UvA-DARE (Digital Academic Repository)

Hepatic amino acid and protein metabolism in non-anorectic moderately cachectic tumor-bearing rats

de Blaauw, I.; Deutz, N.E.P.; Boers, W.; von Meyenfeldt, F.

Publication date
1997

Published in
Journal of hepatology

[Link to publication](#)

Citation for published version (APA):

de Blaauw, I., Deutz, N. E. P., Boers, W., & von Meyenfeldt, F. (1997). Hepatic amino acid and protein metabolism in non-anorectic moderately cachectic tumor-bearing rats. *Journal of hepatology*, 26, 396-408.

General rights

It is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), other than for strictly personal, individual use, unless the work is under an open content license (like Creative Commons).

Disclaimer/Complaints regulations

If you believe that digital publication of certain material infringes any of your rights or (privacy) interests, please let the Library know, stating your reasons. In case of a legitimate complaint, the Library will make the material inaccessible and/or remove it from the website. Please Ask the Library: <https://uba.uva.nl/en/contact>, or a letter to: Library of the University of Amsterdam, Secretariat, Singel 425, 1012 WP Amsterdam, The Netherlands. You will be contacted as soon as possible.

Hepatic amino acid and protein metabolism in non-anorectic, moderately cachectic tumor-bearing rats

Ivo de Blaauw¹, Nicolaas E. P. Deutz¹, Willem Boers² and Maarten F. von Meyenfeldt¹

¹Department of Surgery, Fac. II, University of Limburg, Maastricht, and ²Department of Experimental Internal Medicine, Academic Medical Centre, Amsterdam, The Netherlands

Background/Aims: Cancer cachexia is characterized by loss of lean body mass. Under this condition peripheral proteins are broken down and transferred to visceral organs and the tumor. The liver is the principal organ in the regulation of protein and amino acid metabolism, but liver amino acid kinetics in cancer are unclear. Therefore, we examined the effects of increasing tumor loads on hepatic protein turnover and amino acid handling. **Methods:** A MCA-induced sarcoma was implanted subcutaneously in Lewis rats (200–225 g). Rats were studied when the tumor was 5–15% or 15–30% of body weight. Control rats were sham implanted. Under anesthesia, a primed constant infusion of para-aminohippuric acid and L-[3, 4-³H]-valine was given to calculate hepatic substrate fluxes and protein turnover. Serum α_2 -macroglobulin concentration was measured to determine the acute phase response.

Results: Carcass weight decreased approximately 10% in large-tumor-bearing rats ($p < 0.001$). Liver wet weight increased from 5.5 ± 0.1 (g) to 5.9 ± 0.2 in the small-tumor-bearing group and 7.3 ± 0.3 ($p < 0.001$) in the large-tumor-bearing group, with

minimal changes in water content. Serum α_2 -macroglobulin concentration, essential and gluconeogenic amino acid uptake by the liver increased in large-tumor-bearing animals. This contrasted with reduced liver ammonia uptake and unchanged urea production in tumor-bearing rats. In the small-tumor-bearing group liver protein synthesis increased, whereas protein breakdown remained unchanged. In the large-tumor-bearing group protein synthesis also increased, but protein breakdown decreased to zero.

Conclusions: The study shows that in tumor-bearing rats, liver uptake of essential and gluconeogenic amino acids increases without significant increases in urea or glucose production. Synthesis of both structural and export proteins, e.g. acute phase proteins, increases suggesting that the liver becomes a more efficient nitrogen-sparing and active protein-synthesizing organ during the growth of a malignant tumor.

Key words: Acute phase proteins; Amino acids; Cancer; Gluconeogenesis; Metabolism; Protein turnover; Ureagenesis.

CANCER cachexia is a clinical syndrome characterized by excessive nitrogen loss and increased protein breakdown (1). Peripheral proteins are broken down and amino acids are mobilized and transferred to visceral organs and the tumor (2,3). The liver is a central organ in maintaining amino acid homeostasis (4). The liver is capable of taking up amino acids for the production of defense peptides and proteins, glu-

coneogenesis and ureagenesis (4). Defense peptides and proteins produced in response to cancer are glutathione, acute phase proteins and immune-related proteins of the reticulo-endothelial system, e.g. Kupffer cells (5–8). In addition, previous studies also showed that synthesis of structural proteins of the liver rose in proportion to the tumor burden to maintain and enlarge liver protein mass (9).

It has long been recognized that hepatic gluconeogenesis increases in the progress of cancer (10–14). Augmented glucose production in cancer has been demonstrated to occur from glycerol, lactate and the amino acid alanine (11,12,14). Recently, glutamine has been recognized to contribute to an

Received 15 February; revised 3 June; accepted 12 June 1996

Correspondence: Ivo de Blaauw, Department of Surgery, Diaconessenhuis, P.O. Box 90.052, 5600 PD, Eindhoven, The Netherlands.

Tel.: +31-40-2335933. Fax: +31-40-2455828.

even larger extent to the glucose molecule carbon skeletons than alanine (15). Increased gluconeogenesis thus may be related to the depletion of glutamine observed in several other studies (5). Increased gluconeogenesis has also been held responsible for decreased plasma concentrations of other gluconeogenic amino acids, e.g. serine and threonine (16). If the carbon skeleton of amino acids is used to produce glucose, the amino group is processed to urea by the liver to be removed from the body by the kidney. For this reason, increased urea synthesis in the liver may occur in cancer, explaining in large part the nitrogen loss observed. Contradictory results, however, have been reported in several animal studies. In tumor-bearing mice decreased urea excretion (17,18) and increased nitrogen retention (19) are observed. In addition, decreased plasma concentrations of amino acids of the urea cycle were observed and suggested to reflect a diminished urea cycle activity (16,20).

The present study was undertaken to evaluate the effects of increasing tumor loads in rats on liver amino acid handling. We specifically wanted to evaluate how net amino acid uptake by the liver of the tumor-bearing host contributed to protein turnover, urea synthesis and net glucose release.

Materials and Methods

Animals

Female Lewis rats (200–225 g, Centralized Animal Facilities, University of Limburg, Maastricht, NL) were individually housed in metabolic cages during the experiment after an adaptation period of 3 days. Rats were given standard laboratory rat chow *ad libitum* (SRMA 1210, Hopefarms, Woerden, NL) and subjected to standard 12 hour light-dark cycle periods (7:30 A.M. to 7:30 P.M.) with room temperature maintained at 25°C. The experiments were performed in accordance with the recommendations of the Guide for the Care and Use of Laboratory Animals (21), and approved by the Ethical Committee of Animal Research of the University of Limburg.

