Eur J Cancer Clin Oncol, Vol. 19, No. 5, pp. 671–680, 1983.
Printed in Great Britain.

0277-5379/83/050671-10$03.00/0

Evaluation of Oxidative Metabolism in Tumour-host Livers as a Possible Cause of Energy Loss in Cachectic Sarcoma-bearing Mice*

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Abstract—*Oxygen consumption has been measured in sarcoma-bearing mice, liver cells and tumour tissue. The aim was to determine whether oxidative metabolism in tumour-host livers contributes to the negative energy balance in non-growing animals with a tumour due to insufficient hepatic adaptation of energy consumption. The oxygen uptake in isolated liver cells from freely fed and starved sarcoma-bearing mice showed a* 50% *decrease (depressed by* 322 μmol O_2/hr/g) *compared to freely fed controls, while starvation of control animals reduced the oxygen uptake in the liver cells by* 30–40%. *In host tissues other than the liver, total oxygen uptake was depressed by an average* 27% *(depressed by* 50 μmol O_2/hr/g) 10–11 days *after tumour implantation. In freely fed animals the ratio between oxygen uptake in the tumour-host liver and the host was* 0.13 *and* 0.18 *in sarcoma-bearing and control mice respectively. Depression of oxidative metabolism in tumour-host livers was not associated with ultrastructural alterations in the mitochondria or in other cellular organelles studied by electron microscopy. It is concluded that the negative energy balance in a non-growing tumour-bearing host is not explained by deficient adaptation of the hepatic oxidative metabolism, and that depression of activity metabolism in tumour-bearing animals accounts for depression of the metabolic rate in host tissues other than the liver.*

INTRODUCTION

WE HAVE previously reported that non-growing sarcoma-bearing mice decrease their total energy expenditure during tumour progression [1]. The time course of the decrease in oxygen consumption was similar to that of the decrease in food intake (anorexia) in this animal model [2]. This suggests that malnutrition may be one explanation for adaptation in metabolic rate of tumour-bearing animals. Another way for tumour-bearing animals to save energy for metabolic processes might be to decrease or alter motor activity [3, 4], while the basic metabolic rate in other organs may still be high compared to the decreased overall host energy consumption. In other words, some organs or tissues may not adapt sufficiently to maintain body composition in response to the generally decreased availability of energy precursors in the entire malnourished tumour-bearing animal. This hypothesis is supported by the findings of increased enzyme activities for hepatic glycolysis [5–7], increased hepatic protein synthesis [8–10] and increased net lipolysis in peripheral tissues [11, 12] in tumour-bearing animals compared to well-nourished control animals. The role of the liver in this context can be evaluated by comparing the depression of oxidative metabolism in tumour-host livers with the depression of the total oxidative metabolism in the host.

The aim of this study was to determine whether or not oxidative metabolism in tumour-host liver is a likely explanation for the negative energy balance in weight-losing tumour-bearing animals.

Accepted 28 October 1982.

*This work was supported by grants from the Swedish Cancer Society (project No. 93), the Swedish Medical Research Council (project No. 536), the Assar Gabrielsson Foundation, the Swedish Society of Medical Sciences and the Serena Ehrenström Foundation.

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MATERIALS AND METHODS

Reagents

Collagenase type IV, suitable for isolation of liver cells, and all other reagents were of analytical grade and were obtained from the Sigma Chemical Company, U.S.A.

Experimental model

A methylcholanthrene-induced sarcoma, MCG 101, was used. The tumour was implanted subcutaneously in the flanks of the mice. Non-growing, 3-month-old and weight-stable mice (C57BL/6J) weighing 22–23 g were used as previously described [2, 13, 14]. The animals died from cachexia 15 ± 1 days (mean ± S.D.) after tumour implantation.

