Theory procedure and normal values in the conscious and anaesthetised albino rat. J. Neurochemistry, 28, 897 - 916

SOMOGYI, M. (1945) Determination of blood sugar. J. Biol. Chem., 160, 69 - 73

SPECTOR, A. A. and BRENNENMAN, D. E. (1973) Role of free fatty acids and lipoproteins in the lipid nutrition of tumour cells. In: Tumour lipids, Am. oil chemists society press, pp. 1 - 13

SPECTOR, A. A. and SOBOROFF, J. M. (1971) Effect of chlorophenoxyisobutyrate on free fatty acid utilisation by mammalian cells (35701). P.S.E.M.B., 137, 945 - 947

STANSBIE, D., BROWNSEY, R.W., CRETTAZ, M. and DENTON, R. M. (1976) Acute effects in vivo of anti-insulin serum on rates of fatty acid synthesis and activities of acetyl-Coenzyme A carboxylase and pyruvate dehydrogenase in liver and epididymal adipose tissue of fed rats. Biochem. J., 160, 413 - 416

STEIN, T. P. (1978) Cachexia, gluconeogenesis and progressive weight loss in cancer patients. J. Theoret. Biol., 73, 51 - 59

STEVENS, A. N., MORRIS, P. G., ILES, R. A. and GRIFFITHS, J. R. (1984) Short communication. 5-Fluorouracil metabolism monitored in vivo by ^{19}F NMR. Br. J. Cancer, 50, 113 - 117

STOVROFF, M. C., FRAKER, D. L., SWEDENBORG, J. A. and NORTON, J. A. (1988) Cachectin/tumour necrosis factor : A possible mediator of cancer anorexia in the rat. Cancer Res., 48, 4567 - 4572

STRAIN, A. J., EASTY, G. C. and NEVILLE, A. M. (1980) An experimental model of cachexia induced by a xenografted human tumour. J. Natl. Acad. Sci., 64, 217 - 221

TEREPIKA, A. R. and WATERHOUSE, C. (1956) Metabolic observations during the forced feeding of patients with cancer. Am. J. Med., 20, 225 - 238

THEOLOGIDES, A. (1972) Pathogenesis of cachexia in cancer. Cancer, 29, 484

THOMPSON, M., KOONS, J., TAN, E. T. H. and GRIGOR, M. R. (1981) Modified lipoprotein lipase activities, rates of lipogenesis and lipolysis as factors leading to lipid depletion in C57BL6 mice bearing preputial gland tumour ESR 586. Cancer Res., 41, 3228 - 3232

TISDALE, M. J. (1982) Tumour and host nutrition. Cancer Topics, 3, 113 - 117

TISDALE, M. J. and BRENNAN, R. A. (1983) Loss of acetoacetate coenzyme A transferase activity in tumours of peripheral tissues. Br. J. Cancer, 47, 293 - 297

TISDALE, M. J. and BRENNAN, R. A. (1986) Metabolic substrate utilisation by a tumour cell line which induces cachexia in vivo. Br. J. Cancer, 54, 601 - 606

TISDALE, M. J., BRENNAN, R. A. and FEARON, K. C. (1987) Reduction of weight loss and tumour size in a cachexia model by a high fat diet. Br. J. Cancer, 56, 39 - 43

TISDALE, M. J. (1986) The future : Nutritional pharmacology. Clinics in Oncology, 5, 381 - 405

TISDALE, M. J. (1990) Newly identified factors that alter host metabolism in cancer cachexia. TIPS., 11, 473 - 475

TISDALE, M. J. and BECK, S. A. (1990) Cancer cachexia. International J. Pancreatology. The Humana Press, pp. 141 - 150

TISDALE, M. J. and BECK, S. A. (1991) Inhibition of tumour-induced lipolysis in vitro and cachexia and tumour growth in vivo by eicosapenteanoic acid. Biochem. Pharmacol., 41, 103 - 107

TISDALE, M. J. and LEUNG, Y. C. (1988) Changes in host liver fatty acid synthase in tumour-bearing mice. Cancer Lett., 42, 231 -

VLASSARA, H., SPIEGEL, R. J., DOVAL, D. S. and CERAMI, A. (1986) Reduced plasma lipoprotein lipase activity in patients with malignancy associated weight loss. Horm. Metab, Res., 18, 698 - 703

WAGLE, S. R., MORRIS, H. P. and WEBER, G. (1963) Comparative biochemistry of hepatomas V. Studies on amino acid incorporation in liver tumours of different growth rate. Cancer Res., 23, 1003 - 1007

WARBURG, O. (1930) Metabolism of tumours. Arnold Constable. London

WARNOLD, I., LUNDHOLM, K. and SCHERSTEIN, T. (1978) Energy balance and body composition in cancer patients. Cancer Res., 38, 1801 - 1807

WARREN, S. (1932) The immediate cause of death in cancer. Am. J. Med., 184, 610 - 616

