Potential Sources of Tumor Nitrogen*

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The nitrogen content of a large Walker carcinoma 256 exceeds the amount of nitrogen stored by its host during the period of tumor growth (14). Therefore, part of the nitrogen contained in a tumor must have been derived from the rat's body. Some tissues should then show a decrease in nitrogen content that may be detected by comparing the nitrogen content of the same tissues from tumor-bearing rats and their pair-fed controls of the same age, weight, and sex. The potential sources of tumor nitrogen have been determined in this manner. Apparently, most of the tissues that lose nitrogen during caloric starvation may also lose nitrogen during tumor growth. The liver and spleen, however, acquire nitrogen at least temporarily, while the nitrogen content of the kidney does not change.

METHODS

Rats of the Wistar strain (Carworth) were placed in individual hanging basket-type cages and allowed to eat freely a semisynthetic diet adequate for normal growth, pregnancy, and lactation (13, 14). They were weighed and fed 3 times a week. Water was available at all times. The rats were divided into groups based on similarity in dietary intake and growth rate. One rat, the experimental animal, which was to receive the tumor was allowed to eat freely. The remaining animals in each group, pair-fed controls, were fed only what the experimental rat had eaten in the preceding period. The Walker tumor was implanted aseptically by trocar into the subcutaneous tissues of the inguinal region of the experimental animal. Each pair-fed group was observed at least 2 weeks before the onset of tumor growth. The experimental animals were allowed to die or were killed at various stages of tumor growth, so that earlier effects could be noted. The pair-fed controls were killed at appropriate intervals.

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The liver, heart, spleen, kidneys, lymphoid tissue, and the tumor were excised and freed of all fat and connective tissue. The lymphoid tissue was removed, including the thymus, the cervical lymph nodes, two groups of axillary nodes, the mediastinal nodes, the nodes draining the cecum, the lymphoid chain extending from the cecum to the root of the mesentery, pre-aortic nodes, pelvic nodes, and inguinal nodes. These lymph nodes were found in every rat, although they varied in size and number even in normal rats. The gastrointestinal tract was opened from gastric cardia to anus, cleaned, and then placed in a beaker together with the remainder of the carcass. The carcass and half of each tumor were dissolved directly in 30 per cent potassium hydroxide in 95 per cent ethyl alcohol, but other organs and tissues and the remaining half of each tumor were dried before they were dissolved. Drying was accomplished by covering each tissue with 95 per cent ethyl alcohol, heating in an oven at 105° C. for 48 hours, and drying to constant weight in a desiccator over anhydrous calcium chloride. The solutions were subsequently diluted to volume, and duplicate analyses for nitrogen and total lipids were performed on aliquots as described previously (14). Results were expressed in absolute terms and on a fat-free wet-weight basis. Among rats with tumors of comparable size there was no difference between those rats that were killed and those that died, as far as organ weights and nitrogen content were concerned. No attempt was made to control the dietary intake of the experimental animals; consequently, the varied intake levels of the different groups have caused some scattering of results.

RESULTS

The data were obtained from observations of 39 groups of rats, each group containing two or more animals that ingested identical amounts of diet. Twenty-nine of these groups contained a tumor-bearing rat to which the remaining rats in the group were paired. The remaining ten groups represented normal rats without tumors, each group containing one rat to which the others were paired.

The weight curves of the 29 tumor groups can be divided into two types: a divergent type, in which the tumor-bearing animal continued to gain weight after the onset of progressive tumor growth, despite lowered food intake, while its pair-fed control lost weight; and a parallel type, in which the weight curves of the pair-fed controls were almost parallel to those of the tumor-bearing animals. In the analyses below, no differences were noted between the groups with parallel curves and those with divergent curves.

All graphs have been plotted as a function of tumor weight expressed as a fraction of the total weight of the rat and the tumor (abscissa) and the proportionate difference between the nitrogen content of an organ in a tumor-bearing animal and the nitrogen content of the same organ in the pair-fed controls (ordinate). Thus, the pair-fed control serves as the frame of reference, in order that the influence of dietary intake may be eliminated as far as possible. The scatter exhibited by control groups of normal rats, representing the error of the method, is shown along the vertical axis.

