Lack of Secretion of Serum Protein by Transplanted Rat Hepatomas

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Summary

The participation of the Reuber hepatoma H35 TC and of the Morris hepatomas 5123 TC and 9121 in the production of serum protein was investigated by studying the metabolism of radioactive (14C) amino acids in totally eviscerated tumor-bearing rats. A part of the injected amino acids was oxidized to 14CO2, another part was incorporated into tumor protein, but no secretion of labeled protein into the blood stream could be detected.

Introduction

In addition to studies concerning the regulation of enzyme activity and synthesis in minimal deviation hepatomas (10, 15, 16), it is of interest to investigate how the production and secretion of serum proteins is affected by the change of normal liver to a minimal deviation hepatoma. Morphologic studies of the ultrastructure of the tumor cell (especially of the endoplasmic reticulum-Golgi apparatus system) showed no histologic evidence of secretory activity in the fast-growing Morris hepatoma 3883 (1) whereas the appearance of granules containing large Golgi complexes in the Morris hepatoma 5123 (11) and the Reuber hepatoma H35 (17) was interpreted as a sign of secretory activity (1, 4).

The studies presented in this paper were undertaken to measure the amount of serum protein that might be secreted by the Morris 9121 and 5123 TC and the Reuber H35 TC.

The pharmacodynamics and metabolism of an i.p.-administered mixture of uniformly 14C-labeled leucine, lysine, and histidine and its oxidation to 14CO2 was studied. The hepatomas oxidized about the same portion of the amino acids to 14CO2 as did the liver; furthermore, the specific activity of the tumor protein was comparable to that of the liver. However, no secretion of 14C-labeled protein from the tumor into the blood stream could be detected.

Materials and Methods

ANIMALS AND TUMORS. Holtzman rats were purchased from the Holtzman Rat Company, Madison, Wis.; ACI rats, from Microbiological Associates, Inc., Walkersville, Md. All tumor-bearing animals were provided by Dr. H. P. Morris of the National Cancer Institute, Bethesda, Md. The Reuber hepatoma H35 TC (17) was in Generation 39 and the Morris hepatoma 9121 in Generation 4. Both had been transplanted to male ACI rats. Hepatoma 5123 TC (Generation 40) had been transplanted to male buffalo rats (11). All tumors were implanted s.c. at 4 different sites on the abdomen and thorax. At the time of the operation the total weight of the tumor masses was between 24 and 33 gm. In the larger tumors several blood vessels of the size of the abdominal aorta and vena cava could be observed going to the tumor, indicating a good blood supply. Small necrotic areas in the center of the tumors were seen in only a few cases. All rats were kept for at least 10 days in a room with regulated lighting (light, 9 A.M.-9 P.M.; dark, 9 P.M.-9 A.M.) and fed ad libitum with a diet containing 30% protein (16). Blood samples were taken by opening the thorax under ether anesthesia and puncturing the right ventricle of the beating heart. Each point in the charts describing the labeling of protein corresponds to 1 animal.

TOTAL Eviscerations. Eviscerations were done between 9 A.M. and 1 P.M. under ether anesthesia. Prior to the operation 2 ml of 0.9% NaCl were given i.m. After opening the abdominal cavity by a median incision, the sigmoid colon and then the mesenteric root of the intestine were ligated and severed. The vena cava was ligated about 5 mm above the kidneys and severed. Next, the hepatic veins, the vena cava, and the esophagus were ligated immediately below the diaphragm and severed. After this, the liver, stomach, and intestine could be removed from the abdominal cavity in toto by blunt dissection of the remaining few ligamental connections. Finally, the renal arteries, the veins, and the ureters were ligated and severed and the kidneys were removed. At this point the standard dose of radioactive amino acids was measured into the peritoneal cavity. Then the opening was closed tightly in 2 layers by suturing the abdominal muscles and applying clips to the skin wound. The animals awoke from the anesthesia within 5–20 min. They were nourished with 500 mg/kg of body weight/hr of glucose (3, 19) given i.p. at 30-min intervals as a 5.6% solution. The 1st dose of glucose was given 30 min after the operation.