Study procedure

The tumor was induced by injecting 10 mg methylcholantrene (MCA, Sigma W6501) subcutaneously (22). Afterwards, the tumor was maintained *in vivo* by serially transplanting tissue suspensions through an 18-G needle. MCA tumors are often used in metabolic studies in Fisher 344 rats (12,23) and are known for their rapid growth, invasion of local tissue and

absence of metastatic potential (24). In Lewis rats we previously observed no anorexia and moderate symptoms of cachexia (25).

Two studies were performed. In both experiments rats were randomly divided into three groups: a small-tumor-bearing group (ST), a large-tumor-bearing group (LT) and a control group fed *ad libitum*. In the ST group the tumor was allowed to grow until it reached 5–15% of the host body weight. In the LT group the tumor grew to 15–30% of the host body weight. To avoid effects of time, tumor tissue was transplanted bilaterally on the back in the ST group, whereas it was quadruple-implanted in the LT group. Control rats were sham implanted with saline. Tumor growth was monitored by measuring two

Fig. 1. Three-compartment model to describe *in vivo* membrane transport and protein kinetics of the liver (29,35). Valine enters the liver via the portal and arterial pool and leaves via the hepatic venous pool. Membrane transport rates from the arterial-portal venous pool to the intracellular free amino acid pool ($F_{L,A}$), from the intracellular pool to the hepatic venous pool ($F_{H,L}$) and direct shunting ($F_{H,A}$), are calculated as described previously (29,35). Intracellular disposal and production of valine are calculated simultaneously with membrane transport rates and are for valine related to protein synthesis and breakdown, respectively. Total intracellular appearance of valine (R_{a_i}) is the sum of inward directed membrane transport ($F_{L,A}$) and intracellular production.

orthogonal dimensions and by calculating the volume, assuming the tumor to be a prolate spheroid (26). Rats were housed in metabolic cages; tumor size, body weight and food intake were monitored daily.

In a first experiment ($n=10$ per group) the acute phase response was determined by measuring serum α_2 -macroglobulin (27). Blood was obtained under ether anaesthesia by puncture of the retrobulbar venous plexus.

In a second experiment ($n=16$ per group) liver metabolism was examined as described previously (28,29). In brief, under ether anesthesia and at constant, pre-anesthesia body temperature, a laparotomy was performed. The right renal vein, the mesenteric vein, the portal vein, the hepatic vein and the aorta were cannulated using a 25-gauge needle fixed in a Silastic tube (Silastic Medical Grade tubing 0.051 cm ID, 0.094 OD, Dow Corning Corporation, Midland, MO, USA) and fixed with cyano-acrylate. For flow measurements, the indicator dilution method with *para*-aminohippuric acid (PAH) was used (pH 7.4, iso-osmolaric, Sigma A 1422, St Louis, MI, USA). A primed ($0.15 \text{ ml} \cdot 100 \text{ g bw}^{-1}$, 50 mol/l) continuous infusion ($0.75 \text{ ml} \cdot 100 \text{ g bw}^{-1} \cdot \text{h}^{-1}$, 5 mmol/l) of PAH was infused in the mesenteric vein, using a Minipuls 3 Peristaltic Pump (Gilson Med. Electr. Inc., Villiers-le-Bel, France). A primed ($1 \mu\text{Ci} \cdot 100 \text{ g bw}^{-1}$) constant infusion ($1 \mu\text{Ci} \cdot 100 \text{ g bw}^{-1} \cdot \text{h}^{-1}$) of L-[3, 4- ^3H]valine (^3H -Val, Amersham Int plc, TRK533, Buckinghamshire, UK) and L-[3, 4- ^3H]glutamine (^3H -Gln, NEN NET-551, Mechelen, Belgium) was given in the right renal vein. The results concerning hepatic glutamine turnover are discussed in a separate manuscript. A minimum infusion time of 30 min was needed to reach steady state concentrations for PAH, ^3H -Val and ^3H -Gln (29). In experiment 2 the whole procedure took 40 min before blood was sampled.

Sampling procedure

In the second experiment 1.0 ml blood was simultaneously sampled from the portal vein, hepatic vein and aorta. All blood was collected in heparinized cups (Lithium-Heparin, CB100, Sarstedt, FRG) on ice. The liver was then dissected free, directly freeze-clamped, put in liquid nitrogen and stored at -80°C until further analysis.

For PAH determinations, $50 \mu\text{l}$ heparinized blood was added to $500 \mu\text{l}$ TCA GR 10% (Merck 807, Darmstadt, Germany), vortexed and centrifuged at 8900 g at 4°C (Universac 30RF, Hettich, Tuttlingen, Germany). The supernatant was frozen in liquid

Fig. 2. Food intake of control (top), small-tumor-bearing (middle) and large-tumor-bearing (bottom) rats. All rats were fed ad libitum. No significant changes in food intake between different groups.

nitrogen and stored at -80°C until further analysis. For hematocrit determinations a micro-hematocrit tube (Modulohm 88302, Herlev, Denmark) was filled with heparinized blood and centrifuged at 10000 g at room temperature (Micro Hematocrit Centrifuge, Hawksley Ltd, 891481, UK). Hematocrit was read with a Micro Hematocrit Reader (Hawksley Ltd, UK). Plasma was obtained by whole blood centrifugation at 8900 g at 4°C for 5 min. For ammonia, urea, glucose and lactate determinations, $200 \mu\text{l}$

TABLE 1

Tumor weight, carcass weight, liver wet weight, dry weight, intracellular water content and serum α_2 -macroglobulin concentrations

	Control		Small tumor		Large tumor	
	day 1	day 24	day 1	day 24	day 1	day 24
Tumor weight	–	–	–	20±2	–	48±4
Carcass weight	200±4	200±4	192±3	198±2	199±3	181±2 ^c
Liver						
Wet weight	–	5.5±0.1	–	5.9±0.2	–	7.3±0.3 ^c
Dry weight	–	1.56±0.1	–	1.54±0.05	–	1.90±0.08 ^c
% water	–	71.6±0.5	–	73.9±0.4	–	73.7±0.9
α_2 -Macroglobulin		<50		1019±509 ^a		5300±1040 ^c

Values are grams (mean±SEM) except α_2 -macroglobulin ($\mu\text{g/ml}$) and measured at day of implantation (day 1) and day of experiment (day 24). Detection level α_2 -macroglobulin is 50 $\mu\text{g/ml}$. Statistical analysis by Mann Whitney *U* vs. control group: ^a*p*<0.05, ^b*p*<0.01, ^c*p*<0.001.

plasma was vortexed with 20 μl TCA 50%, put into liquid nitrogen and stored at -80°C . To determine plasma amino acid specific activities and concentrations, 8 mg 5-sulfosalicylic acid (Brunschwig, 10346, Amsterdam, NL) was added to 200 μl plasma, vortexed, frozen in liquid nitrogen and stored at -80°C .

