Metabolic substrate utilization by tumour and host tissues in cancer cachexia

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Utilization of metabolic substrates in tumour and host tissues was determined in the presence or absence of two colonic tumours, the MAC16, which is capable of inducing cachexia in recipient animals, and the MAC13, which is of the same histological type, but without the effect on host body composition. Glucose utilization by different tissues was determined *in vivo* by the 2-deoxyglucose tracer technique. Glucose utilization by the MAC13 tumour was significantly higher than by the MAC16 tumour, and in animals bearing tumours of either type the tumour was the second major consumer of glucose after the brain. This extra demand for glucose was accompanied by a marked decrease in glucose utilization by the epididymal fat-pads, testes, colon, spleen, kidney and, in particular, the brain, in tumour-bearing animals irrespective of cachexia. The decrease in glucose consumption by the brain was at least as high as the metabolic demand by the tumour. This suggests that the tissues of tumour-bearing animals adapt to use substrates other than glucose and that alterations in glucose utilization are not responsible for the cachexia. Studies *in vitro* showed that brain metabolism in the tumour-bearing state was maintained by an increased use of lactate and 3-hydroxybutyrate, accompanied by a 50 % increase in 3-oxoacid CoA-transferase. This was supported by studies *in vivo* which showed an increased metabolism of 3-hydroxybutyrate in tumour-bearing animals. Thus ketone bodies may be utilized as a metabolic fuel during the cancer-bearing state, even though the nutritional conditions mimic the fed state.

INTRODUCTION

Biochemical changes in host tissues frequently occur in cancer patients, and depletion of host adipose tissue and muscle protein in cancer patients is an important parameter determining overall survival [1]. The reason for depletion of host tissues is not known, but is thought to arise from differences in metabolism in the tumour-bearing state. Thus some studies have shown that the daily energy expenditure and the resting metabolic rate are much higher in cancer patients than in controls, even though the energy intake was not significantly different between the two groups [2]. Although anorexia is common in cancer patients [3], numerous studies have documented abnormalities of host metabolism, which together indicate that cancer cachexia is not the same as simple starvation [4].

There are marked alterations in the carbohydrate metabolism of host tissues of both animals and patients with cancer, but how these alterations contribute to the cachexia remains speculative. Glucose metabolism in cancer cells is known to be elevated, owing to an altered membrane transport, which leads to an increased intracellular concentration [5], with consequent increased lactate production by some tumours [6]. This in turn leads to an increased conversion of lactate into glucose in the liver via the Cori cycle, the activity of which is also found to be elevated in cancer patients with progressive weight loss, showing that lactate production rates are higher in these patients [7]. Decreased blood glucose values are often found in tumour-bearing animals [8], suggesting that the glucose availability is not sufficient to provide for both host and tumour. In addition, a decreased plasma insulin level is often found [9], together with an elevated rate of gluconeogenesis from non-carbohydrate precursors [10].

To try to determine the biochemical changes responsible for,

or accompanying, cachexia, we have employed an experimental murine colon adenocarcinoma (MAC16), which produces extensive loss of host body weight, accompanied by decreases in both fat and lean body mass, but without a fall in food intake [11,12]. This tumour is useful for the study of the mechanisms of cachexia, since in some cases growth of the tumour is not accompanied by weight loss [13], suggesting that the presence of the tumour alone is insufficient to produce the effects on host body tissues. In addition, tumours of the same histological type are available which grow without an accompanying cachexia (e.g. MAC13). This study measures changes in glucose utilization in host and tumour tissues in animals with or without cachexia, in order to elucidate if changes in carbohydrate metabolism are related to the cachectic state.

