ABSTRACT

Reutilization of amino acid carbons was evaluated in relation to increased turnover of albumin in tumor-bearing mice. A methylcholanthrene-induced sarcoma (MCG 101) was used in nongrowing mice (C57BL/6J).

Sarcoma-bearing mice developed hypoalbuminemia, but pair-fed controls did not. The hypoalbuminemia was caused by increased albumin degradation rate, measured by injection of Na214CO3, and by exponentially increased deposition of albumin into the tumor compartment. The fractional synthesis rate of albumin was doubled in tumor-bearing mice compared with controls. The translational capacity of albumin synthesis evaluated in vitro was maintained in tumor host livers. The recycling of [14C]leucine carbons was almost extinguished in plasma albumin of sarcoma-bearing mice, while that of control mice contributed to 30 to 40% of the total leucine carbon flux in turned over albumin. The recycling of arginine carbons was also different when measured after simultaneous injection of [guanido-14C]arginine and [2,3-3H]arginine Na+. The hepatic pool of free leucine was increased by 22% in tumor-bearing mice.

It is concluded that increased albumin degradation in cancer may be a disordered event and is earlier and of initially greater quantitative importance than is altered synthesis of albumin for the development of hypoalbuminemia in experimental cancer.

INTRODUCTION

Cancer disease is associated with decreased food intake (anorexia) and negative energy balance measured by loss of nitrogen and lipid content in the carcass of the tumor-bearing host (20, 26, 34). Available information points to the possibility that such metabolic host reactions cannot entirely be ascribed to the anorexia itself (1, 15, 30). It has been established that increased reutilization of amino acids occurs as a compensatory event under conditions of decreased availability of nitrogen in the liver and in the skeletal muscles as well as in cultivated HeLa cells (2, 5, 22). Cancer represents such a situation with increased demand of nitrogen and carbon as indicated by the decreased hepatic catabolism of amino acids among other things (3, 15). Therefore, it was reasonable to assume that cancer disease with its increased energy demand concomitant with decreased energy intake should elicit compensatory mechanisms to save energy, substrates, and nitrogen as normally occur in conditions of pure caloric restriction.

The aim of this study was to measure the degree of hepatic reutilization of amino acid carbons for protein synthesis in one well-defined protein (albumin) isolated from nongrowing and weight-stable sarcoma-bearing mice compared with control animals.

MATERIALS AND METHODS

[U,14C]Leucine, [guanido-14C]arginine, and [2,3-3H]arginine Na+14CO3 were from New England Nuclear, Dreieichenhain, West Germany. Agarose was from Miles Laboratories, Ltd., Stoke Poges, England. Rabbit anti-mouse albumin and mouse albumin were from Cappell Laboratories, Downingtown, Pa. All chemicals and reagents were from Sigma Chemical Co., St. Louis, Mo.

Animal Model and Tumor Implantation

Male and female C57BL/6J mice were used. All experiments were performed in nongrowing, 3-month-old, weight-stable mice (22 to 24 g) as described previously (14). A methylcholanthrene-induced sarcoma, MCG 101, was used. This tumor does not metastasize, and its influence on the host has been characterized (14-17). The tumor tissue was implanted under aseptic conditions, and the controls were sham implanted. The mean survival of the tumor-bearing animals was 14 ± 1 (S.D.) days after tumor implantation at which time tumor dry weight was less than 14% of the dry host carcass weight (minus the tumor), or less than 10% of the dry body weight of the sham-implanted controls. The animals were killed by cervical dislocation. All the animals had free access to Purina chow and tap water ad libitum until they were killed for metabolic studies.

Experiments with pair-fed mice in metabolism cages were performed as described previously (15, 17) in order to elucidate the importance of the progressively decreased food intake for hypoalbuminemia in sarcoma-bearing mice. In these experiments, the animals had free access to tap water. This experimental group of animals will be referred to as "pair-fed controls."

Animal Experimental Protocol

Group 1 (Chart 1). Tumor-bearing, pair-fed, and control mice were kept in metabolism cages. Pair-feeding experiments were performed in detail as described previously (15, 17). Plasma was obtained from the animals by cardiac puncture, and albumin concentration in plasma was quantified by immunoelectrophoresis (12).