TABLE 2

Arterial glucose lactate, ammonia, urea and amino acid concentrations in control, small- and large-tumor-bearing rats

	Control	Small tumor	Large tumor
Glucose	9.7±0.1	8.7±0.4 ^c	7.7±0.2 ^c
Lactate	6.4±0.3	7.4±0.3 ^c	8.4±0.4 ^c
Ammonia	174±10	169±8	161±9
Urea	3.9±0.2	3.9±0.2	3.8±0.1
Glutamine	550±25	577±12	539±28
Glutamate	62±4	82±6 ^a	118±10 ^c
Asparagine	33±2	43±1 ^c	42±2 ^b
<i>Serine/Histidine</i>	189±15	235±7 ^a	199±11
Glycine	180±15	263±18 ^c	276±11 ^c
Threonine	83±4	87±5	70±4 ^b
Citrulline	46±3	52±2	64±17
Alanine	321±18	359±18	358±25
Arginine	118±5	161±6 ^c	150±8 ^c
Taurine	150±9	302±25 ^c	342±37 ^c
α -Aminobutyric acid	22±3	36±2 ^c	48±9 ^c
Tyrosine	61±4	94±4 ^c	113±31 ^c
Valine	117±7	154±6 ^c	168±10 ^a
Methionine	45±2	53±1 ^c	50±2 ^a
Isoleucine	48±3	70±3 ^c	72±4 ^c
Phenylalanine	47±2	66±2 ^c	77±7 ^c
Tryptophan	48±3	38±2 ^c	33±2 ^c
Leucine	73±4	102±4 ^c	98±5 ^c
Ornithine	18±2	37±3 ^c	46±5 ^c
Lysine	291±15	409±15 ^c	356±25 ^b
BCAA	239±13	326±12 ^c	326±14 ^c

Values are presented in $\mu\text{mol/l}$ except glucose, lactate and urea in mmol/l (mean±SEM). Mann Whitney *U* vs. control group: ^a*p*<0.05, ^b*p*<0.01, ^c*p*<0.001. BCAA: Branched Chain Amino Acids (leucine, isoleucine, valine). Essential amino acids in italics, gluconeogenic amino acids in bold (31).

Analysis

Plasma ammonia, urea, glucose, lactate and PAH were determined spectrophotometrically on a Cobas Mira S (Roche Diagnostica, Hoffman-La Roche, Basel, Switzerland) by standard enzymatic methods, using commercially available kits, as described previously (28,30). Plasma amino acid concentrations and specific activity were determined by fully automated high performance liquid chromatography (HPLC), as described previously (31). Serum α_2 -macroglobulin

TABLE 3

Intracellular ammonia and amino acid concentrations in the liver of control, small- and large-tumor-bearing rats

	Control	Small tumor	Large tumor
Ammonia	1681±133	1801±155	1691±78
Glutamine	5221±236	3700±297 ^c	3052±298 ^c
<i>Histidine</i>	408±19	390±22	533±21
Glutamate	5128±237	5066±216	4818±339
Asparagine	68±5	135±28 ^b	103±14 ^b
Serine	404±18	484±45	518±51 ^c
Glycine	3658±128	2674±111	2962±137 ^c
Threonine	758±63	787±173	897±105
Citrulline	99±7	122±8 ^a	128±10 ^a
Alanine	4243±376	4452±294	4563±322
Arginine	261±75	407±79	178±62
Taurine	14006±869	15962±1345	11309±1298 ^a
α -Aminobutyric acid	102±14	165±8	230±23 ^c
Tyrosine	161±12	163±8	201±11 ^a
Valine	238±16	267±15	288±21 ^a
Methionine	133±10	127±11	146±19
Isoleucine	118±8	136±8	141±9 ^a
Phenylalanine	112±4	124±5 ^a	136±7 ^b
Tryptophan	39±2	42±2	41±4
Leucine	228±14	279±13 ^a	284±17 ^a
Ornithine	203±12	272±15 ^b	326±27 ^c
Lysine	884±88	966±155	830±47
BCAA	627±28	713±35 ^a	682±37

Values are presented in $\mu\text{mol/l}$ (mean±SEM). Mann Whitney *U* vs. control group: ^a*p*<0.05, ^b*p*<0.01, ^c*p*<0.001. BCAA: Branched Chain Amino Acids (leucine, isoleucine, valine). Essential amino acids in italics, gluconeogenic amino acids in bold (31).

Fig. 3. Arterial concentrations ($\mu\text{mol/l}$) of total α -amino nitrogen (top, left), gluconeogenic amino acids (top, right), essential amino acids (bottom, left) and non-essential amino acids (bottom, right). Statistical analysis by Mann-Whitney U corrected with Bonferroni procedure: ^a $p=0.05$, ^b $p=0.01$, ^c $p=0.001$.

was determined by single immunodiffusion using monospecific antibodies against rat α_2 -macroglobulin (27). For the determination of tissue ammonia, amino acid concentrations and specific activities, liver tissue was pulverized using a mortar and pestle precooled in liquid nitrogen. The tissue was further homogenized and deproteinized in a Mini-Beadbeater (Biospec products, Bartlesville USA). Approximately 100 mg tissue was added to 400 μl SSA 5%, with 300 mg glass beads (diameter 1 mm, Biospeck Products) and beaten for 30 s. The homogenate was centrifuged at 4°C at 1100 g and the supernatant frozen in liquid nitrogen and stored at -80°C until further determinations.

To determine tissue dry weight and water content, approximately 200 mg pulverized tissue was freeze dried for 24 h in a Speedvac (SC200, Savant Instruments Inc., Farmingdale NY, USA) connected to a refrigerated condensation trap (type RT 400, Savant Instruments Inc., Farmingdale NY, USA).

Calculations

Hepatic plasma flow, flux and intracellular concentrations

Plasma flow across liver was calculated using PAH in the indicator dilution method, as described previously (32,33). Splanchnic flux was calculated by multiplying the mean hepatic plasma flow by hepatic vein-arterial concentration differences. PDV flux is the mean PDV plasma flow multiplied by portal vein-arterial concentration difference. Liver flux was calculated by subtracting portal drained visceral (PDV) flux from the splanchnic flux (32,33). A positive flux indicates net release; a negative flux reflects a net uptake. α -Amino-nitrogen was calculated as the sum of the individual amino acids measured (31). Essential, non-essential and gluconeogenic amino acids are defined as described previously (34). Tissue concentrations of amino acids are expressed in $\mu\text{mol/l}$ and derived by dividing the tissue homogenate amino

Fig. 4. Intracellular concentrations ($\mu\text{mol/l}$) of total α -amino nitrogen (top, left), gluconeogenic amino acids (top, right), essential amino acids (bottom, left) and non-essential amino acids (bottom, right). Statistical analysis by Mann-Whitney *U* corrected with Bonferroni procedure: ^a $p=0.05$, ^b $p=0.01$, ^c $p=0.001$.

acid concentration ($\mu\text{mol/mg}$ wet weight) by the tissue water content.

