Ketone-body metabolism in tumour-bearing rats

Allan M. ROFE, Renze BAIS and Robert A. J. CONYERS*

Metabolic Research Group, Division of Clinical Chemistry, Institute of Medical and Veterinary Science, Frome Road, Adelaide, SA 5000, Australia

During starvation for 72 h, tumour-bearing rats showed accelerated ketonaemia and marked ketonuria. Total blood [ketone bodies] were 8.53 mM and 3.34 mM in tumour-bearing and control (non-tumour-bearing) rats respectively ($P < 0.001$). The [3-hydroxybutyrate]/[acetoacetate] ratio was 1.3 in the tumour-bearing rats, compared with 3.2 in the controls at 72 h ($P < 0.001$). Blood [glucose] and hepatic [glycogen] were lower at the start of starvation in tumour-bearing rats, whereas plasma [non-esterified fatty acids] were not increased above those in the control rats during starvation. After functional hepatectomy, blood [acetoacetate], but not [3-hydroxybutyrate], decreased rapidly in tumour-bearing rats, whereas both ketone bodies decreased, and at a slower rate, in the control rats. Blood [glucose] decreased more rapidly in the hepatectomized control rats. Hepatocytes prepared from 72 h-starved tumour-bearing and control rats showed similar rates of ketogenesis from palmitate, and the distribution of [$1\text{-}^{14}\text{C}$]palmitate between oxidation (ketone bodies and CO_2) and esterification was also unaffected by tumour-bearing, as was the rate of gluconeogenesis from lactate. The carcinoma itself showed rapid rates of glycolysis and a poor ability to metabolize ketone bodies *in vitro*. The results are consistent with the peripheral, normal, tissues in tumour-bearing rats having increased ketone-body and decreased glucose metabolic turnover rates.

INTRODUCTION

Ketone bodies (D-3-hydroxybutyrate and acetoacetate) have several important physiological functions (Robinson & Williamson, 1980) and, notably, during starvation they provide an alternative fuel to glucose, inhibit glucose utilization and act to limit proteolysis and lipolysis (Sherwin *et al.*, 1975; Cahill, 1976; Robinson & Williamson, 1980). Although it is clear that the wasting seen in association with cancer is different from that seen in uncomplicated starvation (Stein, 1978; Conyers *et al.*, 1979a,b; Magee *et al.*, 1979; Brennan, 1981), little is known about the metabolism of ketone bodies in the cancer state.

Some investigators (Schein *et al.*, 1979; Axelrod *et al.*, 1983) have reported normal, and others (Rich & Wright, 1979) less than normal, ketonaemic responses to fasting in cancer patients. Our studies indicate that ketonuria is uncommon in wasted cancer patients (Conyers *et al.*, 1979a), and Axelrod *et al.* (1983) found that the extent of ketonuria in some fasted cancer patients was much lower than would be predicted from the existing ketonaemia.

Few studies have examined ketone-body metabolism in tumour-bearing animals. One report (Sauer & Dauchy, 1983) showed decreased ketonaemia in rats bearing transplanted tumours. However, the mode of transplantation in those studies involved major surgery (Sauer *et al.*, 1982), which in itself may affect ketone-body metabolism. In contrast, there is the observation of increased ketonuria in tumour-bearing rats on parenteral low-carbohydrate nutrition (Cameron & Ord, 1983), and the mention, in passing, of ketonaemia in rats with large tumours (Singh *et al.*, 1980). It is of both biochemical and clinical interest to understand the manner in which

tumour-bearing modifies the metabolic adaptation to starvation. We have examined this question and present data on the effects of tumour-bearing on ketosis in starved rats.

MATERIALS AND METHODS

Animals and tumour

Female DA rats were purchased from the South Australian Government's Department of Agriculture. They were housed singly on sawdust in Perspex cages in an animal house maintained at $22 \pm 1^\circ\text{C}$ with a 14h-light/10h-dark cycle.