MATERIALS AND METHODS

Pure-strain NMRI mice were bred in our own colony and were fed on a rat and mouse breeding diet (Pilsbury, Birmingham, U.K.) and water *ad libitum*. Fragments of either the MAC16 or MAC13 tumour were implanted into the flank of male NMRI mice (starting weight 24–26 g) by means of a trocar, as described in [11,12]. Animals bearing the MAC16 tumour develop weight loss 10–12 days after the transplantation (average tumour weight 200 mg), and, when weight loss developed, the animals were regarded as cachectic (average weight loss 2–4 g). Occasionally tumours grew without the development of weight loss, and these animals were regarded as non-cachectic (average tumour weight 200 mg; about 5 % of the total transplanted). Animals bearing the MAC13 tumour were used 10–12 days after tumour transplantation, when the tumour became palpable (average tumour weight 200 mg). The daily food intake per mouse [63.2 ± 2.5 kJ

Abbreviations used: 2DG, 2-deoxyglucose; 2DGP, 2-deoxyglucose 6-phosphate; [^3H]2DG, 2-deoxy-D-[2,6- ^3H]glucose; [^{14}C]2DG, 2-deoxy-D-[1- ^{14}C]glucose.

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(15.1 ± 0.6 kcal)] in animals bearing the MAC16 tumour did not differ from that of non-tumour-bearing controls [64.0 ± 1.25 kJ (15.3 ± 0.3 kcal)], whereas in animals bearing the MAC13 tumour the daily food intake [68.6 ± 1.25 kJ (16.4 ± 0.3 kcal)] was significantly increased ($P < 0.01$).

Glucose utilization

The extent of glucose utilization by different tissues was investigated by using the 2-deoxyglucose tracer technique [14]. Briefly, animals were starved overnight and throughout the experiment, but given water *ad libitum*. The following morning they were injected intravenously with 30 μ Ci of 2-deoxy-D-[2,6- 3 H]glucose (3 H]2DG; sp. radioactivity 42 mCi/mmol; Amersham International, Amersham, Bucks., U.K.)/kg body wt. in 200 μ l of normal saline. To determine the retention of 2-deoxyglucose 6-phosphate (2DGP) by different tissues, a second intravenous injection of 3 μ Ci of 2-deoxy-D-[1- 14 C]glucose (14 C]2DG; sp. radioactivity 56 mCi/mmol; Amersham International)/kg was administered 35 min after the injection of the 3 H]glucose. Blood was removed from animals by cardiac puncture, while under anaesthesia, at specified time intervals, and the decay of radioactivity in the blood was monitored for 60 min [14]. Blood glucose concentration was determined on whole blood by using the *o*-toluidine reagent kit (Sigma Chemical Co., Poole, Dorset, U.K.). The concentration of radioactivity in the blood was determined on deproteinized neutralized samples with a dual 3 H/ 14 C analyser.

The accumulation of phosphorylated metabolites of 2DG was measured in selected tissues at the 60 min time point [14,15]. Glucose utilization was calculated from the equation [14]:

$$R_g = \frac{C_m^*(T)}{LC} \cdot \frac{\int_0^T C_p^* \cdot dt}{\int_0^T C_p}$$

where R_g is tissue glucose metabolic rate (nmol/min per g), $C_m^*(T)$ is the concentration of phosphorylated metabolites of 2DG in the tissue (d.p.m./g) at $t = 60$ min, C_p is the blood glucose (nmol/ml), C_p^* is the concentration of 3 H]2DG in the blood (d.p.m./ml) and LC (lumped constant) is a dimensionless correction factor for discrimination against 2DG in glucose metabolic pathways and was determined by the method of Ferré *et al.* [16]. The value determined for various tissues, 0.46 [17], corresponds favourably to that previously reported [14–16].