Hepatic protein turnover

Liver *in vivo* protein turnover was calculated in a three-compartment model (29,35). The three compartments involved are the following (Fig. 1): 1) vascular afferent pool (hepatic artery and portal vein); 2) intracellular free amino acid pool; 3) vascular efferent pool (hepatic vein). Disposal and production of valine are calculated out of and into the intracellular free amino acid pool. Disposal of valine was used to estimate liver protein synthesis, as the liver has low valine transaminase activity (36). Similar, valine production can only originate from protein breakdown. Valine is used both *in vitro* and *in vivo* to measure hepatocyte protein turnover (37,38).

Membrane transport rates are calculated as the amino acid flux from the afferent pool to the intracellular pool ($F_{L,A}$), the amino acid flux from the intrac-

ellular pool into the hepatic vein ($F_{L,H}$) and the amino acid flux shunting from afferent vessels into the hepatic vein ($F_{H,A}$).

Statistical analysis

Results are presented as mean $\pm$ SEM. The Mann Whitney *U* test corrected with the Bonferroni procedure was used to test significance between tumor-bearing groups and the control group (39). Significance was considered present at $p < 0.05$.

Results

General response

The presence of MCA tumors did not reduce food intake (Fig. 2), while carcass weight decreased only moderately in the large-tumor-bearing animals (Table 1). Liver weight was unchanged in the small-tumor-bearing animals and increased approximately 30% in the large-tumor-bearing animals after 24 days. Serum

TABLE 4

Hepatic glucose, lactate, ammonia, urea and amino acid fluxes in control, small- and large-tumor-bearing rats

	Control	Small tumor	Large tumor
Flow	2.5±0.5	2.6±0.6	2.7±0.6
Glucose	3184±792	1910±467	3310±487
Lactate	-2940±702	-3659±461	-4235±448 ^a
Ammonia	-310±36	-160±32 ^b	-177±30 ^b
Urea	820±169	456±81	521±57
Glutamine	112±52	-64±34 ^b	37±38
Glutamate	-19±15	108±28 ^c	112±28 ^c
Asparagine	-43±5	57±7	66±6 ^b
<i>Serine/Histidine</i>	-115±19	-166±22	-155±22
Glycine	-201±33	-184±27	-262±30
Threonine	-57±26	-63±8	-68±8
Citrulline	-42±11	-20±7	-56±8 ^c
Alanine	-396±79	-321±63	-545±52 ^d
Arginine	-53±9	-97±14 ^a	-114±15 ^b
Taurine	-52±18	-9±26	-84±45
α -Aminobutyric acid	-11±3	-3±3	-7±4
Tyrosine	-22±5	-18±7	-31±10
Valine	26±8	8±12	-36±18 ^c
Methionine	1±5	-22±7 ^b	-28±5 ^c
Isoleucine	13±4	13±6	-6±6 ^a
Phenylalanine	-3±4	-9±3	-32±8 ^b
Tryptophan	4±7	3±3	-3±4
Leucine	26±14	17±8	-19±13 ^a
Ornithine	3±4	31±10 ^b	-2±12
Lysine	-136±18	-161±31	-208±50
BCAA	64±24	38±24	-62±34 ^b

Values are presented in nmol·100 g bw⁻¹·min⁻¹ (mean SEM). Mann Whitney *U* vs. control group: ^a*p*<0.05, ^b*p*<0.01, ^c*p*<0.001. BCAA: Branched Chain Amino Acids (leucine, isoleucine, valine). Essential amino acids in italics, gluconeogenic amino acids in bold (31).

α_2 -macroglobulin concentration rose with increasing tumor loads. In the large-tumor-bearing rats α_2 -macroglobulin concentrations were elevated, and were comparable to those seen in response to severe acute inflammatory stimuli such as turpentine or surgical trauma (27,40).

Arterial concentrations

Arterial glucose concentrations diminished in the small-tumor-bearing animals and decreased further in the large-tumor-bearing animals (Table 2). Arterial lactate concentrations increased with increasing tumor load.

An arterial hyperaminoacidemia developed in both groups of tumor-bearing rats (Fig. 3, Table 2). In the tumor-bearing rats both essential and non-essential amino acid concentrations increased. In control rats almost 30% of non-essential amino acids consisted of glutamine; this proportion did not change in the presence of a tumor. Concentrations of all gluconeogenic amino acids increased in tumor-bearing rats.

Liver intracellular concentrations

Intracellular ammonia did not vary between controls and tumor-bearing rats (Table 3). Total α -amino acid nitrogen remained unchanged in the small-tumor-bearing animals, but decreased in the large-tumor-bearing animals (Table 3, Fig. 4). However, mostly non-essential amino acids decreased, and of these primarily glutamine and taurine. Essential amino acid concentrations either increased (histidine, valine, isoleucine, phenylalanine, leucine) or remained unchanged (threonine, methionine, tryptophan, lysine) with increasing tumor loads. The sum of essential amino acids increased in the large-tumor-bearing rats.

The sum of gluconeogenic amino acids decreased in the small-tumor-bearing rats and remained reduced in the large-tumor-bearing rats. Urea cycle intermediate amino acids are ornithine, citrulline and arginine. The first two increased in tumor-bearing rats, but arginine concentrations decreased below control values in the large-tumor-bearing rats.

Hepatic plasma flow and flux

Hepatic plasma flow was similar in control and both tumor-bearing animals (Table 4). Glucose release

TABLE 5

Liver valine membrane transport in control, small- and large-tumor-bearing rats

	Control	Small tumor	Large tumor
F _{LA}	88±31	87±50	108±33 ^a
F _{H,L}	105±37	95±56	71±42
F _{HA}	201±31	301±51	325±37 ^b

Values are presented in nmol·valine·100 g bw⁻¹·min⁻¹ (mean±SEM). F_{LA}: inward directed membrane transport rates; F_{H,L}: outward directed membrane transport; F_{HA}: metabolic shunting (see also Fig. 1). Mann Whitney *U* test vs. control group: ^a*p*<0.05, ^b*p*<0.01, ^c*p*<0.001.