Animal groups and experimental procedures

Two groups of sarcoma-bearing mice with appropriate controls were used: (a) freely fed tumour and control animals were used for measurements of oxygen uptake in the total animal, liver cells and tumour tissue from these animals; (b) animals that had been starved for 20 hr before they were killed were used for liver perfusion and subsequent measurement of oxygen uptake in liver cells. Pair-fed control animals [2, 14] were not used in this experiment, since liver cells from starved controls had higher values of oxidative metabolism than freely fed sarcoma-bearing mice. All animals had free access to tap water. All experiments were performed on matched pairs of animals 10–11 days after tumour implantation. Study and control animals were treated identically. Oxygen consumption was measured simultaneously in study and control animals. Liver perfusions with subsequent isolation of cells for measurement of oxygen uptake were also performed simultaneously in each pair of study and control animals.

Liver composition

The content of water, protein, lipid, glycogen and amino acids was determined in fresh liver biopsies as described in detail previously [2]. Briefly, the water content was determined by drying the liver at +40°C to constant weight. The dry weight of tumour tissue was obtained by drying the whole tumour at +80°C to constant weight. Liver tissue was dissolved in 2 M NaOH and the protein content was determined according to Lowry *et al.* [15] with bovine albumin as the standard. The total content of lipids was determined by tissue extraction in chloroform–methanol (1:1), ethanol–acetone (1:1) and finally in pure either. Lipids were dried under a stream of nitrogen gas and the amount of dried lipids was then determined by weighing. Liver tissue was extracted immediately in 30% (w/v) potassium hydroxide for determination of the glycogen content. The amount of glycogen was estimated with the glucose oxidase method (kit from Boehringer Mannheim, West Germany) after extraction and hydrolysis of the glycogen. The hepatic content of twenty amino acids was determined in the clear supernatant after precipitation of the liver tissue proteins with trichloroacetic acid (final conc. 10% w/v), by means of an automatic amino-acid analyser [9].

Liver perfusion and measurement of oxidative metabolism in liver cells

Isolated liver cells, prepared as follows, were used for the estimation of the hepatic oxygen consumption *in vitro*. The animals were anaesthetised with an intraperitoneal injection of pentobarbitone. The abdomen and chest were exposed. A syringe (Brunswik 501-LG) was inserted into the beating heart. The syringe was fixed with a clamp and attached to a polyethylene tube. The animal was perfused through the heart for 10 min with Krebs Ringer bicarbonate buffer solution (pH 7.4) supplemented with glucose (various conc.) and amino acids in physiological concentrations [16], HEPES (20 mmol/l) and collagenase (0.07% w/v). The buffer solution was continuously gassed with oxygen containing carbon dioxide (95:5% v/v). Calcium ions were retained in the buffer since this did not influence the yield or the amount of intact cells. Small incisions were made in the lower front margin of the liver lobes in order to divert the perfusate through the liver. The perfusion rate was 2.2 ml/min. The liver became pale and was completely free from blood cells. The perfused liver was then cut with scissors, transferred to a vessel and incubated in fresh buffer at +37°C (80 strokes/min) under continuous gassing. The buffer solution was supplemented as described above, except that the collagenase concentration was increased to 0.1%. The incubation continued for 30 min. During this incubation the liver disintegrated almost completely. The incubate was filtered through a nylon strainer. Cells were washed twice with fresh buffer solution by centrifugation at 150 *g* for 2 min at +20°C. The cells were counted in the presence of trypan blue under a microscope and recorded as intact cells per unit volume. Cells which excluded trypan blue were regarded as intact and viable in routine procedures [17]. The oxygen consumption was measured polarographically, by means of a Clark oxygen electrode, in an aliquot taken from the suspension of liver cells. Five hundred microlitres of the cell suspension were put into a glass chamber containing the Clark electrode. The

electrode was calibrated with air at +37°C at atmospheric pressure. The oxygen consumption was calculated from the constant rate of reduction of the oxygen tension in the buffer solution around the electrode. No albumin was added to the buffer solution when oxygen consumption was measured since fatty acids were not included in the incubation medium [18]. The incubation medium contained glucose, as indicated elsewhere. The same concentration of glucose as was used in the incubation medium was always used in the perfusate and the buffer during the 30-min collagenase treatment.

