# Liver Nitrogen in Tumor-bearing Rats

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In a previous study of tumor-bearing rats of the Wistar strain (12), it was shown that the growth of a neoplasm elsewhere than in the liver itself was frequently accompanied by an increase in the weight of the liver. It was recognized that the percentage of liver water was increased in such animals (4, 8); but, according to McEwen and Haven (8), the dry livers of rats with subcutaneous implants of carcinosarcoma 256 were not significantly heavier than those of control animals. However, the dry weight may not be a reliable indication of the protein content, inasmuch as Kosterlitz (7) has shown that an alteration in liver nitrogen components may be accompanied by an opposite change in the amount of glycogen or fat or both, with the result that little or no change is detectable in the total dry weight. Recently, a report by Sherman, Morton, and Mider (10) showed that the livers of cancerous rats acquired nitrogen during a part of the period of tumor growth. The present study indicates that, in rats with subcutaneous tumors of varying sizes, (*a*) the nitrogen of the liver increased significantly when large growths were present and (*b*) the degree to which the nitrogen accumulated was proportional to the total mass of the rat—that is, to the weight of the carcass plus the weight of the tumor.

## MATERIALS AND METHODS

Wistar albino rats, 22 males and 14 females, 1–1½ years of age, were used. At 30 days of age the males, from the colony of Dr. Margaret Reed Lewis, had received subcutaneous inoculations of methylcholanthrene (Eastman Kodak Co.). Tumors failed to appear in five of these rats (designated below as "tumors 0 weight"); the remainder developed typical fibrosarcomas. The females were obtained from the stock colony of the Wistar Institute and bore spontaneous mammary tumors (fibroadenomas).

Each rat was killed with ether and weighed, and the tumor removed and weighed. All the growths were well encapsulated, and none was necrotic. The liver was dissected out and handled according to the method of Gurd, Vars, and Ravdin (5). After extraneous fat and connective tissue were trimmed away, the lobes were cut apart and gently pressed to expel excess blood. Two slices were cut from the right lobe, placed in weighing bottles, and promptly weighed. The remainder of the liver was also weighed, and samples were taken for histological preparations. The livers appeared to be normal grossly, except for enlargement in animals with heavy tumors. No pathological changes of importance were seen in other organs, and no metastatic growths were observed.

The slices of liver were dried in an oven and desiccator to constant weight. From the total wet liver weight and the water loss of the duplicate samples, the total dry weight was calculated. Nitrogen determinations were made on the dried slices by a semi-micro-Kjeldahl method. The average of the two nitrogen determinations was used to calculate the nitrogen of the whole dry liver.

## RESULTS

The data for each rat are recorded in Table 1, in which the males and females are listed in descending order of tumor weight. The table also includes the calculated ratios of liver nitrogen to body weight with and without the tumor. The relationship between the relative liver nitrogen and the weight of the tumor is shown in Chart 1. Values for animals with no tumors are represented as horizontal bars on the zero line.

In the upper portion of the graph it may be seen that, with increasing tumor weight, the amount of nitrogen in the liver also increases beyond the amount to be expected from carcass weight alone. There is some indication that this increase in liver nitrogen is more marked in the female rats, but the number of animals involved is too small to draw such a conclusion. In the lower part of the graph it may be seen that the amount of nitrogen in the liver relative to the weight of the carcass plus the tumor remains constant, whether the tumor be large or small.

Sections of the liver examined under the microscope appeared to be normal.[1] Differences observed

Received for publication March 14, 1951.

[1] We are indebted to Dr. John E. Gregory, Professor of Pathology, Hahnemann Medical College and Hospital, for evaluation of sample sections.

in the appearance of the cytoplasm were attributable to variation in glycogen content. The amount and distribution of connective tissue, shown by Mallory and Masson trichrome stains, were alike in all sections. Comparisons of cell size were made by ocular micrometer measurements of the greatest diameter of 500 cells per liver in a number of rats, and nucleocytoplasmic ratios were determined by the method of Chalkley (2). Neither determination showed consistent differences among the rats.

## DISCUSSION

The results reported here suggest a linear, direct relationship between the amount of nitrogen in the liver and the total mass of the organism in which the liver functions. In a normal rat the size of the liver (and, more particularly, its protein content) varies with the body size. This relationship has been interpreted to signify an adjustment to the requirements of the animal's tissues (9, 11). If the tissues of the host are augmented by a neoplasm, apparently the liver nitrogen increases, not in an all-or-none fashion, but in proportion to the sum total of the normal and the tumorous tissue.