TABLE 6

Total intracellular valine turnover and relative contribution of amino acid membrane transport and intracellular protein breakdown (Production)

	Control	Small tumor	Large tumor
R _a	142±40	180±71	174±57
F _{LA} /R _a	0.74±0.07	0.58±0.06 ^a	0.97±0.04
P/R _a	0.26±0.07	0.41±0.06 ^a	0.03±0.04

F_{LA}: inward membrane transport of valine; P: intracellular production from protein breakdown; R_a: total intracellular appearance rate (F_{LA}+production). R_a is in nmol valine·100 g bw⁻¹·min⁻¹, F_{LA}/R_a and P/R_a are the fractions to which membrane transport and protein breakdown contribute to total intracellular appearance rate (see also Fig. 1). Mann Whitney *U* test vs. control group: ^a*p*<0.05, ^b*p*<0.01, ^c*p*<0.001.

Fig. 5. Fluxes of total α -amino nitrogen (top, left), gluconeogenic amino acids (top, right), essential amino acids (bottom, left) and non-essential amino acids (bottom, right) across the liver. A negative flux reflects net uptake, a positive flux reflects net release of amino acids. Statistical analysis by Mann-Whitney U corrected with Bonferroni procedure: ^a $p=0.05$, ^b $p=0.01$, ^c $p=0.001$.

decreased in small-tumor-bearing rats, although not significantly. Lactate uptake increased in the large-tumor-bearing animals. In line with this, we found an increased uptake of gluconeogenic amino acids in the large-tumor-bearing rats (Fig. 5). Alanine uptake accounted for 40–46% of the uptake of all gluconeogenic amino acids by the liver (Table 4).

The uptake of total α -amino acids remained unchanged in the small-tumor-bearing rats, but tended to increase in the large-tumor-bearing rats (Fig. 5). This was due to an increased uptake of essential amino acids. The uptake of non-essential amino acids also only tended to increase. Ammonia uptake decreased in both tumor-bearing rats. Urea release remained unchanged in the small- and large-tumor-bearing animals.

Hepatic protein turnover and valine membrane transport

Liver valine disposal (protein synthesis) increased in the small-tumor-bearing animals, and remained

increased at that level in the large-tumor-bearing animals (Fig. 6). Valine production (protein breakdown) was unchanged in the small-tumor-bearing animals but decreased to zero in the large-tumor-bearing animals. Liver valine flux (difference protein breakdown and synthesis) was thus reduced to zero in the small-tumor-bearing rats by the increased protein synthesis rates. In the large-tumor-bearing animals valine flux became negative (=net protein synthesis) by strongly decreased protein breakdown rates.

Valine inward membrane transport rates (Table 5, $F_{L,A}$) were unchanged in the small-tumor-bearing rats and increased only slightly in the large-tumor-bearing rats. Outward membrane transport ($F_{H,L}$) was not significantly different between the groups. In the large-tumor-bearing group, valine shunting ($F_{H,A}$) increased more than 50%.

To see whether plasma-amino acid membrane transport determines a large fraction of intracellular amino acid turnover, we calculated the total intracellular amino acid appearance rate of valine as the sum

Fig. 6. Intracellular disposal (Fig. 1) of valine (top), production of valine (middle) and flux of valine (bottom) across the liver. Disposal of valine reflects protein synthesis whereas production of valine reflects protein breakdown. Valine flux is the difference between disposal and production (negative: net uptake; positive: net release). Statistical analysis by Mann-Whitney U corrected with Bonferroni procedure: ^a $p=0.05$, ^b $p=0.01$, ^c $p=0.001$.

of valine inward membrane transport plus valine production from intracellular protein breakdown (R_{a_i} , Table 6). Membrane transport determines 74% of all *de novo* appearance of valine in the free intracellular amino acid pool of control rats. Approximately 26% of intracellular amino acid appearance in the hepatocyte is determined by protein breakdown (production). In the small-tumor-bearing rats protein breakdown accounted for a relatively larger amount of total valine appearance rate than in control rats, whereas this decreased to zero in the large-tumor-bearing rats.

Discussion

The liver has a dual role in the development of cancer. It regulates a great part of the intermediary metabolism, particularly related to amino acid and glucose homeostasis. It is, however, also involved in the acute phase response. Processing of amino acids by the liver in cancer therefore serves both the production of defense proteins and the maintenance of metabolic homeostasis. In our rat cancer model no anorexia was observed. The inflammatory and metabolic changes observed can thus only be attributed to the presence of the tumor. However, the unchanged nutrient intake in the presence of a tumor of 5–15% or 15–30% of the body weight must have changed nutrient delivery to non-tumor tissues, with subsequent adaptations in intermediary metabolism. Net carcass weight loss was limited in this cancer model. The current study thus appears to be a reflection of metabolic changes observed in moderate cancer cachexia, e.g. early detected cancer patients with no, or hardly any, nitrogen and weight loss.

Urea production by the liver remained unchanged in both groups of tumor-bearing rats although it tended to decrease ($p=0.07$). The decrease may have remained undetected by the variation of the data (type II error). Citrulline and ornithine are intermediary amino acids of the urea cycle. In a previous study decreased arterial concentrations of these amino acids were found indicative of decreased urea cycle activity in tumor-bearing rats (16). In the current study increased concentrations of these amino acids were observed in arterial plasma and, moreover, in the liver of tumor-bearing rats. We feel that this is more in agreement with decreased urea cycling, as intermediate substrates of the urea cycle appear not to be used. However, it also indicates that changes in amino acid concentrations are difficult to translate into kinetic parameters, and the interpretation of only amino acid concentrations should be done with great care.

Unchanged or possibly decreased activity of the

urea cycle in tumor-bearing animals also explains the increased arterial amino acid concentrations. We previously showed that the presence of the tumor increases peripheral protein breakdown with an enhanced net efflux of amino acids from muscle (41). Thus, mobilization of amino acids in combination with unchanged or decreased urea cycle activity and increased arterial amino acid concentrations, directs amino acid interorgan transfer towards other tissues, e.g. immune cells and tumor tissue.

Arginine is also an intermediary amino acid of the urea cycle. The decreased intracellular arginine concentrations in large-tumor-bearing rats are probably not related to the urea cycle but to the production of nitric oxide: arginine is the precursor of nitric oxide and nitric oxide production by hepatocytes and Kupffer cells increases after stimulation with inflammatory mediators (8).