Group 2 (Chart 2). Tumor-bearing mice and controls were killed at various time after tumor implantation. Albumin was quantified in various tissues to elucidate changes of albumin content in different pools. Tissue was homogenized (10% w/v) in water in the presence of Triton X-100 (0.2% final concentration). The homogenate was centrifuged 105,000 x g for 2 hr. Albumin concentration was measured in the supernatant by means of immunoelectrophoresis, and the tissue content of albumin was then calculated.

Group 3 (Chart 3). Tumor-bearing mice and controls were given i.p. injections of Na214CO3 (7 µCi/g body weight), and disappearance of radioactivity in electrophoretically (agarose electrophoresis) purified albumin was followed during 6 to 48 hr after the isotope injection. In further experiments, study and control mice received [U-14C]leucine (0.2 µCi/g body weight).
Albumin Metabolism in Sarcoma-bearing Mice

Chart 1. Time course of plasma albumin concentration in sarcoma-bearing mice, pair-fed mice, and controls. Parallel lines, 95% confidence interval as determined from all the control animals at Days 0, 5, and 11 (n = 35). The experiments were performed in metabolism cages, one animal in each cage. The time points of measurements were chosen from previous results from corresponding experiments, where we measured body composition, food intake, urea production rate, and nitrogen balance (15). The food intake began to decline 5 to 6 days after tumor transplantation. Values and means of 10 animals in each point except for Days 4, 5, and 6, where each point is the mean of 15 animals. Bars, S.E. O, sarcoma-bearing mice; Δ, pair-fed mice; O, control mice.

The fractional half-life (t1/2) of albumin was calculated as $t_{1/2} = \ln(2)/k$, where k is the slope of disappearance curve of radioactive albumin. In the statistical evaluation, the different slopes were compared. Turnover of plasma albumin was calculated as fractional turnover multiplied by the plasma albumin pool. The pool was calculated from plasma albumin as shown in Chart 1. Values are means of animals in each point. Bars, S.E. Parallel lines, 95% confidence interval as determined from control animals (6 animal each day: Days 0, 2, 5, 7, 9, and 11). Albumin was quantified in tissue homogenates by means of immunoelectrophoresis as described elsewhere (18, 19, 28). Briefly, liver slices were incubated for 2 hr at 37° in Krebs-Ringer bicarbonate buffer solution (pH 7.4), supplemented with all amino acids at concentrations corresponding to 4 times the normal human plasma concentration. This amount of amino acids in the incubation medium gave a constant specific radioactivity of leucine in the extracellular and the intracellular pool throughout incubation (28). Therefore, the specific radioactivity in the incubation medium was used for the calculation of the incorporation rate of leucine into albumin. This incorporation system has been shown to estimate the translational capacity in vitro for the synthesis of hepatic proteins.

Incorporation Experiments

Radioactive precursors were injected i.p. The specific radioactivity in albumin and in urea isolated from blood was measured at the same time points as given separately. In vitro incorporation of amino acids into albumin was measured in incubated liver slices as described in detail elsewhere (18, 19, 28). Briefly, liver slices were incubated for 2 hr at 37° in Krebs-Ringer bicarbonate buffer solution (pH 7.4), supplemented with all amino acids at concentrations corresponding to 4 times the normal human plasma concentration. This amount of amino acids in the incubation medium gave a constant specific radioactivity of leucine in the extracellular and the intracellular pool throughout incubation (28). Therefore, the specific radioactivity in the incubation medium was used for the calculation of the incorporation rate of leucine into albumin. This incorporation system has been shown to estimate the translational capacity in vitro for the synthesis of hepatic proteins.

Radioactivity was measured in albumin isolated from the liver slices (proalbumin plus albumin) with monospecific rabbit antiserum, as described previously in detail (19). Separate control experiments showed that this procedure gave the same result as when albumin was isolated by means of preparative isoelectrofocusing electrophoresis. Albumin was quantified by means of immunoelectrophoresis using mouse serum albumin as a standard (12).
TheAlbumin and urea were isolated as described in "Materials and Methods." The experiments were performed 10 to 11 days after tumor implantation. Values were not statistically significant by Mann-Whitney U test.