The mammary carcinoma was given by the Department of Pathology and Immunology, Monash University, Victoria, Australia. It shows no evidence of glandular differentiation, does not metastasize, grows equally well in male and female rats and can be transplanted either as small pieces of tissue or as a cell suspension. The latter is the preferred method of transplantation, as this markedly decreases the risk of infection. Tumour cells (2×10^7 cells in 0.2 ml of sterile 10 mM-sodium phosphate buffer, pH 7.0, containing 0.15 M-NaCl) were injected subcutaneously into both flanks of each rat under light halothane anaesthesia. Control rats were injected with 0.2 ml of buffer only. After a lag period of 7–10 days, the tumour becomes palpable and grows rapidly, at approx. 1 g/day.

Diet

Rats were fed on a commercial rodent diet (Mouse M & V Cubes; W. Charlick Ltd., Adelaide, SA, Australia) which contains 21% (w/w) protein, 70% (w/w) digestible nutrients and vitamin and mineral supplements. The

* To whom correspondence and reprint requests should be addressed.

metabolizable energy value is 12.9 kJ/g of cubes. When the tumours were established (weight approx. 2 g), the rats were adapted to a meal-feeding regimen of 65 g of food/kg body wt. per day, and this was consumed between 08:30 and 12:30 h. This is equivalent to 85% of the daily intake of control rats if free access to food is allowed. Control rats were meal-fed in an identical manner.

Experimental

Metabolic studies were performed when the tumours were estimated to be 10–15% of the total body weight, and this usually occurred 2–3 weeks after the tumours had become palpable. At 24 h intervals after the last meal, rats were anaesthetized with pentobarbitone (Fauldings, Sydney, Australia: 60 mg/kg body wt., intraperitoneal injection of a solution in water). The abdominal cavity was opened and blood was removed from the descending aorta into a heparinized syringe. At the same time, blood was taken from the posterior vena cava at a point just distal to the left renal vein. The liver was then quickly removed and freeze-clamped in aluminium tongs which had been pre-cooled in liquid N₂. Blood (0.4 ml) was deproteinized with 0.8 ml of 5% (w/v) HClO₄ whereas liver powder (1.0 g) was deproteinized with 4.0 ml of HClO₄. The HClO₄ extracts were centrifuged at 3000 g for 5 min. Metabolites were assayed in neutralized samples of the supernatants as described previously (Rofo & Williamson, 1983). The remainder of the arterial blood was centrifuged at 3000 g for 5 min, and plasma parameters were measured by automated procedures (Sequential Multiple Analyser with Computer; Technicon, Tarrytown, NY, U.S.A.). Liver glycogen was measured by the method of Keppler & Decker (1974). The tumours were dissected free of the surrounding tissues and their wet weights recorded.

Hepatocytes

Hepatocytes were isolated as described previously (Rofo *et al.*, 1980). Viability was 80–90% as assessed by Trypan Blue exclusion. Incubations were performed in silicone-treated glass scintillation vials, with 10⁷ hepatocytes in Krebs–Henseleit (1932) saline containing 2% (w/v) dialysed fatty-acid-free albumin (Sigma, fraction IV), a gas phase of O₂/CO₂ (19:1) and a final volume of 1.0 ml. Substrates were added to the concentrations indicated in the text, with palmitate and octanoate bound

to albumin. After 40 min at 37 °C in a shaking water bath, reactions were stopped with 1 ml of ice-cold 5% (w/v) HClO₄ and treated as for blood. Incubations with [1-¹⁴C]palmitate were performed in duplicate. The incorporation of label into esterified products and ketone bodies was measured as described by Whitelaw & Williamson (1977). The conversion of palmitate into ketone bodies closely paralleled the appearance of label in the HClO₄ supernatant, and this is used here as a measure of the conversion into ketone bodies (see Blumenthal, 1983; Demaugre *et al.*, 1983). CO₂ was also measured by the method of Whitelaw & Williamson (1977), except that modified scintillation vials were used for the incubations. A rubber seal was adapted to hold a suspended cap, to which was added 0.2 ml of Hyamine hydroxide (1 M in methanol) immediately before the addition of HClO₄, which was used to quench the incubation. These additions were made via needles implanted in the rubber seal. After an additional 2 h at 37 °C, the cap containing the Hyamine was removed and placed on its appropriate mini-vial, to which had been added 3.4 ml of scintillant (0.4% 2,5-diphenyloxazole in toluene). The recovery of ¹⁴CO₂ was greater than 95% with NaH¹⁴CO₃ as a standard.