The mechanism responsible for the enlargement of the liver in these circumstances has not been elucidated. Greenstein (4) implied an impairment in the function of the liver presumably owing to a product of the tumor circulating in the blood stream. Homburger (6) described hyperplasia and increased nitrogen of lymphoid tissue in mice with transplanted tumors, and suggested that the adrenal gland might mediate the effect. Campbell and Kosterlitz (1) implicated placental hormones in the increased turnover of radioactive phosphorus and the greater amounts of desoxy- and ribosenucleic acids in the livers of pregnant and lactating rats. In addition to the possibility that hormones or tumor products may be responsible for the enlargement of the liver, some part might be played by a lowered level of the plasma proteins, which

TABLE 1

DATA ON INDIVIDUAL RATS

| Animal | Tumor wt. | Body wt. Plus tumor | Body wt. Minus tumor | Liver wt. wet | Liver wt. dry | | Liver nitrogen (mg.) mg/100 gm body plus tumor | Liver nitrogen (mg.) mg/100 gm body minus tumor |
|---|---|---|---|---|---|---|---|---|
| Male no.: | (gm.) | (gm.) | | (gm.) | | Total | | |
| 1 | 152 | 448 | 296 | 19.23 | 4.36 | 514 | 115 | 173 |
| 2 | 150 | 479 | 329 | 14.17 | 3.83 | 429 | 90 | 130 |
| 3 | 135 | 421 | 286 | 11.57 | 3.09 | 379 | 90 | 132 |
| 4 | 131 | 456 | 325 | 16.07 | 4.10 | 484 | 106 | 149 |
| 5 | 123 | 438 | 315 | 13.32 | 3.61 | 404 | 92 | 128 |
| 6 | 122 | 383 | 261 | 11.39 | 3.05 | 356 | 93 | 136 |
| 7 | 114 | 416 | 302 | 13.35 | 3.86 | 443 | 106 | 147 |
| 8 | 114 | 425 | 311 | 12.71 | 3.34 | 406 | 96 | 131 |
| 9 | 89 | 433 | 344 | 11.84 | 3.49 | 375 | 86 | 109 |
| 10 | 62 | 383 | 321 | 11.27 | 3.04 | 351 | 92 | 109 |
| 11 | 42 | 323 | 281 | 9.61 | 2.74 | 306 | 95 | 109 |
| 12 | 37 | 401 | 364 | 11.13 | 3.40 | 335 | 92 | 84 |
| 13 | 9 | 357 | 348 | 9.00 | 2.51 | 295 | 83 | 85 |
| 14 | 7 | 361 | 354 | 9.85 | 2.86 | 325 | 90 | 92 |
| 15 | 7 | 308 | 301 | 8.14 | 2.00 | 234 | 76 | 78 |
| 16 | 0 | | 404 | 11.82 | 3.70 | 391 | | 97 |
| 17 | 0 | | 329 | 9.21 | 2.78 | 303 | | 92 |
| 18 | 0 | | 382 | 11.15 | 3.47 | 370 | | 96 |
| 19 | 0 | | 326 | 10.02 | 3.07 | 332 | | 102 |
| 20 | 0 | | 306 | 7.77 | 2.35 | 262 | | 86 |
| 21 | 0 | | 379 | 11.49 | 3.60 | 374 | | 99 |
| 22 | 0 | | 392 | 11.74 | 3.84 | 384 | | 98 |
| Female no.: | | | | | | | | |
| 1 | 171 | 482 | 311 | 17.67 | 4.86 | 494 | 102 | 159 |
| 2 | 115 | 362 | 247 | 12.35 | 3.42 | 357 | 99 | 145 |
| 3 | 94 | 408 | 314 | 16.79 | 4.72 | 490 | 120 | 156 |
| 4 | 55 | 319 | 264 | 13.98 | 3.78 | 405 | 127 | 153 |
| 5 | 52 | 340 | 288 | 12.24 | 3.23 | 347 | 102 | 120 |
| 6 | 45 | 303 | 258 | 9.97 | 2.83 | 294 | 97 | 114 |
| 7 | 39 | 296 | 257 | 9.99 | 2.89 | 293 | 99 | 114 |
| 8 | 38 | 308 | 270 | 11.17 | 3.18 | 324 | 105 | 120 |
| 9 | 27 | 314 | 287 | 11.20 | 3.05 | 300 | 96 | 105 |
| 10 | 19 | 314 | 295 | 12.04 | 3.49 | 362 | 115 | 123 |
| 11 | 18 | 260 | 242 | 9.97 | 2.84 | 295 | 113 | 122 |
| 12 | 10 | 295 | 285 | 9.97 | 3.01 | 295 | 100 | 104 |
| 13 | 9 | 273 | 264 | 10.14 | 3.04 | 300 | 110 | 114 |
| 14 | 6 | 257 | 251 | 8.12 | 2.19 | 254 | 107 | 101 |

