Protein Synthesis in Liver and Skeletal Muscle of Mice Bearing an Ascites Tumor

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ABSTRACT

In mice bearing an ascites tumor at an advanced stage of growth, the weight of the gastrocnemius muscle fell, whereas that of the liver increased. Fractional rates of protein synthesis were measured in vivo under conditions designed to minimize uncertainties in the determination of the specific radioactivity of the precursor amino acid pool. Protein synthesis in liver increased in the tumor-bearing mice in comparison with controls either fed ad libitum or pair-fed to the reduced food intake of the tumor-bearing group. In muscle, the rate of protein synthesis fell substantially in comparison to ad libitum-fed controls but was not significantly different from that in a group for which food intake was restricted to that of the tumor-bearing animals.

INTRODUCTION

It is a common observation that many cancer patients lose weight, even when the site of the tumor is not such as to interfere with the function of a vital organ or with the absorption and digestion of food. This condition is often associated with anorexia and general weakness. The use of experimental animal models has allowed the adverse effects on host weight to be analyzed in terms of the individual tissues. There is general agreement between studies involving different types of tumor in rats and mice that, in host animals carrying a large tumor load, there is a loss of weight in skeletal muscle and a gain in the weight of the liver (2, 3, 11, 13, 21, 26, 27). Some of these reports (2, 11, 13, 21, 27) claim to demonstrate, in addition, that the rate of protein synthesis in vivo is decreased in muscle and increased in liver of tumor-bearing animals. However, in most cases the methods used for measuring protein synthesis have not satisfied the essential criterion that, when protein synthesis is determined from the incorporation of radioactive amino acids into tissue protein, the specific radioactivity of the precursor pool of free amino acid must be known throughout the period of measurement (10, 17, 18). A simple method is now available for performing measurements under these conditions in both muscle and liver (7). In this paper, we report data obtained using this method to investigate the effects of an ascites tumor in an advanced stage of growth.

A critical question is the extent to which metabolic changes in host tissues are elicited via changes in nutritional status. Anorexia has been commonly observed both in human cancer patients (4, 20) and in experimental animal models (11, 14, 19). Protein synthesis, particularly in muscle, is sensitive to nutritional supply (7, 17). Therefore, we have investigated the effect of nutritional status by measuring voluntary food intake in mice as tumor growth progresses, and by determining rates of protein synthesis in tissues of control mice pair-fed to the intake of their tumor-bearing counterparts. Some of our results have been presented in preliminary form (23).

MATERIALS AND METHODS

\[\text{L-}[\text{4-}^3\text{H}]\text{Phenylalanine was purchased from Amersham International, Amersham, Bucks, United Kingdom. L-Tyrosine decarboxylase, ninyhydrin, phenylethylamine, and leucylalanine were purchased from Sigma London Chemical Co., Poole, United Kingdom.}

\text{ Animals.} \text{ Adult male mice of either the MF-1 strain (Bantin \& Kingman, Hull, United Kingdom; Experiment 1) or the random-bred Pirbright P (SD) strain (Animal Virus Research Unit, Woking, Surrey, United Kingdom; Experiments 2 to 4) were given i.p. injections of Ehrlich-Lettré ascites cells. Following removal from frozen storage, ascites cells were used for protein synthesis experiments after one (Experiment 1) or 3 (Experiments 2 to 4) passages through the appropriate strain of mouse. Animals were weighed regularly at 8:30 a.m. during the course of tumor growth, and food intake was determined over 24-hr periods from 8:30 a.m. to 8:30 a.m. Food intake was calculated as g consumed/body weight}^{0.75} \text{ (1). Pair-fed animals received an amount of food calculated from the average g consumed per body weight}^{0.75} \text{ by the tumor-bearing animals on the previous day, and were used for protein synthesis measurements on the day after the tumor-bearing group. Pair-fed mice were given their daily allocation of food at 5 p.m. each day so that they were still in the absorptive phase during the morning period when measurements of body weight and protein synthesis were made. This was confirmed by the presence of unabsorbed food in the stomachs when the animals were killed. Animals were kept on a 12 hr-on, 12 hr-off, light-dark cycle at a temperature maintained at 24°. All measurements of protein synthesis were carried out between 9 a.m. and 12 noon to minimize the effects of diurnal variations. Mice were used on Day 9 (Experiment 1) or Day 12 (Experiments 2 to 4) after injection of the tumor.}