Two environmental stimuli are of influence on urea production in tumor-bearing rats. The availability of α -amino nitrogen is a well-described factor which influences liver amino acid uptake and processing (34,42). In particular, low protein diets decrease the availability of amino acids for the liver (42). The uptake of amino acids by the liver thus decreases with subsequent reduced urea production. In our tumor-bearing rats, however, arterial concentrations increased with increasing tumor loads, suggesting a greater availability of amino acids for potential urea cycling. Another important mechanism influencing urea cycling is related to circulating peptides/proteins induced by the presence of the tumor. Hormones and cytokines are known to influence liver urea cycling and protein synthesis in other stress conditions, such as burns, infection and trauma (43). Increased, decreased and unchanged insulin and glucagon levels have been observed in MCA tumor-bearing rats (37,44,45). In addition, increased levels of several cytokines, e.g. TNF α , IL1, IL6, have been observed in the cancer-bearing host (46–50). These cytokines are all able to induce *in vitro* amino acid uptake by hepatocytes (51,52). Involvement of IL6 is further in agreement with the general concept that it is one of the most important mediators of the acute phase response, as observed in the current study (27,50). The positive effects of the increased arterial amino acid concentrations on the urea cycle therefore appear to be counteracted by humoral factors. However, the exact etiology of such a nitrogen-sparing mechanism by the liver in the cancer-bearing host needs further research.

Increased Cori cycling and increased gluconeogenesis have been demonstrated in previous studies of sarcoma-bearing rats (14,45,53). In our study no

changes in net glucose production were observed. This may have been caused by the negative effect of ether anesthesia on gluconeogenesis (54). We, however, also did not measure the exact gluconeogenesis but only the net production of glucose. This will clearly underestimate liver gluconeogenesis, in particular when inflammatory cells of the liver, such as Kupffer cells, are activated and consume glucose.

Lactate and amino acids are major precursors for gluconeogenesis (55). If the carbon skeleton of amino acids is transformed to glucose, the amino group is processed to urea. Amino acids first transfer their amino group to aspartate, which donates one nitrogen group to urea. The second nitrogen of urea is derived from the amide group of glutamine or free ammonia. Gluconeogenic amino acid uptake in control rats is thus more or less in balance with the nitrogen transferred to urea in control rats (Table 4, 850 vs. 820 nmol·100 g bw⁻¹·min⁻¹). In the small-tumor-bearing rats the uptake of gluconeogenic amino acids exceeds the nitrogen used for urea production by 348 nmol·100 g bw⁻¹·min⁻¹, and this surplus increases to 705 nmol·100 g bw⁻¹·min⁻¹ in large-tumor-bearing rats. Thus, it appears that the liver prefers to use gluconeogenic amino acids for other processes, mainly protein synthesis. Acute phase protein synthesis has previously been shown to increase secondary to tumor growth (50). Serum α_2 -macroglobulin is one of the major acute phase proteins in the rat and is a broad spectrum protease inhibitor that can bind cytokines and regulates tissue damage and restructuring (27,56). Increased levels of several cytokines, damage and tumor necrosis are features of our tumor model and may very well explain the raised increased acute phase protein synthesis.

Protein synthesis in the hepatocyte was measured by intracellular valine disposal. This was possible as the enzyme activity of the major other metabolic route, transamination, is very low in liver tissue compared to other tissues (36). Maximal transamination of valine in the rat liver is 75 mmol·100 g bw⁻¹·min⁻¹ and the K_m value is 1.2–2.5 mM (57). Our *in vivo* intracellular valine concentrations are approximately one tenth of the K_m value. Estimated *in vivo* transamination would thus be approximately 4 nmol·100 g bw⁻¹·min⁻¹, which comprises almost half of the intracellular disposal of valine in control rats. However, in the tumor-bearing rats this was only one tenth of total disposal and most of the intracellular valine disposal can thus be accounted for by protein synthesis.

In control rats protein breakdown rates exceeded protein synthesis rates, resulting in a net protein loss with a net efflux of valine. The negative protein bal-

ance of the liver in control rats is related to the experimental conditions. First, all rats are studied postabsorptively. Second, previous studies, although not all (58), suggest that ether anesthesia decreases protein synthesis rates of the liver (59,60). The increased net protein synthesis rates in tumor-bearing rats should thus be interpreted relative to the control rats, and may in absolute terms be an underestimation of the actual protein synthesis rates.

Liver protein synthesis is the sum of export proteins and structural proteins. Serum α_2 -macroglobulin is considered to be the most reactive acute phase protein exported by the liver (27). It has a turnover of approximately 8% per day and a Kd of 820000 (61). Assuming the plasma volume of rats to be 4% of bodyweight (61), the increased serum α_2 -macroglobulin concentrations in the large-tumor-bearing rats equals an additional production of 48 mg α_2 -macroglobulin proteins. This is approximately 27800 nmol valine (62) over approximately 18 days or 0.5 nmol valine $\cdot 100$ g $\text{bw}^{-1} \cdot \text{min}^{-1}$. Although other acute phase proteins (e.g. haptoglobin, ceruloplasmin, α_1 acid glycoprotein) are also produced by hepatocytes in response to inflammation, the discrepancy between the calculated intracellular disposal (44 nmol $\cdot 100$ g $\text{bw}^{-1} \cdot \text{min}^{-1}$) and the theoretical disposal for α_2 -macroglobulin (0.5 nmol), suggests that the majority of the increased protein synthesis reflects increased synthesis of structural proteins of the liver.

The increased protein synthesis in the small-tumor-bearing rats was not accompanied by increased membrane transport rates of valine. This indicates that changes in membrane transport activity were not involved in the regulation of liver protein synthesis. This can also be seen in the relative contribution of amino acid membrane transport and intracellular production to total intracellular amino acid turnover (R_{a_i} , Table 6). The relative contribution of intracellular production increased at the cost of membrane transport rates in the small-tumor-bearing rats. In the large-tumor-bearing rats, however, valine production decreased to almost zero and membrane transport was still sufficiently high to supply valine for protein synthesis. The unchanged valine membrane transport, despite the increased need for intracellular valine in these animals, possibly indicates that amino acid membrane transport of these amino acids in our rat cancer model is working at submaximal activity. These *in vivo* findings are in agreement with *in vitro* observations on hepatocyte system L membrane transport carriers. These studies show that system L membrane transport carriers demonstrate a relatively high activity in hepatocytes, but do not maintain large

concentration differences across cell membranes (63). Furthermore, hepatic system L has been shown not to play any significant role in liver amino acid turnover in the presence of altered amino acid availability due to high- or low-protein diets (42).

In conclusion, the study shows that in tumor-bearing rats, liver uptake of essential and gluconeogenic amino acids increases. Glucose and urea production remain unchanged, whereas protein synthesis of both structural and export proteins, e.g. acute phase proteins, increases. These changes indicate that the liver becomes a more efficient nitrogen-sparing and active protein-synthesizing organ during the growth of a malignant tumor.

