Protein metabolism in the tumour-bearing mouse

Rates of protein synthesis in host tissues and in an Ehrlich ascites tumour at different stages in tumour growth

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We have investigated the time course of the changes in protein metabolism in skeletal muscle and liver in mice during the progression of growth of an Ehrlich ascites tumour. The rate of protein synthesis in muscle begins to fall very rapidly, and the decrease is clearly established by the time the tumour first becomes visible at 4 days after implantation of the cells. Liver protein synthesis increases substantially, and protein breakdown in muscle increases, but the onset of both these changes occurs later than the fall in muscle protein synthesis. A decrease in food intake in these animals occurs very rapidly after introduction of the cells. The fractional rate of protein synthesis in the tumour cells falls from 73 %/day at 5 days to 26 %/day at 12 days after injection, but on an absolute basis the rate of protein synthesis in the tumour at 5 days of growth is very small compared with that in muscle and liver. These results are consistent with the notion that the initial effects on muscle protein synthesis and food intake are brought about by humoral factors rather than as direct consequences of the metabolic demands of the growing tumour.

INTRODUCTION

Cancer patients at an advanced stage of the disease often exhibit a combination of symptoms known as cachexia, including anorexia and severe muscle wasting [1,2]. This cachectic state has been studied in a number of animal models, and several investigations have now demonstrated that rodents bearing a tumour load at an advanced state of growth show a fall in the rate of protein synthesis in skeletal muscle [3–7]. In some cases this is associated with an elevated rate of protein synthesis in the liver [4,6,7], but this is not always observed [3,5].

A number of physiological features of the advanced cachectic state could explain the depressed rate of muscle protein synthesis. First, there is often a pronounced fall in voluntary food intake, leading to a severely malnourished state [1,3,8]. Indeed, in an earlier study we demonstrated that control animals, restricted to the decreased voluntary food intake of a group of mice bearing an Ehrlich ascites tumour at an advanced state of growth, showed a depressed rate of muscle protein synthesis similar to that in the tumour-bearing group [4]. However, this experiment could have over-estimated the effect of the decrease in food intake, since animals paired in this way tend to consume their restricted allocation of diet very quickly, and therefore suffer a much longer period without any food before the experiment than do the tumour-bearing mice, which nibble frequently throughout day and night. A second feature of the cachectic state that could influence muscle protein synthesis is the metabolism of the tumour itself. This could affect the availability of precursors to the host tissues. For example, it has been suggested that a rapidly growing tumour could compete with host tissues for available amino acids, particularly leucine [9]. In addition, many

types of tumour cell, including Ehrlich ascites cells, actively metabolize glucose. It has been suggested that the glucose requirements of a growing tumour in an anorexic host would be supplied increasingly by hepatic gluconeogenesis, and that part of the precursor material for this would be muscle amino acids, at the expense of protein synthesis [10].

We have attempted to resolve some of these questions by investigating the pattern of onset of the changes in protein synthesis in host tissues after the introduction of the tumour rather than waiting until the cachectic condition is fully established. We have looked at this in comparison with the time course of growth and protein synthesis in the tumour itself and with the changes in food intake induced by the tumour. The results indicate that in the mouse bearing an Ehrlich ascites tumour the decreases in muscle protein synthesis and food intake occur very early after tumour implantation, before tumour metabolism could have a direct impact on the substrate supply to host tissues.

MATERIALS AND METHODS

Animals

Male MF-1 mice (5–6 weeks of age) were purchased from OLAC (Bicester, Oxfordshire, U.K.) and placed in individual cages. Ehrlich Lettré cells from frozen stocks were passaged twice before intraperitoneal injection into the mice for protein synthesis studies. Approx. 7×10^6 cells were injected into each mouse. Animals were kept on a 12 h-on/12 h-off light/dark cycle with the temperature maintained at 24 °C. All measurements were carried out between 09:00 and 12:00 to minimize the effects of diurnal variation.