Glucose utilization *in vitro* by tumour cell lines

Both the MAC16 and MAC13 cell lines were derived from the corresponding solid tumours and were maintained *in vitro* in RPMI 1640 culture medium containing 10% (v/v) foetal-calf serum (Gibco Europe, Paisley, Scotland, U.K.) under an atmosphere of 5% CO_2 in air. For measurement of the rate of CO_2 production, the medium, containing cells at a density of 4×10^4 ml $^{-1}$, was supplemented with 0.2 μ Ci of D-[U- 14 C]glucose (sp. radioactivity 270 mCi/mmol; Amersham International)/ml in a flask with a centre well and sealed with a rubber seal. At specified time points 0.3 ml of 0.3 M-NaOH was injected into the centre well, 0.3 ml of 5 M-HClO $_4$ was added to the medium, and the flasks were left overnight. The radioactivity in the alkali was determined with a Packard Tri-carb 2000 CA liquid-scintillation analyser, and the cell number was enumerated with a Coulter electronic particle counter. For determination of glucose consumption and lactate production, cells were resuspended at a density of 4×10^4 ml $^{-1}$, and at specified time intervals medium was removed and the concentration of glucose was determined with the *o*-toluidine reagent kit, and the concentration of lactate by the method of Gutmann & Wahlefeld [18].

Fuel utilization by liver, brain and tumour *in vitro*

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 (2.2 mM), DL-3-hydroxybutyrate (0.1 mM) and lactate (8 mM). In each fuel mixture only one of the three substrates was radioactive. The radiolabelled compounds D-[U- 14 C]glucose (sp. radioactivity 273 mCi/mmol) and L-[U- 14 C]lactic acid (sp. radioactivity 169 mCi/mmol) were purchased from Amersham International, D-(–)- β -hydroxy[3- 14 C]butyric acid (potassium salt; sp. radioactivity 44.3 mCi/mmol) was purchased from New England Nuclear, Stevenage, Herts., U.K. The slices were incubated with 10 ml of fuel mixture containing 2.5 μ Ci of the labelled fuel in a flask containing a centre well and sealed with a Suba-seal. Samples were gassed with O_2/CO_2 (19:1) and incubated at 37 °C for 1 h. At this time 0.3 ml of 5 M-HClO $_4$ was added to the medium to liberate the CO_2 , which was collected in 0.3 ml of 0.3 M-NaOH added to the centre well. After standing for 4 h to allow complete absorption, the contents of the centre well were added to 10 ml of Optiphase Hi-safe 3 scintillation fluid and the radioactivity was determined.

Determination of brain 3-oxoacid CoA-transferase

Brains were homogenized in 10 mM-Tris, pH 7.4, containing 0.25 M-sucrose and 1 mM-2-mercaptoethanol, and enzyme activity was determined in the supernatant fraction prepared by centrifugation at 30000 *g* for 20 min. The rate of acetoacetyl-CoA formation from 0.1 mM-succinyl-CoA was determined by the method of Williamson *et al.* [19].

Effect of tumour-bearing state on $^{14}\text{CO}_2$ production from 3-hydroxybutyric acid

Male NMRI mice bearing either the MAC16 tumour with weight loss or the MAC13 tumour, or non-tumour-bearing controls, were injected intravenously with 50 μ Ci of D-(–)- β -hydroxy[3- 14 C]butyric acid (sp. radioactivity 44.3 mCi/mmol) (Amersham International)/kg and were placed in airtight metabolic cages with the entry air being pumped through CaCO $_3$ (solid) to absorb any CO_2 . Metabolically produced $^{14}\text{CO}_2$ was trapped in ethanolamine/ethoxyethanol (1:4, v/v), and samples were taken at specified time intervals and the radioactivity was determined directly in Optiphase scintillation fluid (FSA Laboratory Supplies, Loughborough, Leics., U.K.).

RESULTS

In order to understand the glucose dynamics of the MAC16 and MAC13 tumours *in vivo*, and the effects on host organs with the development of cachexia, glucose utilization was investigated by the 2DG tracer method [14–16]. Blood glucose levels in animals bearing the MAC13 tumour that had been starved overnight were similar to those in control non-tumour-bearing animals over a 60 min time period (Fig. 1a), whereas animals bearing the MAC16 tumour were hypoglycaemic as compared with either group, irrespective of the development of cachexia, although this was only significant at the 60 min time point. However, the rate of disappearance of the label from 3 H]2DG (Fig. 1b) or from 14 C]2DG (Fig. 1c) did not differ between the four groups. The tissue glucose metabolic rate (R_g) of control mice and animals bearing either the MAC13 tumour or the MAC16 tumour, with or without cachexia, is shown in Table 1. Glucose utilization by the MAC13 tumour was significantly ($P < 0.05$) greater than that by the MAC16 tumour, probably owing to the higher rate of cell replication (doubling time of MAC13 tumour 7 days; doubling time of MAC16 tumour 10–12 days). There was no difference in glucose utilization by the