The hypoalbuminemia in sarcoma-bearing mice was primarily governed by net increased degradation rate (140 μg/hr), as well as a mean a 68-μg/hr amount of albumin was deposited into the tumor tissue compartment over 6 days. The degradation rate of plasma albumin was 347 ± 23 μg/hr, measured between 9 and 11 days after the tumor implantation (calculated from the [14C]carbonate slope in Chart 3). The synthesis rate of albumin in tumor-bearing mice was calculated to be at least 398 μg/hr when the decrease in plasma albumin concentration was down to 18.1 mg/ml (Chart 1), and the net albumin deposition into tumor compartment between 9 and 11 days (Chart 2) was taken into account and added to 347 μg/hr. The synthesis rate of albumin within this period of time was calculated to be higher than the degradation rate. This was consistent with our relative estimates of albumin synthesis measured 1 hr after injection of Na2[14CO3] as shown in Table 1. In these measurements, the synthesis rate of albumin in tumor-bearing mice was calculated as follows. The albumin degradation rate in control mice was 206 ± 12 μg/hr (Na2[14CO3] method). This value equals the synthesis rate in the control mice at steady state conditions. The tumor-bearing animals had doubled synthesis rates in relative terms as compared with controls (Table 1). Therefore, the synthesis rate was 412 μg albumin per hr in the tumor-bearing mice, which should be compared with the value of 398 μg/hr as described above. Increased degradation rate was as important as increased deposition of albumin in the tumor tissue compartment as a governing factor for hypoalbuminemia in tumor-bearing mice, measured between 9 and 11 days after the tumor implantation (166 versus 141 μg/hr; calculated from the slope of tumor growth between 9 and 11 days and the net entrance of albumin into the tumor tissue).

Liver tissue slices were incubated as described in "Materials and Methods." The translational capacity was maintained in tumor host livers (Table 2).

Table 1

<table>
<thead>
<tr>
<th>Time (hr) after isotope injection</th>
<th>Albumin</th>
<th>Urea</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>dpm/μmol</td>
<td>MCG/control</td>
</tr>
<tr>
<td>MCG</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>29,965 ± 845</td>
<td>2.16 ± 0.12</td>
</tr>
<tr>
<td>2</td>
<td>26,780 ± 312</td>
<td>1.86 ± 0.34</td>
</tr>
<tr>
<td>4</td>
<td>23,115 ± 4,745</td>
<td>2.50 ± 0.81</td>
</tr>
<tr>
<td>Controls</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>13,585 ± 390</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>12,350 ± 1,170</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>10,920 ± 845</td>
<td></td>
</tr>
</tbody>
</table>

* p < 0.05 (Mann-Whitney U test).

Table 2

<table>
<thead>
<tr>
<th>Incorporation rate of [14C]leucine</th>
<th>MCG</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>μmol/hr/g albumin</td>
<td>3.39 ± 0.84</td>
<td>3.01 ± 0.48</td>
</tr>
<tr>
<td>mmol/hr/g, wet wt</td>
<td>0.849 ± 0.218</td>
<td>0.785 ± 0.124</td>
</tr>
</tbody>
</table>

* Mean ± S.E.

**Model Counting of Radioactivity**

The radioactivity was counted in a Packard Tri-Carb Model 3320 liquid scintillation spectrometer. Correction for quenching was performed by the external standard method. Protein samples were solved in Soluene 100 (Packard Instrument Co.) and counted in toluene/PPO/POPOP, and aqueous solutions were counted in Insta-Gel (Packard). The discriminating efficiency of tritium in the channel for 14C radioactivity was 99.3%, according to a standard, when both isotopes formed by the external standard method. Protein samples were solved in PPO/POPOP, and aqueous solutions were counted in Insta-Gel (Packard). The concentration of albumin on the 11th day, while the hypoalbuminemia in sarcoma-bearing mice was primarily governed by net increased degradation rate (140 μg/hr), as well as a mean a 68-μg/hr amount of albumin was deposited into the tumor tissue compartment over 6 days. The degradation rate of plasma albumin was 347 ± 23 μg/hr, measured between 9 and 11 days after the tumor implantation (calculated from the [14C]carbonate slope in Chart 3). The synthesis rate of albumin in tumor-bearing mice was calculated to be at least 398 μg/hr when the decrease in plasma albumin concentration was down to 18.1 mg/ml (Chart 1), and the net albumin deposition into tumor compartment between 9 and 11 days (Chart 2) was taken into account and added to 347 μg/hr. The synthesis rate of albumin within this period of time was calculated to be higher than the degradation rate. This was consistent with our relative estimates of albumin synthesis measured 1 hr after injection of Na2[14CO3] as shown in Table 1. In these measurements, the synthesis rate of albumin in tumor-bearing mice was calculated as follows. The albumin degradation rate in control mice was 206 ± 12 μg/hr (Na2[14CO3] method). This value equals the synthesis rate in the control mice at steady state conditions. The tumor-bearing animals had doubled synthesis rates in relative terms as compared with controls (Table 1). Therefore, the synthesis rate was 412 μg albumin per hr in the tumor-bearing mice, which should be compared with the value of 398 μg/hr as described above. Increased degradation rate was as important as increased deposition of albumin in the tumor tissue compartment as a governing factor for hypoalbuminemia in tumor-bearing mice, measured between 9 and 11 days after the tumor implantation (166 versus 141 μg/hr; calculated from the slope of tumor growth between 9 and 11 days and the net entrance of albumin into the tumor tissue).