The residual carcass, of all the tissues studied, lost the most nitrogen during tumor growth because of its comparatively large mass (Fig. 1). A decrease of only 10–15 per cent represents a considerable amount of nitrogen given up to the metabolic pool, and potentially to the tumor. The residue of the tumor-bearing rat always contained significantly less nitrogen than that of its control. This decrease in nitrogen was roughly proportional to the weight of the tumor. The differences in nitrogen content of the normal rats and their pair-fed controls were slight.

Studies of the lymph nodes revealed a constant and striking decrease in their nitrogen content in tumor-bearing animals soon after the inception of tumor growth. Differences as great as 70–90 per cent were observed in some cases (Fig. 2).

The differences observed in the nitrogen content of the heart appeared to be within the error of the method except for some animals with very large neoplasms. Thus, it seems that some nitrogen may be contributed to the metabolic pool by the heart.

No differences between the nitrogen content of the kidneys of tumor-bearing animals and that of their pair-fed controls could be detected.

The rodent spleen is notoriously subject to wide variations, even among normal animals. Control observations indicated a difference in nitrogen content of ± 40 per cent even among rats of the same age, weight, and sex which had ingested identical amounts of the same diet for periods of weeks. The spleens of tumor-bearing rats in general contained appreciably more nitrogen than did their pair-fed

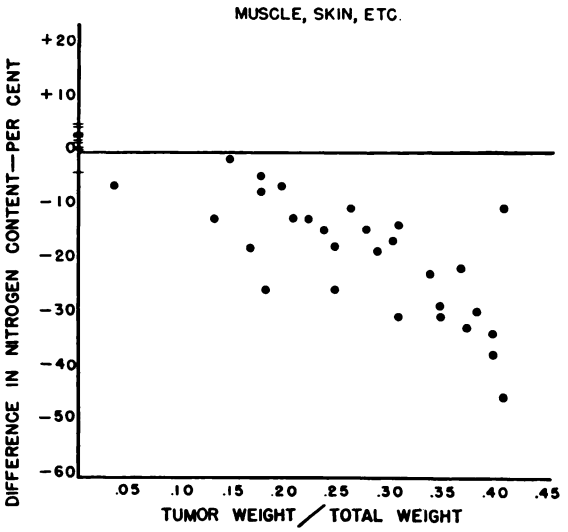

FIG. 1.—Difference in nitrogen content of "residual carcasses" of rats bearing Walker carcinoma 256 and pair-fed control rats of same age and sex at various stages of tumor growth. Dietary intake variable among pairs.

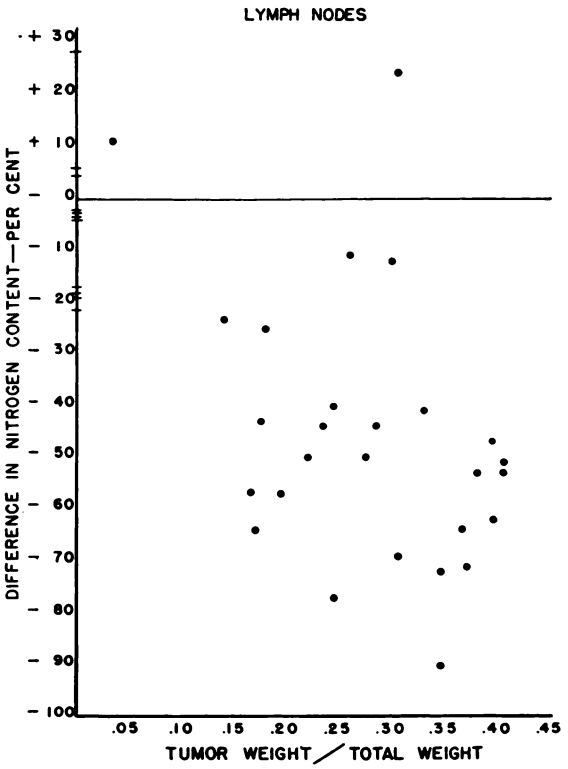

FIG. 2.—Difference in nitrogen content of lymph nodes of rats bearing Walker carcinoma 256 and pair-fed control rats of same age and sex at various stages of tumor growth. Dietary intake variable among pairs.