Acknowledgements

The authors wish to thank Mr. H.M.H. van Eijk, Mr. D.R. Rooyackers and Mrs. H. Sala for analytical help. This research was supported by grant 900-562-125 from The Netherlands Organization for Scientific Research (N.W.O.).

References

1. Kern KA, Norton JA. Cancer cachexia. *J Parenter Enteral Nutr* 1988;12: 286-98.
2. Norton JA, Shamberger R, Stein TP, Milne GW, Brennan MF. The influence of tumor-bearing on protein metabolism in the rat. *J Surg Res* 1981; 30: 456-62.
3. Douglas RG, Shaw JH. Metabolic effects of cancer. *Br J Surg* 1990; 77: 246-54.
4. Christensen HN. Interorgan amino acid nutrition. *Physiol Rev* 1982; 62: 1193-233.
5. Souba WW. Glutamine and Cancer. *Ann Surg* 1993; 218: 715-28.
6. Fahr MJ, Kornbluth J, Blossom S, Schaeffer R, Klimberg VS. Harry M. Vars Research Award. Glutamine enhances immunoregulation of tumor growth. *J Parenter Enteral Nutr* 1994; 18: 471-6.
7. Welbourne TC, King AB, Horton K. Enteral glutamine supports hepatic glutathione efflux during inflammation. *J Nutr Biochem* 1993; 4: 236-42.
8. Billiar TR, Curran RD. Kupffer cell and hepatocyte interactions: a brief overview. *J Parenter Enteral Nutr* 1990; 14: 175S-80S.
9. Warren RS, Jeevanandam M, Brennan MF. Protein synthesis in the tumor-influenced hepatocyte. *Surgery* 1985; 98: 275-82.
10. Holroyde CP, Gabuzda TG, Putnam RC, Paul P, Reichard GA. Altered glucose metabolism in metastatic carcinoma. *Cancer Res* 1975; 35: 3710-4.
11. Lundholm K, Edstrom S, Karlberg I, Ekman L, Schersten T. Glucose turnover, gluconeogenesis from glycerol, and estimation of net glucose cycling in cancer patients. *Cancer* 1982; 50: 1142-50.
12. Inculet RI, Peacock JL, Gorschboth CM, Norton JA. Gluconeogenesis in the tumor-influenced rat hepatocyte: importance of tumor burden, lactate, insulin, and glucagon. *J Natl Cancer Inst* 1987; 79: 1039-46.
13. Burt ME, Lowry SF, Gorschboth C, Brennan MF. Metabolic

- alterations in a noncachectic animal tumor system. *Cancer* 1981; 47: 2138–46.
14. Lowry SF, Foster DM, Norton JA, Berman M, Brennan MF. Glucose disposal and gluconeogenesis from alanine in tumor-bearing Fischer 344 rats. *J Natl Cancer Inst* 1981; 66: 653–8.
 15. Nurjhan N, Bucci A, Perriello G, Stumvoll M, Dailey G, Bier DM, Toft I, Jenssen TG, Gerich JE. Glutamine: a major gluconeogenic precursor and vehicle for interorgan carbon transport in man. *J Clin Invest* 1995; 95: 272–7.
 16. Chance WT, Cao L, Kim MW, Nelson JL, Fischer JE. Reduction of tumor growth following treatment with a glutamine antimetabolite. *Life Sci* 1988; 42: 87–94.
 17. Ekman L, Lundholm KG, Blackburn GL, Grant JP, Young VR, eds. Is the liver or the periphery limiting for hepatic utilization of amino acids in cancer-induced malnutrition? In: *Amino Acids, Metabolism and Medical Applications*. London: John Wright, PSG Inc, 1983: 213–7.
 18. Beck SA, Tisdale MJ. Nitrogen excretion in cancer cachexia and its modification by a high fat diet in mice. *Cancer Res* 1989; 49: 3800–4.
 19. Karlberg HI, Kern KA, Fischer JE. Albumin turnover in sarcoma-bearing rats in relation to cancer anorexia. *Am J Surg* 1983; 145: 95–101.
 20. Krause R, James JH, Humphrey C, Fischer JE. Plasma and brain amino acids in Walker 256 carcinosarcoma-bearing rats. *Cancer Res* 1979; 39: 3065–9.
 21. Waldegrave W, Chairman Council of European Community. Guide for the Care and Use of Laboratory Animals. European Community 1986; Publication 86/609, Brussels, Belgium.
 22. Boyland E, Warren FL. The induction of tumours by methylcholanthrene in two strains of mice. *J Path Bact* 1937; 45: 171–7.
 23. Chen MK, Espat NJ, Bland KI, Copeland EM, Souba WW. Influence of progressive tumor growth on glutamine metabolism in skeletal muscle and kidney. *Ann Surg* 1993; 217: 655–67.
 24. Popp MB, Morrison SD, Brennan MF. Total parenteral nutrition in a methylcholanthrene-induced rat sarcoma model. *Cancer Treat Rep* 1981; 65 (Suppl 5): 137–43.
 25. de Blaauw I, Deutz NEP, von Meyenfeldt MF. Glutamine depletion and increased gut permeability in non-anorectic, non-weight-losing tumor-bearing rats. *Gastroenterology* 1995; 108: A721.
 26. Morrison SD. *In vivo* estimation of size of experimental tumors. *J Natl Cancer Inst* 1983; 71: 407–8.
 27. van Gool J, van Vugt H, Helle M, Aarden LA. The relation among stress, adrenaline, interleukin 6 and acute phase proteins in the rat. *Clin Immunol Immunopathol* 1990; 57: 200–10.
 28. Dejong CHC, Kampman MT, Deutz NEP, Soeters PB. Altered glutamine metabolism in rat portal drained viscera and hindquarter during hyperammonemia. *Gastroenterology* 1992; 102: 936–48.
 29. de Blaauw I, Deutz NEP, von Meyenfeldt MF. *In vivo* amino acid metabolism of gut and liver during short and prolonged starvation. *Am J Physiol* 1996; 270: G298–306.
 30. Dejong CHC, Deutz NEP, Soeters PB. Renal ammonia and glutamine metabolism during liver insufficiency-induced hyperammonemia in the rat. *J Clin Invest* 1993; 92: 2834–40.
 31. van Eijk HMH, Huinck MP, Rooyakkers DR, Deutz NEP. Automated simultaneous isolation and quantitation of labeled amino acid fractions from plasma and tissue by ion-exchange chromatography. *J Chromatogr B Biomed Appl* 1994; 660: 251–7.
 32. Deutz NEP, Reijven PL, Athanasas G, Soeters PB. Post-operative changes in hepatic, intestinal, splenic and muscle fluxes of amino acids and ammonia in pigs. *Clin Sci* 1992; 83: 607–14.
 33. Heeneman S, Deutz NEP. Effects of decreased glutamine supply on gut and liver metabolism *in vivo* in rats. *Clin Sci* 1993; 85: 437–44.
 34. Thomas JH, Gillham B, Thomas J, Gillham B, eds. Plasma amino acids and utilization of amino acids by tissues. In: *Wills' Biochemical Basis of Medicine*, 2nd Edn. London: Wright; 1989: 238–47.
 35. Biolo G, Fleming RY, Maggi SP, Wolfe RR. Transmembrane transport and intracellular kinetics of amino acids in human skeletal muscle. *Am J Physiol* 1995; 44: E75–84.
 36. Shinnick FL, Harper AE. Branched-chain amino acid oxidation by isolated rat tissue preparations. *Biochim Biophys Acta* 1976; 437: 477–86.
 37. Warren RS, Jeevanandam M, Brennan MF. Comparison of hepatic protein synthesis *in vivo* vs. *in vitro* in the tumor-bearing rat. *J Surg Res* 1987; 42: 43–50.
 38. Seglen PO, Solheim AE, Grinde B, Gordon PB, Schwarze PE, Gjessing RM, Poli A. Amino acid control of protein synthesis and degradation in isolated rat hepatocytes. *Ann NY Acad Sci* 1980; 349: 1–17.
 39. Norusis MJ. Statistical Package for Social Sciences (SPSS)/PC+ V4.0 Base Manual for the IBM PC/XT/AT and PS/2. SPSS Inc, 1989; Chicago, IL, USA.
 40. Jennings G, Elia M. The acute-phase response to turpentine-induced abscesses in malnourished rats at different environmental temperatures. *Metabolism* 1992; 41: 141–7.
 41. de Blaauw I, Deutz NEP, von Meyenfeldt MF. Increased whole body glutamine turnover in cancer is not matched by increased hindquarter glutamine release. *Gastroenterology* 1995; 108: A722.
 42. Fafournoux P, Remesy C, Demigne C. Fluxes and membrane transport of amino acids in rat liver under different protein diets. *Am J Physiol* 1990; 259: E614–E625.
 43. Heindorff HA. The hepatic catabolic stress response. Hormonal regulation of urea synthesis after surgery. *Dan Med Bull* 1993; 40: 224–34.
 44. Chance WT, Muggia Sullam M, Chen MH, Murphy RF, Fischer JE. Reversal of tumor-induced biochemical abnormalities by insulin treatment in rats. *J Natl Cancer Inst* 1986; 77: 497–503.
 45. Lowry SF, Norton JA, Gorschboth CM, Brennan MF. Glucose turnover and gluconeogenesis during hypocaloric glucose infusion in tumor-bearing F344 male rats. *J Natl Cancer Inst* 1980; 64: 291–6.
 46. Stovroff MC, Fraker DL, Norton JA. Cachectin activity in the serum of cachectic, tumour-bearing rats. *Arch Surg* 1989; 124: 94–9.
 47. Fearon KCH, McMillan DC, Preston T, Winstanley P, Cruickshank AM, Shenkin A. Elevated circulating interleukin-6 is associated with an acute-phase response but reduced fixed hepatic protein synthesis in patients with cancer. *Ann Surg* 1991; 213: 26–31.
 48. Smith BK, Conn CA, Kluger MJ. Experimental cachexia: effects of MCA sarcoma in the Fischer rat. *Am J Physiol* 1993; 265: E376–R384.