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Measurement of protein synthesis

Protein synthesis was measured *in vivo* by the 'flooding dose' method of Garlick *et al.* [11], but with all animals killed 10 min after the injection of [^3H]phenylalanine, as described previously [12]. The injection solution (150 μmol of phenylalanine) contained approx. 50 μCi of radioisotope in the experiment described in Table 2 and 100 μCi in the remaining Tables. Processing of the tissue samples and measurement of the protein content and of the specific radioactivity of the protein-bound and free [^3H]phenylalanine were as described by Garlick *et al.* [11]. Tissue RNA was measured as described by Ashford & Pain [13].

Measurement of protein degradation

An estimate of the fractional rate of protein degradation (K_d) can be obtained indirectly from the difference between the fractional rates of protein synthesis (K_s) and growth of muscle protein (K_g) from the relationship $K_g = K_s - K_d$. In our experiments the protein mass of the gastrocnemius muscle was determined on each day that protein synthesis was measured, i.e. mostly at 2-day intervals in the tumour-bearing mice. The fractional growth rate for gastrocnemius-muscle protein could thus be estimated on days 2, 4 and 6 of tumour growth, from the average of the growth rates immediately preceding and succeeding those days [13,14]. Since control groups were only examined on days 0 and 9, the growth rate could not be determined in this way, and instead the overall fractional rate of growth of body mass on day 9 was used. The validity of this procedure for normal fed animals has also been discussed previously [13,15].

Measurements on the Ehrlich ascites tumour

Animals were killed and the entire ascitic fluid, containing the tumour cells, was collected into a known volume (2–10 ml) of ice-cold buffer [20 mM-triethanolamine/HCl (pH 7.4)/140 mM-NaCl]. The volume of tumour plus buffer was then measured so that the volume of tumour could be obtained by difference. A measured portion of this suspension was then rapidly frozen for later determination of the specific radioactivities of protein-bound and free phenylalanine, as described above. Residual cells were then washed out of the mouse peritoneum with a further 10 ml of buffer and combined with the original suspension. The cells were collected by centrifugation, washed, and resuspended in the same buffer. Cell number was estimated with a haemocytometer. After removal of the tumour, the remaining carcass was weighed.

RESULTS

Growth and protein metabolism in the tumour

In order to be able to relate the onset of changes in metabolism in host tissues to the behaviour of the tumour, we first examined growth and protein synthesis in the tumour itself over the 12-day period between inoculation of the cells and severe cachexia in the host. Although ascitic tumours could be detected in some animals on day 4 after inoculation, amounts were very small and extremely variable, and consistent results could only be seen from day 5 (Fig. 1). Cell proliferation was rapid until day 10 and then began to slow down. By this time the volumes of ascitic fluid were very large (Table 1). After reaching a maximum around day 6, the density of

Fig. 1. Time course of the growth of Ehrlich Lettré cells in mice

Cells were removed and their numbers measured as described in the Materials and methods section. Each point is the mean $\pm$ S.E.M. of data from 3–7 animals per group.

the cells in the ascitic fluid fell steadily (Table 1). This tendency of the rate of accumulation of ascitic fluid to exceed that of the cells has been observed by others using Ehrlich [16] and other ascites tumours [17,18].

Table 2 shows the rate of protein synthesis in the tumour cells at three time points chosen to represent the early, mid and late phases of tumour growth. The fractional rate of protein synthesis declined considerably over this period, as the cells approached stationary phase. By measuring the RNA/protein ratio, it is possible to analyse this change further in terms of the concentration of ribosomes and the protein-synthesis activity per ribosome [19]. Table 2 shows that the overall decline in protein synthesis as tumour growth progressed was due to decreases in both concentration and activity of the tumour-cell ribosomes. A progressive fall in ribosome activity was previously shown by Henshaw *et al.* [19], studying the growth of a Dunning ascites tumour in rats. However, in lowering their ribosome concentration as the cell growth rate declined, the Ehrlich cells exhibited behaviour distinct from some types of transformed cells, which lose the ability characteristic of normal cells to control ribosome number in response to growth conditions [20].