RADIOACTIVE AMINO ACIDS. Uniformly 14C-labeled leucine (SA, 220–240 mc/m mole), histidine (SA, 240 mc/m mole), and
lysin (SA, 180 mc/m mole), obtained from Schwarz BioResearch, Inc., Orangeburg, N. Y., were used. A mixture containing equal amounts of radioactivity of each amino acid was dissolved in 0.01 N HCl containing 0.9% NaCl and 7.89 µc/100 gm of body weight (50 µc/ml of solution) was administered i.p. as the standard test dose.

TRAPPING OF 14CO2 IN THE EXPIRED AIR. The animals were kept in closed glass tubes of 6 cm diameter and 26 cm length. Fresh air, filtered through 2 x KOH, entered the tube at 1 end; from the far end, the expired air was passed through 2 sequentially placed CO2 traps. As the radioactivity caught in the 2nd trap was less than 0.1% of the activity caught in the 1st trap, the 2nd trap was omitted in later experiments. The CO2 traps were made from 15- x 150-mm test tubes with a sidearm about 30 mm from the top, through which a vacuum was applied to the system. A capillary tube brought the expired air to the bottom of the trap, which contained 2 ml of 1 M hyaminehydroxide in methanol plus 3 ml of dioxane. The traps were changed every 10-30 min when the total liquid volume had been reduced to 1-2 ml by evaporation. The activity was determined as described below. One point in Charts 1 and 2 gives the averaged expired 14CO2/min/100 gm of body weight in a time interval of 20-30 min. The same 8 rats were used throughout the time periods shown.

DETERMINATION OF PROTEIN. Protein was determined by a modification (18) of the procedure described by Lowry et al. (7).

DETERMINATION OF RADIOACTIVITY. The hyaminehydroxide solution from the CO2 traps was added to 10 ml of ANPO and counted in a Packard Tri-Carb liquid scintillation counter with external standardization by a radium source. The measured cpm were corrected with the determined efficiency to dpm. Tissue samples were homogenized with a Potter-Elvehjem type homogenizer with a Teflon pestle in 4 volumes of 0.1 M tris(hydroxymethyl)aminomethane/HCl buffer (pH 7.6) containing 0.15 M sucrose, 0.025 M KCl, and 0.005 M MgCl2. For the determination of amino acids incorporated into protein, 100 µl of homogenate or serum were applied to Whatman No. 3 MM chromatography paper disks (2.3 cm in diameter), which were processed and counted as described by Mans and Novelli (8, 9). For the determination of the total activity in the sera, 100 µl of serum were solubilized with 0.30 ml 1 M hyaminehydroxide in methanol and counted in 10 ml of ANPO.

PREPARATIVE POLYACRYLAMIDE GEL ELECTROPHORESIS. The gel electrophoresis was performed using the apparatus and Tris buffer system described by Jovin et al. (5).

Results

ABSORPTION OF INJECTED AMINO ACIDS. The standard test dose of amino acids was administered i.p. to 2 totally eviscerated female Holtzman rats weighing 263 and 270 gm. After 10 min the

![Chart 1. Oxidation of 14C-amino acids to 14CO2 in 8 intact tumor-free rats.](chart1)

![Chart 2. Oxidation of 14C-amino acids to 14CO2 by tumor-free eviscerated rat and by 6 eviscerated rats bearing the Morris hepatoma 9121.](chart2)

rats were killed with ether and the abdomen was opened. Radioactivity was recovered from the peritoneal cavity by rinsing extensively with 0.9% NaCl. Radioactivity in the peritoneal washings was determined as described under Methods. In 1 animal 85% of the radioactivity was absorbed at 10 min, while in the 2nd rat 60% was absorbed by 20 min. Further evidence for the utilization of the administered amino acids was found by measuring the expired 14CO2.