Functional hepatectomy

Each rat was anaesthetized with a Halothane/N₂O mixture, and a polythene cannula was placed in the carotid artery (Rofo & Williamson, 1983). The abdominal cavity was opened and the liver isolated from the general circulation by the method of Blackshear *et al.* (1974). Blood samples were removed at 10 min intervals via the carotid cannula and treated as above.

Tumour cells

Tumour cells were prepared by dispersing small portions of the solid tumour in Krebs–Henseleit saline with a glass homogenizer with a Teflon pestle (radial clearance 0.5 mm). The viability was 50% as assessed by Trypan Blue exclusion. The cells were then treated and incubated under the same conditions described for hepatocytes.

Biochemicals

All enzymes and biochemicals were purchased from Boehringer Mannheim, Sydney, NSW, Australia. Other chemicals were of analytical-reagent grade and were

Table 1. Body and tumour weights

The feeding conditions are described in the Materials and methods section. The total body weight of the tumour-bearing rats includes that of the tumour. Studies A and B were performed under identical conditions. The results are shown as means $\pm$ S.E.M. ($n = 5$ or 6).

Study	Rats	Total body wt. (g)				Tumour wt. (g)
		Before tumour implant	Before starvation	Starved 48 h	Starved 72 h	
A	Control	159 $\pm$ 3	158 $\pm$ 2	147 $\pm$ 2	–	–
	Tumour-bearing	161 $\pm$ 3	161 $\pm$ 3	148 $\pm$ 2	–	18 $\pm$ 1
B	Control	161 $\pm$ 3	157 $\pm$ 2	–	140 $\pm$ 3	–
	Tumour-bearing	156 $\pm$ 3	162 $\pm$ 2	–	141 $\pm$ 2	20 $\pm$ 2

Fig. 1. Blood metabolite concentrations (mm) in tumour-bearing (▲) and control (△) rats during starvation for 72 h

For experimental details see the Materials and methods section. The results are shown as means $\pm$ S.E.M. ($n = 5$ or 6); * $P < 0.05$, ** $P < 0.001$, for tumour-bearing versus control for the same period of starvation.

obtained from Ajax Chemicals, Adelaide, SA, Australia. Radiochemicals were purchased from Amersham, Sydney, Australia.

Data

The results are reported as means $\pm$ S.E.M.. Statistical significance was calculated by the t test for unpaired means.

RESULTS

Animal and tumour weights

The initial, pre-starvation and starved body weights of the rats are shown in Table 1. Both the control and tumour-bearing rats were able to maintain their total body weights for the duration of the experiment on the meal-feeding regimen described above. If the carcass weight of the rats is considered, however, the tumour-

Table 2. Ketogenesis in isolated rat hepatocytes

Hepatocytes were isolated from 72 h-starved control and tumour-bearing rats and incubated as described in the Materials and methods section. The results are shown as means $\pm$ S.E.M. for eight control and seven tumour-bearing hepatocyte preparations.

Substrate . . .	Ketone-body production (nmol/min per 10^7 cells)				
	Palmitate				Octanoate
Concn. (mm) . . .	0	0.2	0.5	1.0	2.0
Control	14 $\pm$ 3	46 $\pm$ 4	57 $\pm$ 7	68 $\pm$ 10	94 $\pm$ 12
Tumour-bearing	17 $\pm$ 2	35 $\pm$ 4	50 $\pm$ 4	64 $\pm$ 4	83 $\pm$ 7
Rats	Distribution of [1^{14} C]palmitate (%)				
	Ketone bodies	Esterified	CO ₂		
Control	62 $\pm$ 4	26 $\pm$ 4	10.7 $\pm$ 1.1		
Tumour-bearing	62 $\pm$ 4	30 $\pm$ 4	8.0 $\pm$ 0.4		

bearing rats actually lost weight during this time. That is, although the anorectic component of tumour-bearing was controlled by meal-feeding, these animals were nevertheless still cachectic in the sense that the tumour mass was increasing at the expense of host tissue.