**Statistics**

The nonparametric Mann-Whitney U test was used for the statistical evaluation (27). p values lower than 0.05 were considered statistically significant.

**RESULTS**

Saturna-bearing mice had significantly decreased plasma albumin concentration as soon as 4 days after the tumor implantation (Chart 1). The tumor weight was at that time 30 ± 5 (S.E.) mg, or less than 0.2% of the body weight. The sum of albumin content in various host tissues, which approximately equals the exchangeable pool, remained essentially unchanged (Chart 2). The pair-fed control mice had normal plasma concentration of albumin on the 11th day, while the tumor-bearing mice had decreased their concentration 17%. The total body pool of albumin, the total body water, and the albumin degradation rate (evaluated by the [14C]carbonate method; data not shown) were normal 4 to 6 days after tumor implantation in spite of the decrease in plasma albumin. Decrease of plasma albumin (9 to 11 days after the tumor implantation) could be ascribed both to deposition of albumin into the growing tumor tissue compartment and to increased albumin degradation. The tumor content of albumin was 9863 ± 773 μg on the 11th day after tumor implantation. The tumor wet weight at this time was 3868 ± 359 mg (n = 24). The tumor grew exponentially, and the fractional growth rate was 1.78%/hr. The plasma albumin pool decreased from 21.7 mg on the fifth day to 19/9 mg 11 days after tumor transplantation, when the plasma pool was assumed to be 1 ml in these animals (11).

The time course of the specific radioactivity of plasma albumin and plasma urea after i.p. injection of Na2[14CO3] is shown in Table 1. The specific radioactivity of albumin (dpm/μmol albumin) reached its maximum level within 1 hr as reported previously for rats (10, 31). The specific radioactivity of albumin from sarcoma-bearing and control mice increased from 1 to 4 hr (p < 0.10). The translational capacity was maintained in tumor host livers (Table 2).
Time course of the specific radioactivity of $^{14}$C-labeled leucine and of arginine in albumin is shown in Chart 3. From 90 to 100% of the radioactivity in hepatic proteins has been recoverable as leucine after the injection of [3H]leucine (16) and [$^{14}$C]leucine (4). The fractional half-life of plasma albumin was estimated to 29 hr in sarcoma-bearing mice when both [$^{14}$C]leucine and Na$_2$CO$_3$ were used as the labeling precursor (Chart 3). The corresponding values were 76 and 53 hr, respectively, for the controls. This indicates that the net reutilization of labeled leucine carbons was almost extinguished in albumin of sarcoma-bearing mice. The degree of recycling of leucine carbons in albumin of control animals was 30 to 40% of the total leucine flux in turned over albumin.

The degree of recycling of different isotopes in the same amino acid ($^{14}$C and $^{3}$H) of albumin was measured after a single injection of a solution of L-[guanido-$^{14}$C]arginine and L-[2,3-$^{3}$H]arginine with the ratio 15.35 ± 0.34 (S.E.). This ratio was determined from 10 separate aliquots of the double isotope solution before injection into the animals. All animals received the same $^{3}$H/$^{14}$C ratio ± the counting error of radioactivity. The $^{3}$H/$^{14}$C ratio was 42.9 ± 1.9 and 46.2 ± 1.02 measured 48 hr later in electrophoretically purified plasma albumin from individual sarcoma-bearing and control mice, respectively ($p < 0.05; n = 20$). The increase of the ratios from 15.35 to a range of 42 to 46 shows that the non-guanido carbons were recycled extensively, as compared with the guanido carbon in both sarcoma-bearing mice and controls.