49. Ardawi MS. Effects of epidermal growth factor and glutamine-supplemented parenteral nutrition on the small bowel of septic rats. *Clin Sci* 1992; 82: 573–80.
50. Andersson C, Gelin J, Iresjö BM, Lundholm K. Acute-phase proteins in response to tumor growth. *J Surg Res* 1993; 55: 607–14.
51. Andus T, Bauer J, Gerok W. Effects of cytokines on the liver. *Hepatology* 1991; 13: 364–75.
52. Bereta J, Kurdowska A, Koj A, Hirano T, Kishimoto T, Content J, Fiers W, Van Damme J, Gauldie J. Different preparations of natural and recombinant human interleukin-6 (IFN-beta 2, BSF-2) similarly stimulate acute phase protein synthesis and uptake of alpha-aminoisobutyric acid by cultured rat hepatocytes. *Int J Biochem* 1989; 21: 361–6.
53. Singh J, Grigor MR, Thompson MP. Glucose homeostasis in rats bearing a transplantable sarcoma. *Cancer Res* 1980; 40: 1699–706.
54. Philippidis H, Ballard FJ. The development of gluconeogenesis in rat liver. Effects of glucagon and ether. *Biochem J* 1970; 120: 385–92.
55. Hers HG, Hue L. Gluconeogenesis and related aspects of glycolysis. *Annu Rev Biochem* 1983; 52: 617–53.
56. Jennings G, Bourgeois C, Elia M. The magnitude of the acute phase protein response is attenuated by protein deficiency in rats. *J Nutr* 1992; 122: 1325–31.
57. Harper AE, Miller RH, Block KP. Branched chain amino acid metabolism. *Annu Rev Nutr* 1984; 4: 409–54.
58. Sampson DA, Masor M, Jansen GR. Protein synthesis in rat tissues during lactation: no effect of diethyl ether anaesthesia. *Biochem J* 1984; 224: 681–3.
59. Bessesen A, Morland J. Effects of anaesthetics on protein synthesis in isolated rat hepatocytes: inhibition by diethyl ether in contrast to no influence by pentobarbital and fentanyl. *Acta Pharmacol Toxicol Copenh* 1985; 57: 23–9.
60. Bessesen A, Smith Kielland A, Gadeholt G, Morland J. Reduced synthesis of hepatic and plasma proteins in rats during diethyl ether anaesthesia. *Acta Pharmacol Toxicol Copenh* 1984; 54: 241–6.
61. James WPT, Coward WA, Waterlow JC, Stephen JML, eds. Metabolism of plasma proteins. In: *Nitrogen Metabolism in Man*. London: ASC; 1981: 457–73.
62. Beitel GJ, Luft AJ, Panrucker DE, Lorscheider FL. Structural analysis of acute-phase α_2 -macroglobulin. *Biochem J* 1986; 238: 359–64.
63. Kilberg MS. Amino acid transport in isolated rat hepatocytes. *J Membr Biol* 1982; 69: 1–12.