Plasma and urine biochemistry

Both the tumour-bearing and control rats appeared healthy and active throughout the study. Nevertheless, plasma and urine values were assessed in the rats at 0, 48 and 72 h of starvation to examine the effects of tumour-bearing on the clinical status of these animals. There were a number of significant changes in the plasma values of tumour-bearing rats, but these were generally minor and still within the normal reference ranges for the laboratory rat (Mitraka & Rawnsley, 1977). These changes included increased [Ca^{2+}] and [globulins] and decreased [albumin], which are consistent with tumour-bearing (results not shown).

Similar rates of Na^+ , K^+ and Cl^- excretion were observed in both groups of rats after 48 and 72 h of starvation, whereas urea excretion was increased by 65% at 72 h in the tumour-bearing rats ($P < 0.05$). The excretion of ketone bodies in the 48 h-starved tumour-bearing rats was 8-fold that of the control rats. At 72 h, the excretion of ketone bodies in the tumour-bearing rats was unchanged ($21.0 \pm 4.7 \mu\text{mol}/24 \text{ h}$; $n = 5$) from that at 48 h and still 3-fold greater than that in the control rats ($6.2 \pm 1.0 \mu\text{mol}/24 \text{ h}$; $n = 5$, $P < 0.01$). Total nitrogen excretion was not measured, but it is probably increased in the tumour-bearing rats, for the increased loss of ketone bodies would be accompanied by an increased excretion of NH_4^+ ions (see Owen *et al.*, 1983).

Blood metabolite concentrations

The most outstanding finding was the marked increase in blood [ketone bodies] in the tumour-bearing rats, reaching a mean concentration of 8.53 mm after 72 h of starvation, compared with a mean value of 3.34 mm in the control animals (Fig. 1). Blood [acetoacetate] was

Table 3. Arterio-venous differences in blood metabolite concentrations

Blood was sampled simultaneously from the abdominal aorta and the posterior vena cava (see the Materials and methods section). The difference in concentration between the metabolites in the artery and vein is taken as a measure of substrate use by the tumour, compared with the changes observed in the control. The results are shown as means $\pm$ S.E.M. (controls, $n = 13$; tumour-bearing, $n = 11$): * $P < 0.05$.

	Arterio-venous metabolite concentration difference (mM)			
	Glucose	Lactate	Alanine	Ketone bodies
Control	0.47 ± 0.26	-0.38 ± 0.16	-0.05 ± 0.03	0.38 ± 0.12
Tumour-bearing	0.31 ± 0.12	-0.71 ± 0.14	$0.13 \pm 0.03^*$	0.85 ± 0.32

increased more than 4-fold in the tumour-bearing rats at 72 h, with a [3-hydroxybutyrate]/[acetoacetate] ratio approaching 1:1, compared with 3:1 in the control rats. Blood [lactate] was increased in the tumour-bearing animals, a particularly common finding in cancer (Sauer & Dauchy, 1983). [Lactate]/[pyruvate] ratios were slightly but not significantly increased in the tumour-bearing rats (results not shown). Fed tumour-bearing rats were hypoglycaemic compared with controls (Fig. 1), and blood [glucose] decreased with progressive starvation in both tumour-bearing and control rats. There was no difference in the plasma [non-esterified fatty acids] between the two groups at any time, and only a non-significant increase in blood [glycerol] in the tumour-bearing rats at 72 h (results not shown). In the tumour-bearing rats the blood [alanine] was significantly increased before starvation, the same at 24 and 48 h, and again significantly increased when compared with that in the controls at 72 h (Fig. 1).

Liver metabolite concentrations

Livers were freeze-clamped at intervals during starvation and metabolite concentrations were measured. The only consistent difference in liver metabolite concentrations between the tumour-bearing and control rats during starvation was the significant increase in [total ketone bodies] (tumour-bearing, 4.21 ± 0.54 , $n = 6$; control, 2.54 ± 0.14 , $n = 5$; $\mu\text{mol/g wet wt. liver at 72 h}$; $P < 0.05$). Unlike in the blood, the [3-hydroxybutyrate]/[acetoacetate] ratio was not significantly different from that in the control rats. Before starvation liver [glycogen] was 35% lower in the tumour-bearing rats ($P > 0.05$).