Oxygen uptake in total animals

Oxygen uptake was measured with 3–4 animals in a glass metabolic cage (3.5 l, Delman, Maywood, IL) equipped with a thermometer, water and food feeder and faeces–urine separator. The air outlet was at the bottom of the cage, while the inlet was in the top of the lid. An air-mixer was used to lower the gradients of CO_2 and O_2. A unit for drying gas, a flow meter (Omniflow, Flow Technology, Inc.) and a gas analyser (Servomex Oxygen Analyser OA 184, Taylor Servomex Ltd) were used for reproducible measurements. The gas analyser consisted of two separate paramagnetic analysers mounted inside a common case. One reference analyser measured air from the inlet of the animal cage, the other from the outlet. The difference in oxygen concentration was converted into a millivolt signal of 10 mV for a difference in oxygen concentrations of 1%.

The average oxygen uptake (mmol O_2/100 g/24 hr) was calculated from continuous computerised measurements over 24 hr in tumour-bearing and control animals. Full details of this experimental system will be published elsewhere.

Estimation of oxygen uptake in the tumour

Numerous small tissue specimens (5–7 mg wet weight) were excised from the peripheral viable parts of the tumour and immediately incubated separately for measurements of oxygen uptake as described above for liver cells. The total tumour was extirpated and weighed and the amount of tissue necrosis was assessed by weighing. The oxygen uptake *in vivo* in the entire tumour was then calculated, allowing for tumour necrosis and assuming that the oxygen uptake was even throughout the tumour in viable tumour tissue.

Electron-microscopic methods

The mouse liver was fixed by perfusion with 3% (w/v) glutaraldehyde in 0.1 M cacodylate buffer at pH 7.2, as described above. Small pieces of liver tissue were then kept in the solution for 4 hr at +4°C, washed in cold buffer and post-fixed in 1% w/v OSO_4 for 2 hr, then dehydrated in ethanol, embedded in Epon 812 and cut in an LKB Ultratome III. Special staining for glycogen was performed according to Thiery [19].

Computation of partition of oxygen uptake

Computation of partition of oxygen uptake between the host, tumour-host liver and tumour was done as described by Morrison [20]. The oxygen uptake in the total animal was determined by continuous measurement during 24 hr. The oxygen uptake in the liver was calculated by multiplying the oxygen uptake in isolated liver cells by the liver weight. The oxygen uptake in the tumour was calculated by multiplying the oxygen uptake in tiny tissue samples by the viable tumour mass. The oxygen uptake in the host was calculated as the oxygen uptake in the total tumour-bearing animal minus that in the tumour. In some experiments we also calculated the depression of oxygen uptake in host tissue minus that in tumour-host liver. The advantages and limitations of this approach have been discussed by Morrison [20].

Statistics

The non-parametric Mann–Whitney *U* test was used for the statistical evaluation [21]. *P*-values below 0.05 were considered significant.

RESULTS

Isolated liver cells from sarcoma-bearing mice consumed significantly less oxygen under all experimental conditions than cells from control animals (Table 1). The results are shown in relation to dry tumour weight. Starvation itself had no effect on oxygen uptake in liver cells from sarcoma-bearing mice, but it seemed to reduce the oxygen uptake in liver cells from control animals. This was concluded from experiments with cells isolated and incubated without any exogenous substrate. The *in vitro* rate of oxygen consumption in the entire tumour-host livers was estimated to be 366 μmol/hr, compared to 720 μmol/hr in the control livers when the increased water content in the tumour-host livers was allowed for (Table 2). These values were calculated from the mean uptake of oxygen *in vitro* (Table 1), assuming that 10^6 liver cells corresponded to 1 mg of liver tissue wet weight *in vivo*. It has been verified in our laboratory that this is a reasonable approximation (unpublished results). The recovery of intact liver cells from tumour-influenced livers tended to be higher than that from control livers ($P < 0.10$, Table 1). This may be due to an altered intercellular matrix, more susceptible to collagenase, in the tumour-host livers. The relative amount of intact cells

Table 1. Oxygen consumption in isolated liver cells from sarcoma-bearing mice and control animals under different experimental conditions