OXIDATION OF INJECTED 14C-AMINO ACIDS TO 14CO2. The standard dose of labeled amino acids was administered to intact and eviscerated tumor-bearing and intact tumor-free rats weighing 150-230 gm. Chart 1 shows the production of 14CO2 in 8 normal ACI male rats. The average maximum rate of excretion of radioactive CO2 was about 7.5 X 103 dpm/min/100 gm of body weight and was reached within 60 min after the injection. Chart 2 gives the rate of 14CO2 production in 6 eviscerated ACI male rats bearing the hepatoma 9121 and in 1 eviscerated female Holtzman rat without any tumor. The average maximum rate for the eviscerated group with the hepatoma 9121 was the same as in normal rats. The rate of the production of 14CO2 in the liverless, tumorless animal, however, was considerably lower. The maximum rate of production of 14CO2 in a male ACI rat having

* The preparative polyacrylamide gel electrophoresis was performed in the laboratory of Dr. P. Feigelson at Columbia University, New York, N. Y.
both liver and a hepatoma 9121 (not shown in a chart) was $17 \times 10^3$ dpm/min/100 gm of body weight.

These results suggest that (a) in normal animals the liver and associated viscera are the major sites of the production of $^{14}$CO$_2$ from the labeled amino acids, and (b) the tumor 9121 oxidizes about the same amount of amino acids as the liver. Similar results were obtained in parallel studies with 4 rats bearing the Reuber hepatoma H35 TC.

The ratio of protein-$^{14}$C to nonprotein-$^{14}$C in the serum. The protein and nonprotein radioactivity per ml of serum at several times after administration of the standard dose of amino acids is shown in Chart 3 for normal rats and in Chart 4 for totally eviscerated rats bearing 9121 hepatomas. The nonprotein radioactivity per ml of serum was obtained by subtracting the protein radioactivity from the total radioactivity. The peak of the nonprotein activity was about 10 times higher in the liverless, tumor-bearing group than in the normal rats. Its duration was about 60 min in both groups.

Chart 3. Protein and nonprotein radioactivity in the serum of normal male ACI rats after i.p. injection of $^{14}$C-amino acids. Each pair of symbols at a given time point represents data from 1 rat; 8 rats were used.

Chart 4. Protein and nonprotein radioactivity in the serum of eviscerated male ACI rats bearing Morris hepatomas 9121 after i.p. injection of $^{14}$C-amino acids. Each pair of symbols at a given time point represents data from 1 rat. Note that the scale of the ordinate is 10 times higher than in Chart 3.

Chart 5. Specific activity of the protein of various organs of intact rats after i.p. administration of the standard dose of $^{14}$C-amino acids. The various symbols at a given time point represent data from 1 rat.

Chart 6. Specific activity of serum protein of intact rats, of eviscerated rats, and of eviscerated tumor-bearing rats after i.p. administration of $^{14}$C-amino acids. The intact tumor-free rats and the eviscerated rats bearing hepatoma 9121 were males of the ACI strain. The eviscerated rats without tumors were female Holtzman rats. Each symbol represents data from 1 rat.

Kinetics of the labeling of protein in different organs of intact rats. Chart 5 shows the specific activity of the protein in several organs of 8 normal rats at different times after the injection of the standard dose of labeled amino acids. The liver showed the fastest labeling with a maximum after 50–60 min. Skeletal muscle was labeled very slowly. The labeling of the serum protein had an initial lag of about 20 min, as described earlier for albumin by Peters (13, 14). Between 1 and 2 hr a shift
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The specific activity of the serum protein in the liver to the proteins in the serum took place.

Aliquots of the sera of the animals killed after 1, 1.5, 2, and 3 hr were pooled and analyzed by preparative polyacrylamide gel electrophoresis (5). About 95% of the labeled protein put on the column was recovered during elution as albumin-\(^{14}C\).