Hepatocytes

Hepatocytes prepared from 72 h-starved tumour-bearing and control rats showed similar rates of ketogenesis from 1 mM-palmitate and 2 mM-octanoate (Table 2). Furthermore, varying the concentration of palmitate in the medium did not reveal any difference in the rates of ketogenesis in hepatocytes isolated from the tumour-bearing and control rats. In some catabolic disease states (e.g. infection; see Beisel & Wannemaker,

1980) the hepatic metabolism of fatty acids is changed, such that the partition of long-chain fatty acids between oxidation (CO_2 and ketone bodies) and re-esterification is altered in favour of the latter. However, there was no evidence of this in the present study, where the amounts of [$1\text{-}^{14}\text{C}$]palmitate converted into ^{14}C -labelled ketone bodies, $^{14}\text{CO}_2$ and ^{14}C -labelled esterified products were the same in hepatocytes prepared from either tumour-bearing or control rats. The rate of gluconeogenesis from lactate was also similar in both experimental groups.

Tumour cells

In general, tumour cells appear to lack the ability to metabolize ketone bodies (Magee *et al.*, 1979; Tisdale & Brennan, 1983), even though extraction of ketone bodies by tumours *in vivo* has been reported (Sauer & Dauchy, 1983). The ability of the carcinoma to use 3-hydroxybutyrate and acetoacetate was investigated in isolated cell suspensions. Glucose was rapidly metabolized ($2.8 \mu\text{mol/h per } 10^7 \text{ cells}$) to lactate. In comparison, the rate of disappearance of 3-hydroxybutyrate and acetoacetate was less than 10% of this rate, and could be accounted for by the appearance of acetoacetate and of 3-hydroxybutyrate respectively. This effect is in keeping with the very low activity of 3-hydroxybutyrate dehydrogenase in this tumour ($< 0.1 \text{ unit/g wet wt.}$).

Arterio-venous differences

An assessment of the venous outflow from the tumour indicated that most of the blood entered the posterior vena cava distal to the renal veins. Blood was sampled from this point, and metabolite concentrations were compared with those in the abdominal aorta to obtain information on the extent of substrate use by the tumour. The arterio-venous differences are shown in Table 3. The arterial [alanine] was higher than that in the vein of the tumour-bearing rats, suggesting that the tumour utilizes alanine. In the control rats, the venous [alanine] was marginally higher than that in the artery, which is consistent with the release of alanine from muscle. A comparison of other arterio-venous differences suggests that less glucose and more ketone bodies are used and more lactate is released in the periphery plus tumour in the tumour-bearing rats.

Functional hepatectomy

Functional hepatectomy produced profound changes in the concentration of blood metabolites (Fig. 2a). The overall rates of increase in the blood [lactate], [alanine], [glycerol] and [non-esterified fatty acids] were similar in tumour-bearing and control rats, though the blood [lactate] and [alanine] increased more rapidly during the first 5 min after hepatectomy in the tumour-bearing rats. The similar increases in [non-esterified fatty acids] and, in particular, [glycerol] suggest that the rates of lipolysis in tumour-bearing and control rats were also comparable. Blood [glucose] decreased more rapidly in the control rats, but the reverse was true for blood [ketone bodies], which decreased by an average of 3.3 mM in the tumour-bearing rats, compared with 1.7 mM in the control rats. In the tumour-bearing rats, the decrease in blood [ketone bodies] was entirely due to decreased [acetoacetate]; the [3-hydroxybutyrate] actually increased slightly during the first 10 min after hepatectomy (Fig. 2b). It is likely that some acetoacetate is converted into 3-hydroxybutyrate in the kidney and peripheral tissues,

Fig. 2. Changes in blood metabolite concentrations (mm) after functional hepatectomy in tumour-bearing ($\blacktriangle$) and control ($\triangle$) 72 h-starved rats

For experimental details see the Materials and methods section. The results are shown as means $\pm$ S.E.M. ($n = 6$).

and thus the results do not give a true indication of the rate of use of individual ketone bodies.

DISCUSSION

Tumour-bearing is associated with a wide range of metabolic disturbances (Brennan, 1981), of which the previously unreported excessive ketonaemia observed here in starved tumour-bearing rats is yet another. Possible mechanisms for the hyperketonaemia and, in particular, the marked increase in [acetoacetate] include a decreased clearance of ketone bodies in the peripheral tissues or increased hepatic ketogenesis, with the latter being due to either an increase in the ketogenic capacity of the liver or increased lipolysis in adipose tissue.