Animals and incubation procedure	Oxygen uptake (nmol O_2/min/10^6 intact cells)	No. of intact cells ($\times 10^6$/ml)	Protein content (μg/ml)	Tumour dry weight (mg)
Ad libitum, 12 mmol/l glucose				
T (10)	5.8 ± 0.9**	2.42 ± 0.33	141 ± 12	675 ± 23
C (10)	10.8 ± 1.6	1.79 ± 0.26	157 ± 22	
Ad libitum, 8 mmol/l glucose				
T (4)	5.5 ± 0.6**	—	—	664 ± 31
C (4)	12.3 ± 1.3	—	—	
Ad libitum, without exogenous glucose				
T (6)	7.0 ± 0.9*	1.28 ± 0.20**	—	669 ± 27
C (6)	12.9 ± 1.5	0.72 ± 0.10	—	
Starvation without exogenous glucose				
T (8)	5.0 ± 0.8*	1.49 ± 0.30	—	644 ± 40
C (8)	7.5 ± 0.8	0.96 ± 0.15	—	

Mann-Whitney U test: * $P<0.05$; ** $P<0.01$.
Liver cells were isolated and counted as described in Material and Methods. Two study-groups were used, with control mice treated under identical conditions: (a) sarcoma-bearing mice and controls eating *ad libitum*; and (b) sarcoma-bearing mice and controls starved for 20 hr before the experiments, but with free access to tap water. The buffer solution used for liver perfusion, isolation of the cells and incubation contained exogenous glucose in the concentrations given. Mean ± S.E., number of animals within parentheses. T = sarcoma-bearing mice; C = controls.

Table 2. Composition of liver tissue in sarcoma-bearing mice and controls in per cent of wet weight

	H_2O	Protein	Amino acids	Glycogen	Lipids
Sarcoma-bearing mice	71.4 ± 1.0(10)	18.8 ± 0.5(14)	0.31 ± 0.02(9)	1.0 ± 0.2(33)	6.0 ± 0.4(15)
Controls	67.1 ± 0.3(10)	19.2 ± 0.6(15)	0.26 ± 0.01(9)	3.8 ± 0.4(34)	5.8 ± 0.3(15)
	$P<0.025$	n.s.	$P<0.05$	$P<0.01$	n.s.

The liver wet weight did not differ between the groups. Mean ± S.E., number of animals within parentheses.

after the isolation procedures did not differ between the groups (viable cell count 80–85%). The protein content in the cell suspension could not be used as the reference, since debris and intact cells occurred despite filtration and washing of the cell fraction. Addition of exogenous pyruvate (200 μmol/l), glucose (0–12 mmol/l) and insulin in physiological concentrations (250 μU/ml) or insulin in high concentrations (25 mU/ml) to the incubation medium did not influence the constant rate of oxygen consumption within short periods of time (5–10 min). Addition of ADP (2.1×10^{-4} M) to the incubation medium did not influence the constant rate of oxygen consumption in cells from sarcoma-bearing mice or controls, which it did in isolated liver mitochondria (data not shown). This was regarded as support for the view that the cell membrane was not freely permeable to charged components after collagenase perfusion.

The liver wet weight was 1.0–1.1 g in freely fed sarcoma-bearing and control mice. The composition of tumour-host and control livers is shown in Table 2. The hepatic concentrations of different amino acids have been reported elsewhere [9]. Light and electron microscopy were used to evaluate the morphological appearance of the liver cells. The electron-microscopic examination indicated that the hepatocytes of the tumour-bearing animals differed from those of the control animals in the following ways (Fig. 1A and B): the glycogen content was markedly reduced. The smooth endoplasmic reticulum appeared increased throughout the cytoplasm and the granular endoplasmic reticulum seemed to be moderately increased. The lipid inclusions were increased in number and size and lysosomes were more common. The nucleus and the various membranes appeared normal. The electron-microscopic appearance of the hepatocytes was in accordance with the biochemical analysis of the liver tissue from tumour-bearing animals and controls (Table 2).