Protein metabolism in tissues of the host

In our previous work [4], and that of a number of laboratories investigating the effects on host metabolism of a variety of tumours [3,5–7], it was demonstrated that muscle protein synthesis is severely depressed by the time the growth of the tumour has reached an advanced stage. Table 3 shows the results of a study designed to determine the time of onset of this decline in muscle protein synthesis. Measurements were made in groups of animals at various times between 2 and 9 days after the injection of Ehrlich ascites cells. Control groups were examined on the day on which the tumour-bearing groups were injected (day 0) and on the day on which the last tumour-bearing group was killed (day 9). An effect of the tumour on the fractional rate of protein synthesis was clearly apparent by day 4, and the results suggest that a small decline from the rate seen in the day-0 control had even occurred by day 2, though the difference was not statistically significant. Thus an effect on muscle protein

Table 1. Growth of Ehrlich ascites tumours in mice

Determinations were as described in the Materials and methods section. Each value is the mean $\pm$ S.E.M. of values from the number of mice shown.

Day after tumour implantation...	5	6	8	10	11	12
Initial body wt. (g)	29.2 $\pm$ 0.7	27.1 $\pm$ 1.7	28.8 $\pm$ 0.8	26.4 $\pm$ 0.7	27.8 $\pm$ 0.7	30.3 $\pm$ 1.2
Final body wt. (g)	34.0 $\pm$ 1.0	32.0 $\pm$ 1.4	39.5 $\pm$ 4.4	37.0 $\pm$ 4.4	42.3 $\pm$ 1.2	40.9 $\pm$ 1.6
Carcass wt. (g)	31.7 $\pm$ 0.7	29.6 $\pm$ 1.2	32.8 $\pm$ 1.4	24.3 $\pm$ 1.9	26.7 $\pm$ 1.3	23.2 $\pm$ 0.9
Tumour volume (ml)	0.87 $\pm$ 0.25	1.65 $\pm$ 0.31	4.6 $\pm$ 0.8	10.8 $\pm$ 2.2	14.1 $\pm$ 1.1	17.0 $\pm$ 1.3
10 ⁻⁸ $\times$ Tumour cell density (cells/ml)	1.43 $\pm$ 0.21	1.80 $\pm$ 0.19	1.42 $\pm$ 0.18	0.80 $\pm$ 0.14	0.55 $\pm$ 0.08	0.62 $\pm$ 0.08
No. of mice per group	5	4	8	4	7	4

synthesis could be seen at a time when the tumour mass was barely detectable, if at all. As the tumour grew, the rate of protein synthesis in muscle continued to decline. The RNA/protein ratio also began to fall, but only after day 6. Thus the early effect, on day 4, was due to a decrease in the ribosomal activity, as reflected in the rate of protein synthesis per unit of RNA in the tissue.

When the rates of protein degradation were calculated as described in the Materials and methods section (Table 3), they were not greatly changed over the first 4 days of tumour growth, but showed an elevation on day 6. Thus the fall in protein synthesis appears to be initiated in advance of any change in protein breakdown in skeletal muscle. This difference was also seen in another ex-

periment (results not shown), in which the onset of the increase in protein breakdown was not seen until even later (day 8 of tumour growth).

In our earlier study [4] we attempted to address the question of whether the fall in muscle protein synthesis in tumour-bearing mice was the result of the decreased voluntary food intake in these animals. When we compared rates of gastrocnemius-muscle protein synthesis in mice on day 12 of tumour growth with that of a group pair-fed on the same low food intake, we found no significant difference. However, the design of this type of experiment is open to criticism (see the Introduction), and other workers, studying the effects of different tumours in mice and rats, have reported data indicating

Table 2. Rates of protein synthesis in Ehrlich ascites tumours at different stages of growth in mice

Determinations were carried out as described in the Materials and methods section. Data are means $\pm$ S.E.M. of values from the number of mice shown in parentheses. *Significantly different from both day-5 and day-8 groups ($P < 0.001$).