Peripheral clearance

The rate of ketone-body metabolism in peripheral tissues correlates directly with the prevailing blood concentration (Bates *et al.*, 1968; Reed *et al.*, 1984), except at high blood [ketone bodies], when a maximal rate of ketone-body utilization in peripheral tissues is reached (Bates, 1972). The blood concentration at which this maximum occurs appears to be lower for acetoacetate and of the order of 1–2 mM (Bates, 1972). This [acetoacetate] was not achieved in the control rats (0.80 mM) with 72 h of starvation, but was exceeded in the tumour-bearing rats (2.12 mM) after 48 h of starvation. Although the accumulation of acetoacetate in the blood of tumour-bearing rats may reflect this low clearance maximum, it does not imply that the rate of acetoacetate clearance is decreased in tumour-bearing rats relative to the controls. Indeed, the results of the experiments with functionally hepatectomized rats (Figs. 2a and 2b) and the arterio-venous differences (Table 3) clearly indicate that ketone-body and, notably, acetoacetate clearance is increased in starved tumour-bearing rats compared with the starved controls. Acetoacetate appears to be the preferred substrate in most tissues (Robinson & Williamson, 1980) and the rapid decrease in [acetoacetate], but not [3-hydroxybutyrate], after functional hepatectomy in the tumour-bearing rats supports this view, though it is probable that some of the acetoacetate disappearance can be accounted for simply by the conversion of acetoacetate into 3-hydroxybutyrate in peripheral tissues.

Therefore, although decreased peripheral clearance of ketone bodies is considered to be a major mechanism for hyperketonaemia in starved normal (i.e. control) animals (Bates *et al.*, 1968; Balasse, 1979), this same mechanism appears not to operate in the tumour-bearing rat. It is concluded that, for starved tumour-bearing rats to sustain the increased rate of ketone-body clearance observed here, they must also have increased rates of ketogenesis.

Ketogenesis

The major regulation of ketogenesis is now considered to operate at the entry of long-chain fatty acyl-CoA into the mitochondria (McGarry & Foster, 1980), with the depletion of hepatic [glycogen] being an important signal for the liver to enter a ketogenic mode (McGarry *et al.*, 1973). The lower hepatic glycogen observed here and by others (Cameron & Ord, 1983; Singh *et al.*, 1980) in tumour-bearing rats explains the more rapid appearance of ketosis, but not the sustained hyperketonaemia, during starvation in tumour-bearing rats. The studies *in vitro* with isolated hepatocytes do not support the notion of increased rates of ketogenesis or altered distribution of palmitate carbon between the pathways of fatty acid oxidation and esterification in tumour-bearing rats (Table 2). However, the tumour-bearing rats had increased hepatic [ketone bodies], which is consistent with increased ketogenesis. We have also observed increased hepatic [ketone bodies] in C57/BL6j mice bearing B16 melanomas, and these mice also exhibited increased ketonaemia in the first 24 h of starvation (Rofo *et al.*, 1985). Furthermore, we have demonstrated increased ketonaemia in tumour-bearing mice fed on long-chain triacylglycerols, showing the importance of substrate supply (Magee *et al.*, 1979). In normal animals the hormonal milieu and the rate of fatty acid supply to the

liver are known to modify the rate of ketogenesis (Robinson & Williamson, 1980). Our studies show that there is ample ketogenic capacity in tumour-bearing rats, mice and even humans (Conyers *et al.*, 1979b), but the dynamic nature *in vivo* of fatty acid supply to, and extraction by, the liver in the tumour-bearing host remains to be defined.