Fig. 1. Plasma glucose concentration (a) and the disappearance of [^3H]2DG (b) or [^{14}C]2DG (c) from the blood of non-tumour-bearing animals (■), MAC13-tumour-bearing animals (○) and animals bearing the MAC16 tumour in the presence (●) or absence (□) of weight loss

Animals were starved overnight and then injected with 30 μCi of [^3H]2DG/kg and 35 min later with 3 μCi of [^{14}C]2DG/kg, and serial blood samples were removed at time intervals. Each point represents the mean $\pm$ S.E.M. of four or five animals. *, $\dagger$, $P < 0.005$ for difference from non-tumour-bearing controls by Student's t -test.

MAC16 tumour in the presence or absence of cachexia (Table 1). The R_g values for testes, colon, spleen, kidney and particularly brain ($P < 0.005$) were significantly decreased in animals bearing the MAC16 tumour. The large decrease in the R_g 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 the various organs to glucose utilization in tumour-bearing animals is shown in Table 2. The magnitude of the contribution to glucose utilization by the various organs depends on both the R_g value and the weight of a particular

organ. In all tumour-bearing animals the tumour was the second major consumer of glucose after the brain. This placed a high demand on the host, and was accompanied by a marked decrease in glucose utilization by the brain. In animals bearing the MAC13 tumour, glucose consumption by the brain decreased by an amount equal to that used by the tumour, whereas, in animals bearing the MAC16 tumour, brain glucose consumption was decreased to a greater extent than glucose consumption by the tumour. In animals bearing the MAC16 tumour, glucose consumption was also significantly decreased in epididymal fat-pads, testes, colon, spleen and kidney, but was not related to the presence of cachexia.

To investigate whether cachexia altered the retention of 2DGP

Table 1. Tissue glucose metabolic rate (R_g) in non-tumour-bearing mice, or in animals bearing the MAC13 tumour or the MAC16 tumour in the presence or absence of cachexia

The glucose metabolic rate (R_g) was determined from the decay of [^3H]glucose in the blood over 60 min after injection of 30 μCi of [^3H]2DG/kg body wt. as described in the Materials and methods section. The results are mean values $\pm$ S.E.M. for five animals per group and are expressed as nmol of glucose per min/g. Values that are significantly different by Student's t test from control values are indicated by: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.005$, **** $P < 0.001$. Values for MAC16-tumour-bearing animals that are significantly different by paired t test from those for MAC13-tumour-bearing animals are indicated by: $\dagger P < 0.05$, $\dagger\dagger P < 0.001$.

Organ	Control	MAC13-bearing	MAC16-bearing (cachectic)	MAC16-bearing (non-cachectic)
Brain	1270 $\pm$ 113	933 $\pm$ 68*	653 $\pm$ 43***	837 $\pm$ 35*
Lungs	184 $\pm$ 25	253 $\pm$ 19	160 $\pm$ 15	197 $\pm$ 22
Liver	73 $\pm$ 19	61 $\pm$ 3	—	—
Kidney	119 $\pm$ 5	109 $\pm$ 5	32 $\pm$ 7****	33 $\pm$ 4****
Spleen	326 $\pm$ 28	322 $\pm$ 21	192 $\pm$ 13*	149 $\pm$ 22****
Colon	236 $\pm$ 29	325 $\pm$ 9	145 $\pm$ 16**	182 $\pm$ 8
Adipose	43 $\pm$ 4	46 $\pm$ 6	42 $\pm$ 22	26 $\pm$ 1
Testes	237 $\pm$ 23	205 $\pm$ 17	82 $\pm$ 12*	140 $\pm$ 8***
Tumour	—	1652 $\pm$ 139	925 $\pm$ 78†	735 $\pm$ 47††

Table 2. Organ glucose utilization rate in non-tumour-bearing mice, or in animals bearing the MAC13 tumour or the MAC16 tumour in the presence or absence of cachexia

The results (calculated as for Table 1) are mean values $\pm$ S.E.M. for five animals per group and are expressed as nmol of glucose used/min per tissue. Values that are significantly different by Student's t test from control values are indicated by: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.005$, **** $P < 0.001$.