Day after tumour implantation		Protein synthesis (%/day)	RNA/protein ratio (mg/g)	Protein synthesis/RNA (g/day per g of RNA)	Sp. radioactivity of acid-soluble phenylalanine (d.p.m./nmol)
5	(8)	72.8 $\pm$ 7.7	62 $\pm$ 2.5	11.6 $\pm$ 0.42	847 $\pm$ 33
8	(8)	58.1 $\pm$ 4.3	56 $\pm$ 1.7	10.4 $\pm$ 0.35	898 $\pm$ 13
11	(7)	26.1 $\pm$ 3.6*	40 $\pm$ 1.9*	6.5 $\pm$ 0.73*	804 $\pm$ 39

Table 3. Rates of protein synthesis and degradation in gastrocnemius muscle of mice at different stages of tumour growth

Determinations were carried out as described in the Materials and methods section. Data are means $\pm$ S.E.M. of values from eight animals per group. *Significantly different from both groups of control animals ($P < 0.001$). Abbreviation: nd, not determined.

Group	Day after implantation	Protein synthesis		RNA/protein (mg/g)	Growth rate (%/day)	Protein degradation (%/day)
		(%/day)	(g/day per g of RNA)			
Control	0	7.79 $\pm$ 0.49	13.0 $\pm$ 0.55	6.02 $\pm$ 0.24	nd	
Control	9	6.76 $\pm$ 0.26	11.7 $\pm$ 0.51	5.68 $\pm$ 0.14	1.06	5.70
Tumour-bearing	2	6.59 $\pm$ 0.46	11.5 $\pm$ 0.4	5.68 $\pm$ 0.19	-0.15	6.74
	4	5.13 $\pm$ 0.21*	9.15 $\pm$ 0.21*	5.64 $\pm$ 0.16	-0.93	6.06
	6	4.27 $\pm$ 0.29*	8.60 $\pm$ 0.57*	4.81 $\pm$ 0.15*	-4.75	9.02
	9	3.20 $\pm$ 0.27*	7.18 $\pm$ 0.60*	4.42 $\pm$ 0.17*	nd	

Table 4. Food intake and muscle protein synthesis at early stages in tumour growth

Results are means $\pm$ S.E.M. of values for groups of 5–7 animals. Animals were injected with tumour cells or 0.9% NaCl (for controls) on day 0. Protein synthesis was measured in the gastrocnemius muscle at the beginning of each day indicated. The food-intake data are for the 24th immediately preceding the protein-synthesis measurements. Significance of difference from corresponding control group: * $P < 0.005$; ** $P < 0.01$. Abbreviation: nd, not determined.

Day after tumour implantation	Food intake (g/day)		Protein synthesis (%/day)	
	Control	Tumour-bearing	Control	Tumour-bearing
0	5.11 $\pm$ 0.23	–	6.11 $\pm$ 0.41	–
1	5.28 $\pm$ 0.20	4.77 $\pm$ 0.11*	nd	nd
2	4.63 $\pm$ 0.06	3.48 $\pm$ 0.22**	6.30 $\pm$ 0.34	4.62 $\pm$ 0.39*
4	5.05 $\pm$ 0.17	3.54 $\pm$ 0.23**	6.37 $\pm$ 0.56	4.39 $\pm$ 0.34**

Table 5. Rates of protein synthesis in hearts of mice at different stages of tumour growth

Determinations were carried out as described in the Materials and methods section. The numbers of observations per group are given in parentheses. Each determination was performed on the pooled heart muscle of two identically treated animals, as there was insufficient material in a single heart. Statistical significance of differences: * $P < 0.05$, ** $P < 0.01$ compared with day-0 control group; † $P < 0.05$, †† $P < 0.01$ compared with day-9 control group.