Lipolysis

The rate of fatty acid supply to the liver is important in determining the rate of ketogenesis in normal animals (Robinson & Williamson, 1980). Altered lipid metabolism appears to be common in rodent-tumour systems, with observations of increased blood lipaemia and [non-esterified fatty acids] (Mider, 1951; Kralovic *et al.*, 1977), decreased lipoprotein lipase activity in adipose tissue (Thompson *et al.*, 1981) and decreased hepatic fatty acid synthesis (Lanza-Jacoby *et al.*, 1984) being reported. These observations suggest that the tumour-bearing host may exhibit conditions which favour fatty acid mobilization and catabolism and, in the presence of food deprivation, ketogenesis. Although the tumour-bearing rats described here showed no significant differences in blood [non-esterified fatty acids] or [glycerol] from the control rats during starvation and after functional hepatectomy, these findings do not preclude differences in fatty acid supply and utilization in the tumour-bearing rats compared with the controls. More dynamic studies of fatty acid turnover in tumour-bearing rats are needed to determine if such changes do indeed occur.

Overview

Hyperketonaemic states of the magnitude observed here in the tumour-bearing rats have also been reported in association with phlorrhizin-induced diabetes (Ruderman *et al.*, 1974) and the combination of starvation and pregnancy (Rudolf & Sherwin, 1983; Scow *et al.*, 1964). In both cases, hypoglycaemia is thought to be the major contributing factor to the hyperketonaemia, and in the present study the utilization of glucose by the tumour undoubtedly contributes to the prevailing hypoglycaemia and hyperketonaemia in the tumour-bearing rats. It is possible that this hypoglycaemia, accompanied by either low plasma [insulin] or insulin resistance, places peripheral tissues of tumour-bearing rats at a disadvantage with respect to glucose uptake. In contrast, the more marked decrease in blood [glucose] in the control rats after hepatectomy reflects increased glucose metabolism by peripheral tissues. The very high blood [ketone bodies] in the tumour-bearing rats may accentuate the insulin resistance seen in established starvation (Robinson & Williamson, 1980) and cancer (Stein, 1978). That the peripheral tissues (but not the tumour) of tumour-bearing rats have a greater reliance on non-carbohydrate fuels is indicated by the rapid decrease in [ketone bodies] after hepatectomy.

Whether the hyperketonaemia observed here confers any advantage on the host in terms of decreasing the negative nitrogen balance of tumour-bearing by decreasing the excessive oxidation of branched-chain amino acids in the muscle (Sherwin *et al.*, 1975) remains to be determined. The effects of ketone bodies in this regard are somewhat controversial (Miles *et al.*, 1983). It seems clear, however, that the metabolic state observed in the tumour-bearing rats is different from that seen in normal starvation and warrants further study with regard to

the roles of lipolysis, ketogenesis and ketone-body metabolism.

We gratefully acknowledge the technical assistance of Sue Porter and the support of the Anti-Cancer Foundation of the Universities of South Australia.