\text{Measurements of Protein Synthesis.} \text{ Mice were given i.v. injections of a flooding dose of L-[4-}^3\text{H}]\text{phenylalanine (1 ml of 150 mCi} \text{g body weight). For each experimental group, injections were given to 10 mice; 4 were killed at 2 min and 6 killed at 10 min after injection of the isotope. The animals killed after 2 min, in conjunction with those at 10 min, served to define the time course of specific radioactivity of the free pools of phenylalanine during the measurement period (6, 17). Tissues were rapidly removed into liquid nitrogen. Processing of tissues, measurement of the specific radioactivity of free and protein-bound phenylalanine, and calculation of protein synthesis rates were performed as described by Garlick et al. (6). The protein content of the tissues was determined by the method of Lowry et al. (12).}
RESULTS

Chart 1 shows the effect of the ascites tumor on food intake during the later stages of growth. A substantial fall was seen when the data were expressed as food intake per animal (Chart 1A) or as food intake per g body weight (not shown). Chart 1B shows the same data expressed in terms of the metabolic body mass, i.e., per g body weight^0.75. This exponent has been found to give a good correlation between energy expenditure and body weight in adult animals (1). Since it seemed very probable that such a large decrease in food intake could itself affect the rate of protein synthesis, we set up control groups of tumor-free mice pair-fed to the intake of the tumor-bearing groups.

Table 1 shows the effect of the ascites tumor at an advanced stage of growth on the body weight and on the individual weights of the gastrocnemius muscle in the tumor-bearing animals relative to controls fed ad libitum. In contrast, there was little difference in the weight of the gastrocnemius muscle between the tumor-bearing mice and groups of controls pair-fed to them. In all experiments, there was an increase in the liver weight in comparison with either pair-fed or ad libitum controls. Thus, the effect of pair-feeding control mice to the intake of the tumor-bearing animals mimics the effect of the tumor on muscle weight but has the opposite effect on the liver.

Many investigators, studying the effects of a variety of different types of tumor on host metabolism, have observed increases in liver mass (see "Introduction"). It is possible that changes in cellular composition of the liver may contribute to this effect, but as yet we have no detailed histological information. It is clear from Table 1 that the increase we observe in liver mass is largely reflected by the total protein content. There is a slight suggestion in the data of a comparatively trivial fall in the protein mass per g wet weight of the liver in the tumor-bearing mice relative to ad libitum-fed controls. This could be due to minor changes in the glycogen, fat, or water content of the tissue.

Table 2 shows the results of 2 experiments in which rates of protein synthesis in vivo in tissues of tumor-bearing mice were compared with those in controls fed ad libitum. It is clear that, in skeletal muscle, the fractional rate of protein synthesis falls substantially in the tumor-bearing mice. In the liver, on the other hand, there is an increase in the rate of synthesis. In order to see the extent to which the decreased food intake of the tumor-bearing animals contributes to the effects on protein synthesis, measurements were made on additional control animals pair-fed to the dietary intake of the tumor bearers. The regimen used for pair-feeding is described in "Materials and Methods." The results are shown in Table 3. In muscle, the restricted diet itself caused

Table 1

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<tr>
<th>Body weights, tissue weights, and protein content in control, tumor-bearing, and pair-fed mice</th>
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<td><strong>Experiment 3</strong></td>
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<td><strong>Experiment 4</strong></td>
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a Mean ± S.E. of no fewer than 9 observations.
DISCUSSION

Our results confirm earlier reports that muscle protein synthesis is impaired and liver protein synthesis elevated in host animals carrying an advanced tumor load. Unlike several of these earlier reports, however, our data give a good quantitative indication of the magnitude of the effect, since protein synthesis was measured under conditions in which the effect of uncertainties in the magnitude of the effect, since protein synthesis was measured from the incorporation of \([3\text{H}]\)-phenylalanine administered either i.v. or i.p. The rates obtained were 28.2 ± 1.8 %/day (S.E.) and 31.9 ± 3.9 %/day, respectively. These rates compare well with those obtained by Henshaw et al. (9) for a Dunning ascites tumor in the later stages of growth in rats.