Group	Day after implantation		Protein synthesis		RNA/protein (mg/g)
			(%/day)	(g/day per g of RNA)	
Control	0	(4)	11.1 $\pm$ 0.26	10.8 $\pm$ 0.09	10.3 $\pm$ 0.27
	9	(3)	12.3 $\pm$ 0.50	11.5 $\pm$ 0.35	11.1 $\pm$ 0.39
Tumour-bearing	2	(4)	10.7 $\pm$ 0.66	10.8 $\pm$ 0.51	10.1 $\pm$ 0.31
	4	(4)	11.2 $\pm$ 0.60	11.7 $\pm$ 1.2	9.91 $\pm$ 1.0
	6	(4)	9.68 $\pm$ 0.15**††	9.19 $\pm$ 0.41*†	10.6 $\pm$ 0.38
	9	(4)	10.2 $\pm$ 0.41†	8.73 $\pm$ 0.37**††	11.7 $\pm$ 0.06

that anorexia is not solely responsible for the fall in muscle protein synthesis in tumour-bearing animals [5,6,21]. As an alternative approach to this problem, we have examined changes in muscle protein synthesis and food intake over the first 4 days of tumour growth in an attempt to compare the time of onset of the changes in these two parameters. However, the results (Table 4) show that significant decreases in both food intake and the fractional rate of protein synthesis had already occurred by day 2 of tumour growth.

Table 5 shows the changes in protein synthesis in the heart between 2 and 9 days of tumour growth, and it is clear that this tissue is more resistant than skeletal muscle. Only at later stages of tumour growth was the rate of protein synthesis significantly decreased, and the extent of the change was very small. This relative resistance of protein synthesis in heart is reminiscent of that observed in other stress conditions [22,23].

Table 6 shows the effects of tumour growth on protein synthesis in the liver. As demonstrated in our previous

Table 6. Rates of protein synthesis in liver of mice at different stages of tumour growth

Determinations were as described in the Materials and methods section. Results are means $\pm$ S.E.M. of values for eight mice per group. *Significant difference from both control groups ($P < 0.001$).

Group	Day after implantation	Protein synthesis		RNA/protein (mg/g)
		(%/day)	(g/day per g of RNA)	
Control	0	85.3 $\pm$ 2.6	19.8 $\pm$ 0.54	43.2 $\pm$ 1.0
	9	83.1 $\pm$ 3.2	19.4 $\pm$ 0.70	43.0 $\pm$ 0.88
Tumour-bearing	2	77.8 $\pm$ 4.7	18.5 $\pm$ 0.97	41.9 $\pm$ 0.96
	4	93.9 $\pm$ 6.5	18.5 $\pm$ 1.10	50.7 $\pm$ 1.1*
	6	124 $\pm$ 6.0*	20.9 $\pm$ 0.73	59.1 $\pm$ 2.0*
	9	145 $\pm$ 6.0*	20.4 $\pm$ 0.98	71.1 $\pm$ 0.87*

Table 7. Rates of protein synthesis in the spleen of mice at different stages of tumour growth

Protein-synthesis data are means $\pm$ S.E.M. of values from groups of eight mice. Significance of difference from day-0 control group: * $P < 0.05$, ** $P < 0.001$. Significance of difference from day-9 control group: †† $P < 0.001$.

Group	Days after implantation	Protein synthesis		RNA/protein (mg/g)	Growth (%/day)
		(%/day)	(g/day per g of RNA)		
Control	0	79.2 $\pm$ 7.2	12.4 $\pm$ 0.6	63.5 $\pm$ 4	
	9	53.9 $\pm$ 3.4*	8.47 $\pm$ 0.66**	64.2 $\pm$ 2	
Tumour-bearing	2	85.8 $\pm$ 6.00††	13.4 $\pm$ 0.8††	64.5 $\pm$ 2	25.6
	4	108 $\pm$ 0.5***††	12.6 $\pm$ 0.8††	87.3 $\pm$ 5***††	22.9
	6	104 $\pm$ 3.0***††	12.4 $\pm$ 0.5††	85.0 $\pm$ 4***††	12.6
	9	126 $\pm$ 8.0***††	9.79 $\pm$ 0.95*	13.5 $\pm$ 10***††	1.2

work [4], protein synthesis was increased in livers of animals carrying advanced Ehrlich ascites tumours. The experiment shown in Table 6 provides new information on the response of the liver at earlier stages of tumour growth. No significant increase in hepatic protein synthesis was seen until after day 4, with a sharp rise occurring between days 4 and 6. Thus the change in liver first becomes apparent slightly later than that in muscle. Analysis of the effect in terms of ribosome concentration and activity shows the activity to remain unchanged. Thus it appears that the increase in hepatic protein synthesis largely results from a rise in the ribosome concentration.