WATERHOUSE, C., KEMPERMAN, J. H. and STORMONT, J. M. (1964) Alterations in triglyceride metabolism as produced by dietary change. J. Lab. Clin. Med., 63, 605 - 620

WATERHOUSE, C., JEANPETRE, N. and KEILSON, J. (1979) Gluconeogenesis from alanine in patients with progressive malignant disease. Cancer Res., 39, 1968 - 1972

WATERHOUSE, C. (1981) Oxidation and metabolic interconversion in malignant cachexia. Cancer Treatment Reports, 65, (Supplement) 61 - 66

WATSON, A. (1990) PhD Thesis, Aston University, Birmingham

WEBER, G. (1977) Enzymology of cancer cells (part 2) N. Engl. J. Med., 296, 541 - 555

WEINHOUSE, S. (1951) Studies on the fate of isotopically labelled metabolites in the oxidative metabolism of tumours. Cancer Res., 11, 585 - 591

WEINHOUSE, S. (1953) Metabolism of neoplastic tissue. V. Fatty acid oxidation in slices of transplanted tumors. Cancer Res., 13, 367 - 371

WEINHOUSE, S. (1972) Glycolysis, respiration and anomalous gene expression in experimental hepatomas : G. H. A. Clowes Memorial Lecture. Cancer Res., 32, 2007 - 2016

WEINHOUSE, S., LANGAN, J., SHATTON, J. A. and MORRIS, H. P. (1973) Fatty acids as metabolic fuels of cancer cells. In : Tumour Lipids, Am. Oil Chemists Society Press (1973) pp. 14 - 20

WHITE, D. A., MIDDLETON, B. and BAXTER, M. (1984) Hormones and metabolic control. Arnold press

WIELAND, O. (1974) Glycerol. UV-method. Methods of enzymatic analysis, volume 3, pp. 1404 - 1409. Ed. Bergmeyer. H. U.

WILLIAMS, L. R. and COHEN, M. H. (1978) Altered taste thresholds in lung cancer. Am. J. Clin. Nutr., 31, 122 - 125

WILLIAMSON, D. H. (1981) Utilisation of ketone bodies by mammalian tissues. In: Regulation of ketone-body metabolism. 592nd Meeting of the Lipid Group/Regulation in metabolism group, Joint Colloquium. London. pp. 346 - 347

WILLIAMSON, D. H., BATES, M. W., PAGE, M. A. and KREBS, H.A. (1971) Activities of enzymes involved in acetoacetate utilisation in adult mammalian tissues. Biochem. J., 121, 41 - 47

WILLIAMSON, D. H. and MELLANBY, J. (1974) D-(-)-3-Hydroxybutyrate. In : Methods of enzymatic analysis, 4, pp. 1836 - 1839

WILLSON, J., WEESE, J., WOLBERG, W. and SHUG, A. (1983) Differences between normal and cancerous colon in carnitine and CoA levels. Proc. Am. Assoc. Cancer Res., 24, 170

WISEMAN, G. and GHADIALLY, F. N. (1955) Studies in amino acid uptake by RD3 sarcoma cell suspensions *in vitro*. British J. Cancer, 9, 480 - 485

YOUNG, V. R. (1977) Energy metabolism and requirements in the cancer patient. Cancer Res., 37, 2336 - 2347

ZIELKE, H. R., OZAND, P. R., TILDON, J. T., SEVDALIAN, D. A. and CORNBLATH, M. (1976) Growth of human diploid fibroblasts in the absence of glucose utilisation. Proc. Natl. Acad. Sci. USA., 73, 4110 - 4114

APPENDIX

List of publications

Abstracts

MULLIGAN, H. D. and TISDALE, M. J.(1990)
Alterations in glucose metabolism in tumour-induced cachexia. 31st Annual meeting of the British Association for Cancer Research and the 5th Annual meeting of the Association of Cancer Physicians, University of Sussex, U.K., 19 - 22 March.

MULLIGAN, H. D. and TISDALE, M. J. (1991)
Increased lipid utilisation in the cachectic state. Science Link '91, ICI pharmaceutical Division, Macclesfield, Cheshire, U.K.

Papers

BECK, S. A., MULLIGAN, H. D., and TISDALE, M. J. (1990)
Lipolytic factors associated with murine and human cancer cachexia. J. Natl. Cancer Inst., 82, 1922 - 1926

MULLIGAN, H. D. and TISDALE, M. J. (1991) Lipogenesis in tumour and host tissues in mice bearing colonic adenocarcinomas. Br. J. Cancer, 63, (in press)

MULLIGAN, H. D. and TISDALE, M. J. (1991) Metabolic substrate utilisation by tumour and host tissues in cancer cachexia. Biochem. J., (in press)

Aston University

Content has been removed for copyright reasons

Aston University

Content has been removed for copyright reasons