The mean rate of oxygen consumption in freely

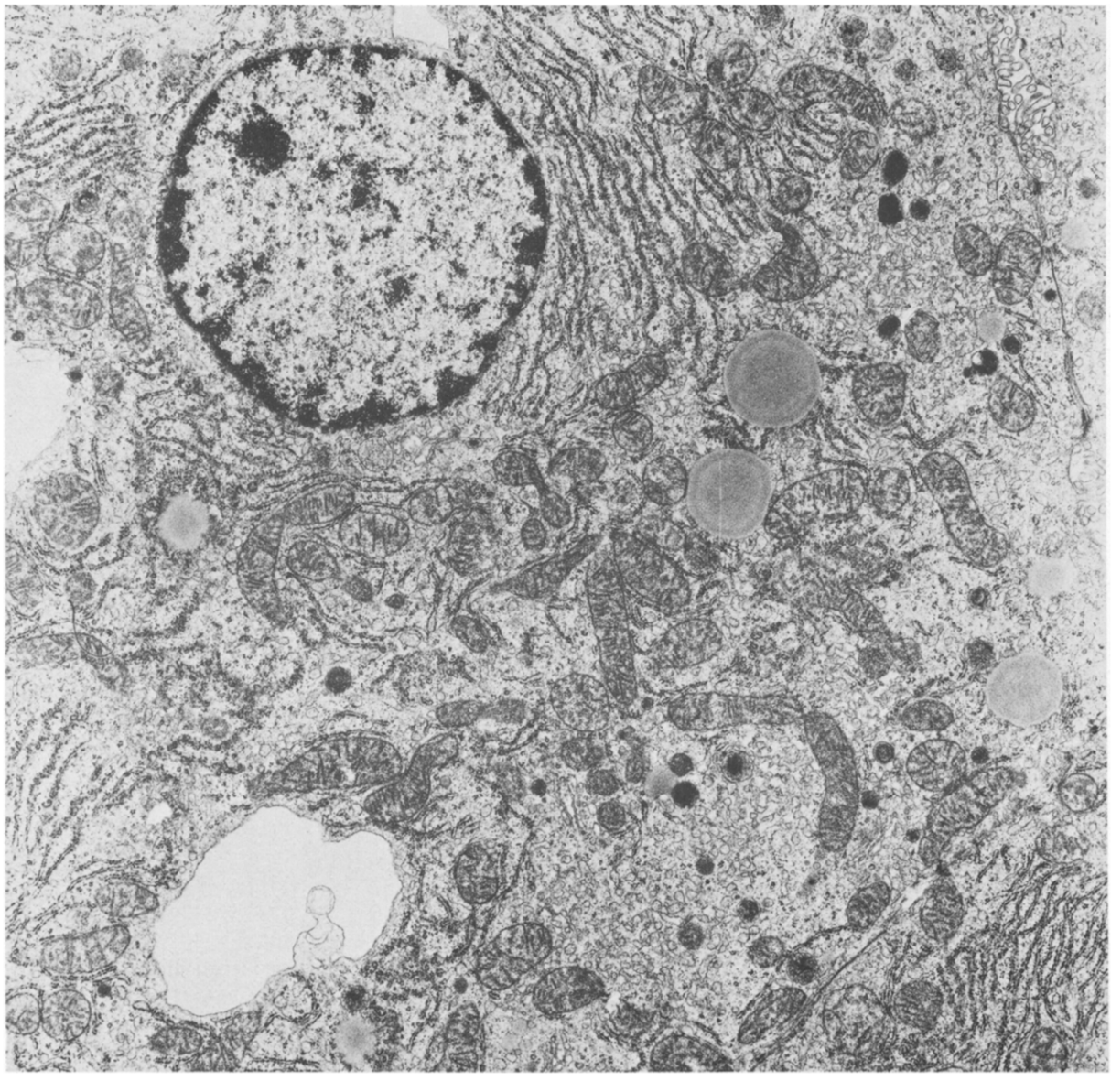

Fig. 1 (*A*)