During tumour growth the protein content of the spleen increased by a factor of 4–5-fold (Table 7). Similar, though less dramatic, growth was seen by Radcliffe *et al.* [24] in rats bearing a methylcholanthrene-induced sarcoma. The growth response was associated with a stimulation of the fractional rate of protein synthesis in the tissue, which was clearly apparent by day 4 of tumour growth (Table 7). Both the ribosome concentration and activity were elevated.

DISCUSSION

In this study we have investigated the time of onset of a number of the major changes in host-tissue protein metabolism that we and others have observed previously in animals bearing an advanced tumour. Even though the model that we have used involves very rapid tumour growth, we see marked differences in the time at which the consequent changes first become apparent. The fall in protein synthesis in skeletal muscle occurs at a very early stage, probably by 2 days, and certainly by 4 days after the inoculation of the tumour. Under our conditions, however, the tumour itself is hardly visible at this time. We have made rough estimates of the absolute rates of protein synthesis in the tumour, liver and skeletal muscle at various stages of tumour growth (Table 8). At 4 days the absolute rate of protein synthesis in the tumour is very low relative to those estimated for skeletal muscle or liver. These results make it seem extremely unlikely that in this tumour model the initiation of the fall in muscle protein synthesis is a direct response to the metabolic demands of the tumour, either for amino acids [9] or for glucose production by gluconeogenesis at the expense of muscle protein [10]. However, it is possible

that the further decrease in muscle protein synthesis seen between days 4 and 9 of tumour growth (Table 3) does help to support such demands. By day 9 the rate of protein synthesis by the tumour is up to about 50% of that in skeletal muscle (Table 8).

The fractional protein-synthesis rate in liver is only slightly increased, if at all, on day 4. The rise is most pronounced between days 4 and 6, and thus occurs too late to be a major factor in triggering the decline in protein synthesis in skeletal muscle, as has been suggested for another tumour model [25]. The results in Table 3 indicate that the rate of muscle protein degradation only shows a convincing increase around day 6 of tumour growth, i.e. later than the decline in protein synthesis in

Table 8. Estimates of absolute rates of protein synthesis in the liver, skeletal muscle and tumour

Results are calculated from the mean values for liver and muscle protein synthesis (taken from Tables 3 and 6). The absolute rates of protein synthesis were obtained by multiplying the fractional synthesis rates given in these Tables by the protein mass. The assumptions made were: (a) total muscle mass of control animals is 40% of body weight; (b) total skeletal-muscle mass falls in tumour-bearing mice by the same proportion as that of gastrocnemius muscle; (c) values for protein mass/g of tissue in the gastrocnemius muscle are representative of the skeletal muscle in the whole body. The fractional rate of protein synthesis in the Ehrlich ascites tumour was estimated by extrapolation from the data in Tables 1 and 2 to give values for days 4, 6 and 9 of tumour growth. The protein mass of the tumour was calculated on the basis that 3.5 ± 10^6 cells contain 1 mg of protein. The absolute rate of protein synthesis in the tumour was then calculated by multiplying these two values.

Group	Day	Protein synthesis (mg/day)			
		Liver	Muscle	Tumour	Total
Control	0	287	143	—	430
	9	258	133	—	391
Tumour-bearing	2	247	129	—	378
	4	284	96	9	389
	6	399	82	15	496
	9	502	54	26	582

this tissue. Unlike the effect on protein synthesis, the onset of increased proteolysis coincides both with the increase in liver protein synthesis and with a rapid and significant increase in tumour mass, and it could therefore be causally related to the metabolic demands of these processes.