REFERENCES

- Axelrod, L., Halter, J. B., Cooper, D. S., Aoki, T. T., Roussel, A. M. & Bagshaw, S. L. (1983) *Metab. Clin. Exp.* **32**, 924–937
- Balasse, E. O. (1979) *Metab. Clin. Exp.* **28**, 41–50
- Bates, M. W. (1972) *Am. J. Physiol.* **222**, 462–467
- Bates, M. W., Krebs, H. A. & Williamson, D. H. (1968) *Biochem. J.* **110**, 655–661
- Beisel, W. R. & Wannemaker, R. W. (1980) *J. Parenter. Enteral Nutr.* **4**, 277–285
- Blackshear, P. J., Holloway, P. A. H. & Alberti, K. G. M. M. (1974) *Biochem. J.* **142**, 279–286
- Blumenthal, S. A. (1983) *Metab. Clin. Exp.* **32**, 971–976
- Brennan, M. F. (1981) *N. Engl. J. Med.* **305**, 375–382
- Cahill, G. F. (1976) *Clin. Endocrinol. Metab.* **5**, 397–415
- Cameron, I. L. & Ord, V. A. (1983) *Cancer Res.* **43**, 5228–5234
- Conyers, R. A. J., Need, A. G., Durbidge, T., Harvey, N. D. M., Potezny, N. & Rofo, A. M. (1979a) *Med. J. Aust.* **1**, 398–399
- Conyers, R. A. J., Need, A. G., Rofo, A. M., Potezny, N. & Kimber, R. J. (1979b) *Br. Med. J.* **1**, 1146
- Demaugre, F., Buc, H., Girard, J. & Leroux, M.-P. (1983) *Metab. Clin. Exp.* **32**, 40–48
- Keppler, D. & Decker, K. (1974) In *Methods in Enzymatic Analysis* (Bergmeyer, H. U., ed.), vol. 3, pp. 1127–1131, Academic Press, New York and London
- Kralovic, R. C., Zepp, E. A. & Cenedella, R. J. (1977) *Eur. J. Cancer* **13**, 1071–1079
- Krebs, H. A. & Henseleit, K. (1932) *Hoppe-Seyler's Z. Physiol. Chem.* **210**, 33–66
- Lanza-Jacoby, S., Lansey, S. C., Miller, E. E. & Cleary, M. P. (1984) *Cancer Res.* **44**, 5062–5067
- Magee, B. A., Potezny, N., Rofo, A. M. & Conyers, R. A. J. (1979) *Aust. J. Exp. Biol. Med. Sci.* **57**, 529–537
- McGarry, J. D. & Foster, D. W. (1980) *Annu. Rev. Biochem.* **49**, 395–420
- McGarry, J. D., Meier, J. M. & Foster, D. W. (1973) *J. Biol. Chem.* **248**, 270–278
- Mider, G. B. (1951) *Cancer Res.* **11**, 821–829
- Miles, J. M., Nissen, S. L., Rizza, R. A., Gerich, J. E. & Haymond, M. W. (1983) *Diabetes* **32**, 197–205
- Mitruka, B. M. & Rawnsley, H. M. (1977) *Clinical Biochemical and Haematological Reference Values in Normal Experimental Animals*, pp. 117–126, Masson Publishing, New York
- Owen, O. E., Caprio, S., Reichard, G. A., Mozzoli, M. A., Boden, G. & Owen, R. S. (1983) *Clin. Endocrinol. Metab.* **12**, 359–379
- Reed, W. D., Baab, P. J., Hawkins, R. L. & Ozand, P. T. (1984) *Biochem. J.* **219**, 15–24
- Rich, A. J. & Wright, P. D. (1979) *J. Parenter. Enteral Nutr.* **3**, 350–354
- Robinson, A. M. & Williamson, D. H. (1980) *Physiol. Rev.* **60**, 143–187
- Rofo, A. M., James, H. M., Bais, R., Edwards, J. B. & Conyers, R. A. J. (1980) *Aust. J. Exp. Biol. Med. Sci.* **58**, 103–116
- Rofo, A. M., Porter, S. J., Bais, R. & Conyers, R. A. J. (1985) *Br. J. Cancer* **52**, 619–623
- Rofo, A. M. & Williamson, D. H. (1983) *Biochem. J.* **212**, 231–239
- Ruderman, N. B., Ross, P. S., Berger, M. & Goodman, M. N. (1974) *Biochem. J.* **124**, 639–651
- Rudolf, M. C. J. & Sherwin, R. S. (1983) *Clin. Endocrinol. Metab.* **12**, 413–428
- Sauer, L. A. & Dauchy, R. T. (1983) *Cancer Res.* **43**, 3497–3503
- Sauer, L. A., Stayman, J. W. & Dauchy, R. T. (1982) *Cancer Res.* **42**, 4090–4097
- Schein, P. S., Kisner, D., Heller, D., Blecher, M. & Hamosh, M. (1979) *Cancer* **43**, 2070–2076
- Scow, R. O., Chernick, S. S. & Brinley, M. S. (1964) *Am. J. Physiol.* **206**, 796–804
- Sherwin, R. S., Hendler, R. G. & Felig, P. (1975) *J. Clin. Invest.* **55**, 1382–1390
- Singh, J., Grigor, M. R. & Thompson, M. P. (1980) *Cancer Res.* **40**, 1699–1706
- Stein, T. P. (1978) *J. Theor. Biol.* **73**, 51–59
- Thompson, M. P., Koons, J. E., Tan, E. T. H. & Grigor, M. R. (1981) *Cancer Res.* **41**, 3228–3232
- Tisdale, M. J. & Brennan, R. A. (1983) *Br. J. Cancer* **47**, 293–297
- Whitelaw, E. & Williamson, D. H. (1977) *Biochem. J.* **164**, 521–528

Received 25 June 1985/23 August 1985; accepted 17 September 1985