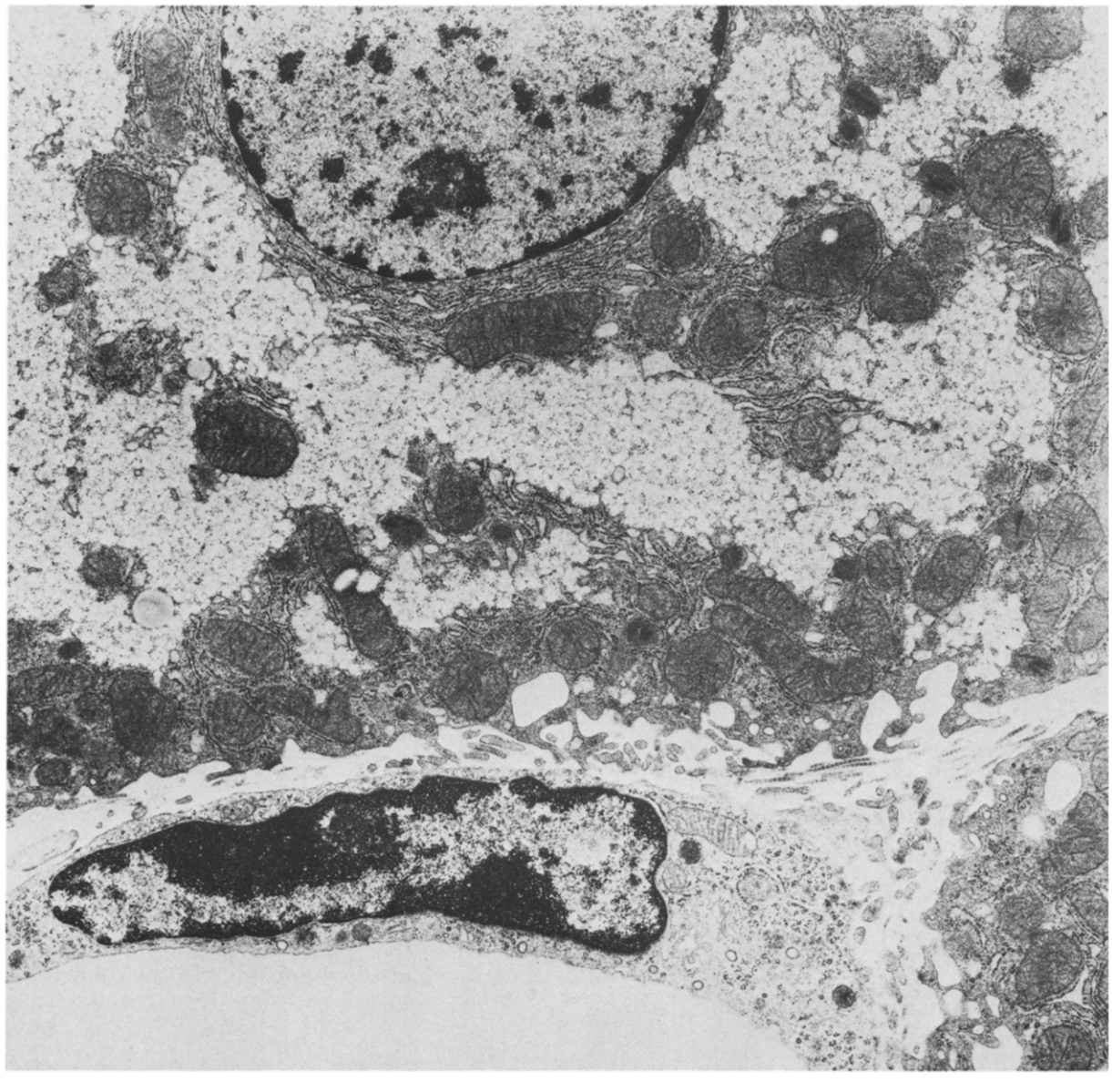

Fig. 1 (*B*)

Fig. 1. *The electron-microscopic appearance of hepatocytes from a sarcoma-bearing mouse* (*A*) *and from a control mouse* (*B*) (× 11,466).

fed sarcoma-bearing mice was 12.6 ± 1.1 mmol O_2/hr/100 g body weight 10–11 days after tumour implantation, compared to 17.5 ± 0.3 mmol/hr/100 g in the controls ($P < 0.01$). The time course of oxygen consumption in the sarcoma-bearing mice has been reported elsewhere [1]. The oxygen uptake in tumour tissue, measured in tissue specimens from five different tumours, was 0.48 ± 0.05 μmol O_2/min/g wet weight. Tumour necrosis accounted for 17 ± 1% of the total tumour mass in these tumours. The tumour wet weight was 3.52 ± 0.26 g (mean ± S.E.) and the fractional growth rate was 1.78%/hr (data not shown). The mean oxygen uptake in the entire tumour was calculated to be 84 ± 9 μmol/hr 10–11 days after tumour implantation.