**THE KINETICS OF LABELING SERUM PROTEIN IN ANIMALS BEARING TUMORS.** The specific activity of serum protein of normal intact rats and of tumor-free eviscerated rats was compared with that of eviscerated rats bearing 1 of the following transplanted hepatomas: 9121, 5123 TC, or H35 TC. The animals were killed at different times after administration of the standard dose of labeled amino acids, and the specific activity of the serum protein was determined (Chart 6). The specific activity of the serum protein of normal rats (Charts 5, 6) rose rapidly and reached a peak of about 2700 dpm/mg of protein at 2 hr after the administration of the amino acids. In contrast, the eviscerated tumor-free rats showed very little radioactivity in serum protein; the average specific activity after 2 hr was only 150 dpm/mg of protein. The specific activity of the serum protein of the tumor-bearing eviscerated groups was even less; in no case did it exceed \(\frac{1}{10}\) of the maximum SA of the serum proteins of the intact tumor-free group within 2 hr.

**COMPARISON OF THE SPECIFIC ACTIVITY OF TUMOR AND SERUM PROTEINS.** Chart 7 shows the specific activity of hepatoma and serum protein of the same animals at different times after the administration of the standard dose of labeled amino acids. Within 2 hr the specific activity of the tumor protein was as much as 14 times greater than that of the serum protein, suggesting that the tumor used the injected labeled amino acids for the synthesis of its proteins but did not secrete any protein into the blood serum. The low value for the activity of the tumor protein at 180 min was probably caused by an insufficient blood supply to the tumor in that particular rat. However, the specific activity of the serum protein of that same rat at 180 min after injection was the highest of all serum protein measured, indicating again that there was no correlation between the labeling of the proteins of the tumor and the serum.

**COMPARISON OF THE AMOUNT OF AMINO ACIDS INCORPORATED INTO TOTAL LIVER AND TOTAL TUMOR WITH SPECIFIC ACTIVITY OF SERUM PROTEIN.** The specific activity of the protein from hepatomas (Chart 7) was somewhat lower than that from the liver (Chart 5). However, the total mass of the hepatomas was greater than that of the liver. For the secretion of metabolic products, the total mass is of interest. Chart 8 gives the incorporation of labeled amino acids into the total liver and tumor protein after administration of the standard test dose. The total amount of radioactivity incorporated into protein was about twice as high in the tumors.

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Chart 9 demonstrates how the total amount of radioactive amino acid incorporated into (a) liver and (b) tumor protein compared with the specific activity of the serum proteins in (a) animals with liver but without tumor and (b) animals with tumor but without liver. The ratio of the total radioactivity of tumor protein to the specific activity of the serum protein was up to 1220 times greater than the ratio of the total radioactivity of the liver protein to the specific activity of the serum protein and declined very little with time.

This could only be explained if the tumor secreted essentially no serum proteins into the blood stream.

**Discussion**

The present study demonstrated that, unlike the liver, the 3 minimal deviation hepatomas investigated secreted little or no protein into the blood stream. The experiments gave no explanation for the failure of secretion of serum protein by the hepatomas. As shown in Charts 8 and 9, the tumors incorporated substantial amounts of radioactive amino acids into protein within the tumors. It might be that serum proteins are synthesized in the tumor but not released, as suggested by Essner and Novikoff (2) for the hepatomas 5123 and H35. These workers reported the accumulation of electron-dense material in cytoplasmic granules and suggested that it might be a secretory product of the cell. As a possible reason for the accumulation, either a defect in the secretory mechanism of the cell or the production of an abnormal protein in the tumor cell was proposed (2). Changes in the electrophoretic mobility in starch gel for soluble tumor and serum proteins of rats bearing the hepatoma 5123 have been described recently (6, 12). The other alternative would be that no serum proteins at all are produced in the hepatoma cells and that only the proteins concerned with tumor structure and metabolism are made. A direct investigation on the nature of the proteins synthesized in the hepatoma cells is necessary to clarify this question.

**References**

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