Organ	Control	MAC13-bearing	MAC16-bearing (cachectic)	MAC16-bearing (non-cachectic)
Brain	549 $\pm$ 41	356 $\pm$ 33**	224 $\pm$ 14****	214 $\pm$ 11****
Lungs	47 $\pm$ 9	47 $\pm$ 5	27 $\pm$ 5	34 $\pm$ 3
Liver	70 $\pm$ 14	63 $\pm$ 4	—	—
Kidney	43 $\pm$ 2	37 $\pm$ 2	8 $\pm$ 2****	6 $\pm$ 1****
Spleen	26 $\pm$ 2	34 $\pm$ 3	13 $\pm$ 2***	7 $\pm$ 1****
Colon	54 $\pm$ 7	54 $\pm$ 6	28 $\pm$ 4**	28 $\pm$ 3**
Adipose	17 $\pm$ 2	19 $\pm$ 4	7 $\pm$ 2*	6 $\pm$ 1****
Testes	35 $\pm$ 3	27 $\pm$ 3	11 $\pm$ 1****	19 $\pm$ 2****
Tumour	—	189 $\pm$ 5	157 $\pm$ 13	94 $\pm$ 22

Table 3. Labelling of 2DGP in organs of non-tumour-bearing mice or in animals bearing the MAC13 tumour or the MAC16 tumour in the presence or absence of cachexia

The results are mean values $\pm$ S.E.M. for five animals per group and are expressed as the $^3\text{H}/^{14}\text{C}$ ratio of the 2DGP. Values that are significantly different by Student's *t* test from control values are indicated by: * $P < 0.05$, ** $P < 0.01$. Values for MAC16-tumour-bearing animals that are significantly different by paired *t* test from those for MAC13-tumour-bearing animals are indicated by † $P < 0.01$, and between cachectic and non-cachectic MAC16-tumour-bearing animals by ‡ $P < 0.01$.

Organ	$^3\text{H}/^{14}\text{C}$			
	Control	MAC13-bearing	MAC16-bearing (cachectic)	MAC16-bearing (non-cachectic)
Brain	6.8 $\pm$ 0.6	8.9 $\pm$ 0.3*	9.4 $\pm$ 1.0*†	5.6 $\pm$ 0.2
Lungs	7.0 $\pm$ 0.6	9.5 $\pm$ 1.0	15.3 $\pm$ 2.1**†	6.3 $\pm$ 0.5
Liver	12.3 $\pm$ 5.6	6.2 $\pm$ 0.6	—	—
Kidney	3.5 $\pm$ 0.2	4.4 $\pm$ 0.2	5.8 $\pm$ 2.1	4.3 $\pm$ 1.3
Spleen	7.9 $\pm$ 0.6	10.3 $\pm$ 0.8*	6.9 $\pm$ 2.0	6.5 $\pm$ 0.2
Colon	5.8 $\pm$ 0.5	9.4 $\pm$ 1.0	8.6 $\pm$ 2.6	5.4 $\pm$ 0.7
Adipose	9.3 $\pm$ 2.0	10.8 $\pm$ 1.2	9.2 $\pm$ 0.32	7.0 $\pm$ 1.4
Testes	10.3 $\pm$ 1.2	13.8 $\pm$ 0.8*	15.4 $\pm$ 1.3*	8.7 $\pm$ 0.3
Tumour	—	13.4 $\pm$ 0.8	12.6 $\pm$ 1.4‡	8.5 $\pm$ 0.5