The depression of oxidative metabolism in tumour-host liver cells compared to control liver cells was 51%. The mean depression of net oxygen uptake in host tissues other than the liver was 27%. The partition of oxygen uptake between host, liver and tumour is shown in Table 3. The depression of oxidative metabolism in the host liver was 354 μmol O_2/hr (322 μmol O_2/hr/g). It can therefore be calculated that a further 879 μmol O_2/hr (average 50 μmol O_2/hr/g) must have been saved in other host tissues or organs (3964 μmol/hr (control animals) – [2731 (host) – 354 (depression in host liver)] = 879 μmol/hr).

DISCUSSION

Carcass weight loss in tumour-bearing animals is due to translocation of nitrogen and energy-containing substrates from host compartments to the tumour [2]. It is also possible that energy is expended by other means in tumour-host compartments due to altered tumour-host metabolism caused directly or indirectly by the malignant tumour. Previous findings of high glucose [22], lactate [23], alanine [24] and glycerol turnover [25] in cancer patients, as well as similar metabolic changes in tumour-bearing animals [26], support this hypothesis. Findings of increased enzyme activities for hepatic glycolysis [6, 7], increased hepatic protein synthesis [8–10] and increased net lipolysis in peripheral tissues [11, 12] have indicated a possibility that the tumour-host liver may be one organ where energy may be expended at high rates in the host compartment compared to both freely fed and pair-fed controls. This hypothesis has been evaluated in the present work, where we have mainly used the technique described by Morrison for evaluation of the partition of oxygen uptake between host, tumour-host liver and tumour [20]. The partition of oxygen uptake has been calculated from continuous measurements in the tumour-bearing animal minus the uptake in the liver and the tumour. This means that the accuracy of these calculations is dependent on estimates of liver and tumour metabolism. In this study we have actually measured the potential capacity of the tumour-host hepatocytes to consume oxygen, which may exceed the *in vivo* oxygen uptake by the cells since it may well be that the pO_2 in the tissues of the tumour host is lower than normal. It must therefore be questioned whether estimates of this kind are relevant to the *in vivo* situation. The rates of oxygen consumption of liver cells in this study agreed with those previously reported for isolated rat liver cells and perfused rat liver [18]. Isolated hepatocytes also maintain *in vivo* levels of ATP [18], and the calculated oxygen uptake in the tumour-host liver was 13% of the total uptake in the tumour-bearing animals (Table 3). The corresponding value for controls was 18%.

This agrees with studies in which hepatic oxygen uptake in relation to total body uptake has been evaluated by direct measurements *in vivo* [18]. At present there is no suitable method for measuring total oxidative metabolism in subcutaneous tumours. We have therefore measured oxygen uptake in tumour-tissue specimens and calculated the entire tumour oxygen uptake, allowing for tumour necrosis, as described by Morrison [20]. Our estimate of oxygen consumption in the tumour was three times the measured *in vivo* uptake of 0.13 μmol O_2/min/g in a fibrosarcoma reported by Gullino [27]. That tumour had a doubling time of 61 hr, compared to 57 hr for the tumour in this study. Our value for tumour oxygen uptake (0.48 μmol/min/g) may be an overestimate since we have assumed that the oxygen uptake was that high throughout the

Table 3. Partition of oxygen consumption between the tumour-host liver, the tumour and the host

	Oxygen uptake (μmol/hr)					Depression of oxidative metabolism (μmol/hr) in the host due to:	
	Total animal	Tumour-host liver	Tumour	Host	Liver host	tumour-host liver	unidentified
T	2815 ± 321(10)	366 ± 26(10)	84 ± 9(5)	2731 ± 320	0.13	~354	~879
C	3964 ± 179(10)	720 ± 37(10)	—	—	0.18	—	—