The hepatic tissue pool of leucine was increased by 22 ± 2% ($n = 21; p < 0.01$) in sarcoma-bearing mice compared with controls.

**DISCUSSION**

It has been concluded that decreased synthesis and increased degradation of albumin are the cause of hypoalbuminemia in human cancer (23, 24, 29, 32), while animal studies have shown that increased degradation is a more prominent feature (7–9, 24). One animal study has previously implied increased synthesis of albumin in growing tumor-bearing rats, but that particular experimental design did not allow estimates of specific radioactivity of [s$^{35}$S]methionine in the tissue pool(s) (7). In our study, most results are in favor of an increased albumin synthesis, which to some extent balanced an increased degradation and exponentially increasing deposition of albumin into the tumor tissue compartment. In contrast to our results, Waterlow et al. (33) have reviewed that no convincing evidence exists in literature about compensatorily increased synthesis of albumin. It has been suggested that normally the synthesis of albumin is close to its maximum level. However, circumstantial evidence exists showing that not all of the hepatocytes are simultaneously active in albumin synthesis (13). The mechanism behind increased synthesis of albumin in sarcoma-bearing mice is unclear, but it may depend on the expanded hepatic pool of free amino acids (21, 25). Therefore, it is possible that the flow of amino acids from the degradative sites in the skeletal muscles supported the synthesis of albumin more than the synthesis of certain other hepatic proteins of which some had increased and some had decreased synthesis (Ref 16; unpublished results). Our present results are consistent with our previous report showing a negative correlation between wasting of peripheral tissue (body weight index), and the in vitro synthesis of albumin in cancer patients (18).

Anorexia was an additional factor for the hypoalbuminemia in sarcoma-bearing mice. Pair-fed mice had normal plasma concentration of albumin (Chart 1) but significantly decreased body water content (15). Therefore, it could be calculated that the circulating amount of albumin decreased significantly in pair-fed mice even if the concentration remained unchanged, provided that decrease in water content was confined to the extracellular space also.

Malnutrition and hypocaloric intake influence the synthesis rate of albumin (24) and lead to increased reutilization of amino acids (2, 5, 22, 33). In contrast, the results in this study show that the recycling of amino acid carbons was decreased in albumin with increased turnover, combined with host condition of negative energy and nitrogen balance (15). The decreased recycling of leucine carbons in tumor-bearing animals shows that amino acids from degraded albumin were not channeled into the pathway for resynthesis of albumin to the same extent as in controls. This means that amino acids from degradative sites of albumin are probably not randomly distributed into hepatic pools of amino acids. This suggests that such amino acids are preferentially channeled into other pathways, perhaps for tumor protein synthesis. This suggestion is based on the fact that no tissue other than the tumor, the liver, and the spleen had increased synthesis and on the assumption that the turnover rate of protein is the denominator for the probability of reutilization of an amino acid for peptide formation. The reutilization of arginine carbons was also different. It is not likely that the decreased reutilization of amino acid carbon in sarcoma-bearing mice was simply due to increased dilution of isotopes in the hepatic pool(s), since the results were similar when the flux of label in the intermediary metabolic pathways was evaluated with different isotopes within the same amino acid [guanido-$^{14}$C]arginine and [2,3-$^{3}$H]arginine at the same time.

Seen together, our data suggest that increased synthesis of albumin is triggered by some unknown factor(s), but the increased synthesis rate was not sufficient to support both deposition of albumin in the tumor compartment and the increased degradation of albumin. It is not likely that increased albumin degradation was meant to supply the liver with amino acids or to save energy for more urgent metabolic processes, since the albumin synthesis was increased. This suggests that increased albumin degradation in cancer, and perhaps also in other conditions, may be a disordered event; clarification of the mechanisms must await further experiments.

**REFERENCES**

I. Karlberg et al.

Reutilization of Amino Acid Carbons in Relation to Albumin Turnover in Nongrowing Mice with Sarcoma

Ingvar Karlberg, Lars Ekman, Staffan Edström, et al.


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