in the various organs, a sequential double-labelling technique was applied, followed by an analysis of the two labels in 2DGP. Since there was a marked initial decay of the precursor in the blood, the bulk of the [^3H]2DGP was synthesized in the tissues during the initial 35 min of the labelling period, and the $^3\text{H}/^{14}\text{C}$ ratio of tissue 2DGP was measured at the end of the experiment. Loss of 2DGP from the tissue would affect the ^3H component of the ratio more than the ^{14}C component, and therefore the $^3\text{H}/^{14}\text{C}$ ratio of 2DGP in the tissues was a measure of the retention of 2DGP, i.e. a low ratio indicated a high rate of loss. Since 10 times as much ^3H radioactivity was administered as ^{14}C , the $^3\text{H}/^{14}\text{C}$ ratio would be expected to be near 10 if both radioisotopes were incorporated into 2DGP at the 60 min time period and there was no loss of DGP. This was true for most control tissues, except for lung, colon, brain and kidney, in which the ratios were much lower (Table 3). The lower ratio for brain has previously been reported [14], and arises from an increased rate of loss of 2DGP from this tissue. When compared with control animals and MAC16-tumour-bearing animals without cachexia, the $^3\text{H}/^{14}\text{C}$

ratio for testes, lung and brain was significantly higher in cachectic animals bearing the MAC16 tumour (Table 3). This suggests a slow incorporation of 2DG into 2DGP, a low rate of utilization and, assuming that the energy requirements remain constant, that the tissues of cachectic animals adapt to use metabolic substrates other than glucose.

The decrease in glucose utilization by organs in tumour-bearing mice must be accompanied by an alteration in the preference of the substrate for energy production. To determine the preferred metabolic substrate, the utilization of glucose, lactate and 3-hydroxybutyrate was determined by using a substrate mixture with a high lactate concentration. The results for liver, brain and tumour are shown in Table 4. At the concentration employed, lactate was the most important oxidative substrate for the liver in control and tumour-bearing animals, with the rate of utilization by liver slices from animals bearing the MAC13 tumour being significantly ($P < 0.005$) elevated over that of non tumour-bearing controls (Table 4). Both glucose and 3-hydroxybutyrate consumptions by the liver were low, and, although the former did not change in the tumour-bearing state, consumption of 3-hydroxybutyrate was significantly higher ($P < 0.001$) in animals bearing the MAC13 tumour.

Lactate was an important metabolic fuel for the tumour, and the rate of utilization was significantly greater ($P < 0.001$) in the MAC13 tumour. Although the glucose consumption rate was not significantly different for the two tumour types, the rate of utilization 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, possibly owing to the supra-physiological lactate concentration, whereas in the brain of animals bearing the MAC16 tumour with cachexia, the rate of glucose utilization was decreased by two-thirds ($P < 0.05$) and was also significantly less than that in brains of animals bearing the MAC13 tumour ($P < 0.005$). This metabolic deficit produced by a decreased glucose consumption was accompanied by a marked increase in both lactate ($P < 0.05$) and 3-hydroxybutyrate ($P < 0.01$) utilization in the brains of animals bearing the MAC16 tumour, when compared with non-tumour-bearing controls. This increased 3-hydroxybutyrate utilization in the brains of tumour-bearing animals is probably due to an increase in the level of 3-oxoacid CoA-transferase (EC 2.8.3.5), the rate-limiting enzyme in ketone-body utilization. Thus animals bearing both the MAC16 and MAC13 tumours show a significant ($P < 0.05$) elevation of brain 3-oxoacid CoA-transferase activity (1.8 ± 0.2 and 1.5 ± 0.1 $\mu\text{mol}/\text{min}$ per mg of protein respectively) as compared with non-tumour-bearing controls (1.2 ± 0.1 $\mu\text{mol}/\text{min}$ per mg of protein).

Table 4. Utilization *in vitro* of glucose, 3-hydroxybutyrate and lactate by slices of liver, brain and tumour from control mice (A) or from animals bearing either the MAC13 tumour (B) or the MAC16 tumour (C)

The results are mean values $\pm$ S.E.M. for five animals per group and are expressed as nmol of CO_2/h per g. Values that are significantly different by Student's *t* test from control values are indicated by: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.005$, **** $P < 0.001$. Values for MAC16-tumour-bearing animals that are significantly different by paired *t* test from those for MAC13-tumour-bearing animals are indicated by: † $P < 0.05$, †† $P < 0.005$, ††† $P < 0.001$. In each fuel mixture only one of the three substrates was radioactive.