Mean ± S.E., number of animals within parentheses. T = sarcoma-bearing mice, C = controls.

whole tumor tissue. Another explanation may be that we measured the rate of O_2 uptake by the tumour tissue in air, i.e. under a high pO_2, whereas the transplanted tumour *in vivo* is known to be very poorly supplied with oxygen [28, 29]. However, an overestimation in this respect is not critical for the conclusion drawn in this study, i.e. that the adaptation in oxidative metabolism in tumour-host liver is not low compared to that of other host tissues. Application of the value reported by Gullino [27] in our computations would not affect the conclusions. Our results also show that the mean rate of oxidative metabolism was highest in the liver, followed by the average oxygen uptake in other host tissues, and that tumour tissue had the lowest oxidative metabolism.

Isolated cells from tumour-host livers had decreased oxygen consumption compared to controls under all experimental conditions, i.e. (a) with food intake *ad libitum*; and (b) after 20 hr of starvation, as well as with and without exogenous substrates in the perfusion and incubation medium. The mechanisms behind this finding cannot be deduced from this study. Studies of isolated mitochondrion function in tumour-host livers have been controversial and do not allow general conclusions [26, 30–32]. Measurements of hepatic mitochondrial enzymes in our sarcoma-bearing mice have shown normal maximum enzyme activities (V_{max}) for alanine-amino tansferase, 3-OH-acyl-CoA dehydrogenase, citrate synthase and cytochrome *c* oxidase in liver tissue homogenates [33]. We therefore conclude that changes in maximum activities of respiratory enzymes do not explain the decreased oxygen consumption in tumour-host livers. The electron-microscopic appearance did not provide evidence that the decrease in oxidative metabolism was due to a decreased number or major structural changes in the mitochondria. Ghadially and Parry reported a normal mitochondrial appearance in liver tissue from rats with small benzanthracene-induced sarcomas. However, in the terminal stages of the tumour growth pathological swelling of many liver mitochondria was seen in that study [34]. It is not likely that our results were dependent on an increased susceptibility of the tumour liver cells to the isolation procedures, since the number of intact cells did not differ in the two groups. Moreover, the lack of effect of exogenous ADP in concentrations that stimulated respiratory activity in isolated hepatic mitochondria suggests that cells excluding trypan blue were not freely permeable to charged co-factors of importance for the regulation of respiratory activity. Small but important changes in the various cell membranes cannot be excluded as the reason. Another possible mechanism may be the decreased food intake (anorexia) in sarcoma-bearing mice, which we have reported [2]. It has previously been reported that incubated liver cells from starved rats had constant respiratory activity, despite the lack of liver glycogen, when the rats had been starved for 48 hr [18]. This means that the substrates used for the oxidative metabolic pathway were principally fatty acids and amino acids. It also shows that the level of endogenous substrates can support high *in vitro* oxidative metabolism in isolated liver cells. This may explain why rapid variations in the glucose and pyruvate concentration in the incubation medium and the addition of insulin in our system had almost no immediate effect on the constant rate of uptake of oxygen.

In conclusion, this study supports the view that negative energy balance in weight-losing sarcoma-bearing mice is not explained by deficient adaptation of oxidative metabolism in tumour-host livers. The finding that liver cells from starved tumour-bearing mice had the same oxidative metabolism as freely fed tumour-bearing mice while starved controls had slightly higher values is also in line with this hypothesis. This suggests that host liver metabolism was already maximally depressed in the fed state. The insufficient adaptation of carcass energy consumption leads to a negative energy balance, which explains the progression of cachexia. The site(s) and the metabolic pathway(s) for this energy consumption remain to be identified.

This study and other experiments in our laboratory [33] also show that a high metabolic rate in tumour-host livers is not a general phenomenon. This may otherwise be concluded from previous studies which have reported increased energy-generating hepatic enzyme activities with high substrate affinities [5, 6, 7, 26]. Furthermore, this study suggests that depression of 'activity metabolism' [20], which has been reported to be lower than would be expected from the body weight and food intake of the host [20], may account for depression of metabolic rate in host tissues other than the liver.

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