are known to be provided by synthesis in the liver (3, p. 52). Greenstein (4) stated that blood proteins tend to be lowered in tumor-bearing hosts. Possibly they may be withdrawn from the circulation at an increased rate for the needs of the growing tissue, and, as in other homeostatic mechanisms, thus provide the stimulus for their increased production by the liver. Under such circumstances, according to Addis and his colleagues (9, 11), the nitrogen (protein) of the liver is increased.

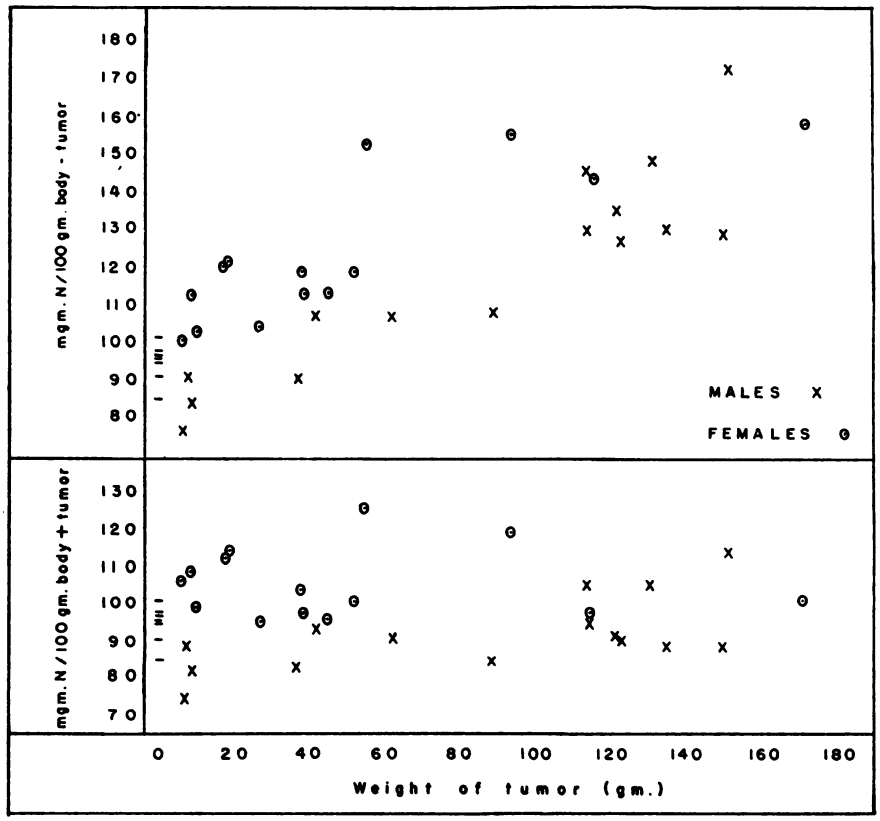


CHART 1.—The nitrogen content of the liver per 100 grams of body weight is plotted against the size of the tumor. In the upper portion of the graph, the nitrogen content is calculated per 100 grams of body weight minus the weight of the tumor, while in the lower portion the nitrogen content is based on the body weight plus the tumor weight.

## SUMMARY

Twenty-two male Wistar albino rats, inoculated subcutaneously with methylcholanthrene, and fourteen females of the same strain, with spontaneous mammary tumors, were sacrificed at various stages of tumor size (0–171 gm.). The growths were encapsulated, and no metastases were seen. Body weight and tumor weight, wet and dry weight of the liver, and nitrogen content of the dry liver were determined. From these data the nitrogen of the liver, relative to body weight without the tumor and to body weight plus tumor weight, was calculated.

It was found that the nitrogen content of the liver increased proportionally with the total mass of the animal. In rats bearing large tumors, the relative amount of liver nitrogen was greater than expected when computed according to the weight of the carcass without the tumor; but, if calculated according to the weight of the carcass plus the weight of the tumor, the relative nitrogen content of the liver was the same as in normal rats.

## ACKNOWLEDGMENT

We wish to express our gratitude to Dr. Margaret Reed Lewis of the Wistar Institute for her interest and help in this work.

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