Organ	Substrate								
	Glucose			3-Hydroxybutyrate			Lactate		
	A	B	C	A	B	C	A	B	C
Liver	28 $\pm$ 2	25 $\pm$ 5	25 $\pm$ 2	0.3 $\pm$ 0.05	0.6 $\pm$ 0.1****	0.3 $\pm$ 0.05††	157 $\pm$ 13	367 $\pm$ 55***	238 $\pm$ 12
Brain	340 $\pm$ 156	237 $\pm$ 36	96 $\pm$ 21*††	1.1 $\pm$ 0.3	1.8 $\pm$ 0.2	2.9 $\pm$ 0.4**†	276 $\pm$ 9	701 $\pm$ 166*	562 $\pm$ 92*
Tumour	—	92 $\pm$ 43	61 $\pm$ 7	—	2.5 $\pm$ 0.2	0.8 $\pm$ 0.2†††	—	306 $\pm$ 44	52 $\pm$ 7†††

Fig. 2. Production of $^{14}\text{CO}_2$ from D-(—) β -hydroxy[3- ^{14}C]butyric acid by non-tumour-bearing animals (■) and animals bearing the MAC13 (○) and MAC16 (●) tumours

* $P < 0.05$ and † $P < 0.05$ compared with non-tumour-bearing animals by Student's t test. The results are expressed as means $\pm$ S.E.M. for six animals per group.

Production of $^{14}\text{CO}_2$ after intravenous administration of D-(—)3-hydroxy[3- ^{14}C]butyrate was rapid in all groups of animals and was significantly ($P < 0.05$) greater in animals bearing both the MAC16 and MAC13 tumours (Fig. 2). This suggests that in tumour-bearing animals brain metabolism is supported by an increased utilization of 3-hydroxybutyrate, as occurs in starvation, and this appears to be independent of the development of cachexia.

DISCUSSION

Glucose is an important metabolic substrate, particularly for solid tumours [20], possibly owing to a poor vascularization, which would militate against oxidative metabolism. In a study of energy production by a rapidly growing hepatoma cell line, it was estimated that about 60% of the total ATP was derived from glycolysis and 40% from oxidative phosphorylation [21]. In the present study the glucose consumption by the colon adenocarcinomas of the MAC series has been shown to be high ($0.7\text{--}1.1 \mu\text{mol/min per g}$) and comparable with that of the brain ($0.8 \mu\text{mol/min per g}$). Interestingly, the rate of glucose utilization by the MAC16 tumour was lower than that by the MAC13 tumour and independent of the production of cachexia in the animal, suggesting that the high glucose consumption by the tumour is not sufficient to explain the loss of host body compartments.

The tumour-bearing state also induces profound changes in glucose utilization by host organs, and in particular by the brain, where glucose utilization is severely depressed. Previous studies [22] have shown that glucose consumption by murine peritoneal-exudate macrophages is suppressed by pleural effusions, ascites fluids and sera from patients with advanced primary lung and gastric cancers. Inhibition of glycolysis was ascribed to an inhibition of the rate-limiting key enzyme in the glycolysis pathway, D-fructose-6-phosphate 1-phosphotransferase, although the nature of the inhibitory factor is not known. Certainly, suppression of glucose utilization by host organs would be advantageous to the tumour, in view of the high glucose utilization. A decreased glucose uptake in the peripheral tissues of cancer patients has also been reported [23], possibly owing to a decreased insulin response, although this would not explain the decreased glucose utilization by the brain.