We have also estimated the rate of protein synthesis (%/d) by the tumor cells in vivo. We were concerned that there may be a delay in the penetration of an i.v. injected isotope into the ascitic fluid surrounding the cells. Therefore, we compared the rates of protein synthesis measured from the incorporation of \([3\text{H}]\)-phenylalanine administered either i.v. or i.p. The rates obtained were 28.2 ± 1.8 %/day (S.E.) and 31.9 ± 3.9 %/day, respectively.

In addition, because the measurement time was short, the rates obtained for the liver represent the total protein synthetic activity under conditions in which the effect of uncertainties in identifying the correct precursor pool was minimized (6, 10, 17).

A fall in protein synthesis of similar magnitude to the effect of the tumor. However, in liver the dietary restriction had no effect on the rate of protein synthesis; thus, the difference between tumor-bearing and control animals was maintained.

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It seems unlikely that this complex pattern, in which protein synthesis in tumor-bearing animals shows a decrease in muscle and a rise in liver, could be a simple consequence resulting from anorexia. Under more drastic conditions of nutritional deficiency in tumor-free animals, such as starvation (7, 10, 17) and protein deficiency (7, 16, 18), protein synthesis falls in both muscle and liver. Our experiments with pair-fed mice (Table 3) confirmed that the reduced food intake of the tumor-bearing animals cannot provide a simple explanation of the effect of the tumor on liver protein synthesis. In muscle, on the other hand, the rates of protein synthesis in the pair-fed controls were decreased to an extent similar to those in the tumor-bearing mice, suggesting that malnutrition, or a consequence of it such as low insulin levels, may be involved in the response. This result differs from that of Kawamura et al. (11), who found that protein synthesis in muscle of rats bearing a fibrosarcoma was decreased substantially more than that in pair-fed controls. This may be a genuine difference in response to the 2 types of tumor, but the interpretation is complicated by the fact that Kawamura et al. (11) starved both their tumor-bearing and control rats overnight immediately before carrying out their determinations of rates of protein synthesis. There is also some uncertainty in the interpretation of our pair-feeding experiments. We calculated the amount of food to be given to each pair-fed animal from the average amount consumed by the tumor-bearing mice, adjusted to be the same per kg body weight. It is recognized as a means of normalizing food intakes for adult animals of different body weights (1). However, it may not be completely appropriate in the present instance. If the tumor has an elevated rate of metabolism by comparison with normal mouse tissue, then we may be underestimating its contribution to food utilization. Alternatively, as the tumor weight includes a high content of water in the ascitic fluid, our method may overestimate the contribution of the tumor. However, the alternative methods of either pair-feeding the same intake per mouse or feeding the same per kg body weight would suffer from the same uncertainty of interpretation; in the former case, the amounts of food given to the pair-fed mice would be increased and, in the latter, reduced relative to that given in the present experiments. In all cases, the amount given to the pair-fed group would be substantially lower than the ad libitum intake of a normal mouse. Thus, although we cannot be certain that all of the depression of protein synthesis in muscle of tumor-bearing mice is caused by the reduction in food intake, we must conclude that malnutrition is responsible for a large part of the effect.

Since malnutrition alone cannot explain the overall effects of the tumor load on protein metabolism, particularly in the liver, it is necessary to seek other contributory mechanisms. Decreased levels of circulating insulin (8) and thyroid hormones (4) have been reported in various tumor-bearing states. Again, these changes would be consistent with decreased protein synthesis in muscle (5, 22) but not with increased liver weight and protein synthesis (16, 24). Perhaps a more likely possibility is an elevation of glucocorticoid levels, resulting from the stress of the tumor load. It is known that the administration of high doses of corticosterone to normal rats reduces protein synthesis in muscle (25). This effect would be consistent with a mechanism involving the diversion of amino acids from muscle to liver as part of the input for an elevated rate of gluconeogenesis. It is of interest in this context that the administration of glucocorticoids to rats subjected to protein-energy malnutrition has been reported to result in reduced muscle weight in conjunction with increased growth of the liver (15), a situation at least superficially similar to that induced by the tumor load in this study. It is hoped that further investigations will elucidate the role of endocrine changes in mediating the effects of tumor growth on host protein metabolism.

REFERENCES

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