We have not been able to discern unambiguously whether or not the fall in food intake occurs before the decrease in muscle protein synthesis is initiated. However, it is clear that both of these effects occur very early in tumour growth. In other tumour models, decreased muscle protein synthesis has been observed in the absence of (or before) a fall in food intake [5,6,21]. It is noteworthy in this context that Quesada *et al.* [26] also observed a very early metabolic effect in mice inoculated with Ehrlich Lettré cells. Just 1 day after tumour implantation the animals showed a pronounced fall in food intake and increases in glutamine concentration in liver, kidney and plasma.

The effects that we see in both muscle and liver are very similar to those seen in endotoxin-treated rats [27,28]. Recent elegant work by Ash & Griffin [27] has shown that the fall in muscle protein synthesis in endotoxin-treated animals is still seen when they are fed with a normal energy intake by total parenteral nutrition. Those results, and those in the present paper, would be consistent with current suggestions that food intake and muscle protein metabolism may each be affected by cytokines such as tumour necrosis factor (TNF) α or interleukins produced by macrophages in response to the invasion of a host by a tumour or an infectious agent [29]. Severe cachectic symptoms have been induced in nude mice by implantation of tumour cells transfected with the human TNF α gene, which secreted the factor continuously [30]. Goldberg and colleagues have reported that a factor produced by macrophages is able to induce increased rates of proteolysis in isolated muscle preparations, but neither they [31,32], nor others [33], could reproduce this effect with recombinant interleukin-1 or TNF α , whether administered *in vivo* or added directly to the muscles *in vitro*. However, a recent report [34] demonstrated that injection of recombinant TNF α into rats produced very similar effects on protein metabolism to those seen in the tumour-bearing mice in this paper, i.e. a rapid decrease in protein synthesis in skeletal muscle and an increase in protein synthesis in the liver. Clearly a great deal of work is needed to untangle the contributory effects of hormones, cytokines and nutritional changes in bringing about the derangements of protein metabolism seen in cancer cachexia.

There is a clear consensus that protein synthesis decreases considerably in skeletal muscle of tumour-bearing rodents, but the effects on protein synthesis in liver reported in different studies vary considerably. In contrast with our results, Emery *et al.* [5] observed a pronounced decrease in hepatic protein synthesis, measured by two different methods, in immunosuppressed mice bearing a tumour grown from a human hypernephroma, and Kawamura *et al.* [3] found the presence of a fibrosarcoma to have no effect on liver protein synthesis in rats. A number of factors may be involved here. Firstly, the response of the liver may be related to the rate of growth of the tumour. For example, Paxton *et al.* [6] found an increase in hepatic protein synthesis in rats bearing a fast-growing Morris hepatoma, but not in those bearing a slower-growing tumour.

Similarly, Tayek *et al.* [7] showed no change in livers of rats bearing a Yoshida ascites tumour, but observed elevated rates in rats carrying a Novikoff ascites tumour, which, though smaller than the Yoshida tumour, exhibited a higher protein-synthesis rate at the time of the experiment. Secondly, the method used here includes production of plasma proteins in the overall estimate of protein synthesis in liver. The growth of the tumour used in this work involves a rapid expansion in the volume of ascitic fluid, and early work showed this to contain a high concentration of albumin [17,35]. Thus the resultant shifts in body water may be associated with an increased body pool of plasma proteins, and work with a sarcoma model has suggested that albumin synthesis increases under these circumstances [25]. Indeed, we have found similar increases in liver protein-synthetic activity in pregnant mice, which also need to increase their whole body pool of plasma proteins [12]. Thus at least part of the effect in liver may be a normal response to the growth of a 'parasitic' load (not specifically a tumour) that affects the fluid balance of the animal. On this argument, types of tumour that have lesser effects on the whole-body pool of plasma proteins may have less impact on hepatic protein metabolism. Thirdly, the effect that we see in Table 6 resembles that seen in animals subjected to a variety of stress conditions that are frequently associated with increased synthesis of acute-phase proteins by the liver, and this may contribute to the elevated rate of hepatic protein synthesis seen in our animals.

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