The decreased glucose utilization by peripheral tissues in the tumour-bearing state suggests an increased dependence on fat as

an energy source. Certainly, in patients with malignant cachexia, a greater proportion of oxidative metabolism appears to arise from fatty acids, particularly when exogenous glucose is available [24]. The inability of glucose loading to suppress oxidation of fatty acids in cachectic patients suggests that there is a disturbance in normal homeostatic mechanisms involving the utilization of glucose and other important fuel sources. We have recently observed an increased lipogenesis from glucose in host tissues in animals bearing both the MAC16 and MAC13 tumours (H. D. Mulligan & M. J. Tisdale, unpublished work). This suggests that glucose is diverted away from host tissues, not only for direct utilization by the tumour, but also as an important starting material for the synthesis of substances that may be required for tumour growth. In addition, if some glucose is not oxidized directly, but is first converted into fat before supplying utilizable energy, there is a significant energy cost to the organism, which could lead to loss of body weight if energy intake is not increased.

In order to determine the predominant metabolic fuel used by tumour and host tissues, we have employed an assay system *in vitro* using slices of brain, liver and tumour fed with a fuel mixture of glucose (2.2 mM), D-3-hydroxybutyrate (0.05 mM) and lactate (8 mM) [25]. This system paralleled the study *in vivo* using 2DG, in that, although glucose was the predominant metabolic fuel for the brain in non-tumour-bearing animals, its importance in oxidative metabolism was decreased in the tumour-bearing situation, especially in animals bearing the cachexia-inducing 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 [26]. Thus the brains of cachectic animals bearing the MAC16 tumour resemble those during starvation, although the food intake is not decreased [11,12]. In the rat, the major factor influencing the rate of utilization of ketone bodies has been suggested to be the concentration in the blood [19]. However, although extensive mobilization of adipose tissue is observed in animals bearing the MAC16 tumour, no elevations in plasma or urinary levels of ketone bodies have been detected [11]. In addition, in the situation *in vitro*, the concentrations of glucose, lactate and 3-hydroxybutyrate in the incubation medium remained constant, suggesting that the enzymes which regulate ketone-body utilization in the brain of tumour-bearing animals may be altered. This hypothesis was confirmed by measurements of the levels of 3-oxoacid CoA-transferase, the rate-limiting enzyme in ketone-body utilization, which was shown to be significantly enhanced in animals bearing both the MAC16 and MAC13 tumours. In contrast, the level of acetoacetyl-CoA thiolase was not significantly different between tumour-bearing ($18.3 \pm 4.1 \mu\text{mol/min per mg}$ of protein) and non-tumour-bearing ($22.0 \pm 6.6 \mu\text{mol/min per mg}$ of protein) animals.

We have previously shown that 3-oxoacid CoA-transferase is also present in the MAC16 tumour, but at decreased levels compared with normal colon [27]. The activity of the enzyme in liver is low or non-detectable, and coincides with a low rate of utilization of 3-hydroxybutyrate by liver slices *in vitro*, whereas the rate of oxidation by tumour tissue was 3–10 times higher. For the MAC13 tumour, lactate was the most important metabolic fuel at the high concentration employed, and lactate has also been shown to be utilized by a number of rat tumours *in vivo* [28]. Studies *in vitro* with the two tumour cell lines showed that, although the total glucose consumption did not differ, oxidation to CO_2 was greater in MAC13 cells ($P < 0.01$), whereas lactate production was higher in MAC16 cells ($P < 0.001$). This suggests that there may be some difficulty in the oxidation of reduced nicotinamide nucleotides by the MAC16 tumour.

Thus the high metabolic demand for glucose placed on the host in the tumour-bearing state is accompanied by a decreased

utilization by host organs, principally the brain, accompanied by utilization of ketone bodies as an alternative metabolic fuel. Utilization of ketone bodies appears to result from an increased activity of 3-oxoacid CoA-transferase without an alteration in substrate availability. This change appears not to be specific for the cachectic state, although the increased metabolic demand on the host is associated with an increased food intake in animals bearing the MAC13 tumour, whereas animals bearing the MAC16 tumour have the same food intake as non-tumour-bearing controls. Thus cachexia could arise from an inability of the host to adapt to the increased metabolic requirements of the tumour-bearing state.

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