controls. The difference was more pronounced among rats with tumors constituting 15–30 per cent of the total weight than among rats with larger neoplasms. This suggests that a transitory period of splenomegaly, probably due to extramedullary hematopoiesis, may result from tumor growth. Some rats bearing the largest tumors had decidedly shrunken spleens. There is no direct evidence that these spleens were ever large, but this may be inferred from failure to find comparable changes among normal rats or those with Walker tumors of moderate size.

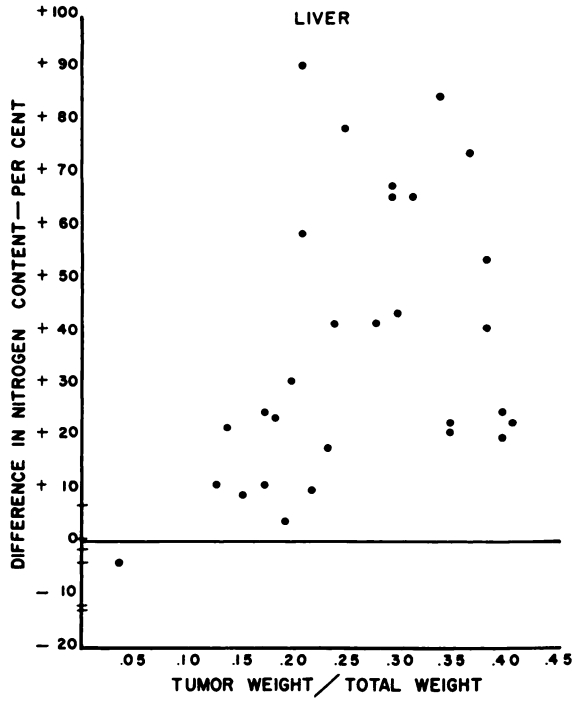

FIG. 3.—Difference in nitrogen content of lymph nodes of rats bearing Walker carcinoma 256 and pair-fed control rats of same age and sex at various stages of tumor growth. Dietary intake variable among pairs.

The livers of tumor-bearing animals usually contained significantly more nitrogen than those of their pair-fed control rats (Fig. 3). The greatest increases were in the range of the medium-sized tumors (15–30 per cent), while there seemed to be a return toward the control values when the tumors reached more than 35 per cent of total weight. That this was an actual hypertrophy is shown by the high values for liver nitrogen per gram of fat-free body weight (rat plus tumor) and even higher values for liver nitrogen per gram of carcass weight (rat minus tumor). Just as in the graph for liver nitrogen, and the graphs for liver nitrogen per gram fat-free body weight and for liver nitrogen per gram fat-free carcass weight, the nitrogen values are higher in tumor animals. Furthermore, Table 1 shows that in tumor-bearing animals liver nitrogen forms a larger proportion of total nitrogen than in the pair-fed controls and in normal rats. It is to be noted, also, that in those pair-fed animals representing partial starvation, the ratio of liver nitrogen to total nitrogen was low. This is in marked contrast to the results with tumor-bearing animals.

The scatter in Figure 3 is ascribed to differences in dietary intake of individual groups of rats, for the degree of anorexia varies. Larger groups of rats were studied in which each rat ate the same amount of diet. Only those rats whose tumors grew at approximately the same rate were used. Tumor-bearing animals and their nontumorous pair-fed controls were sacrificed at suitable intervals and the nitrogen content of the livers compared. A typical experience is depicted in Figure 4, which supports our contention that the liver may lose nitrogen during the period of precipitous weight loss preceding death.

There was close correlation between nitrogen content and both wet and dry weights of the various organs. In other words, the same differences were found on the basis of weights as on the basis of nitrogen content, and the same conclusions could have been drawn from weight data alone. The livers of tumor-bearing animals, however, contained consistently more water (74.3 per cent ± 0.3 per cent) than those of the pair-fed controls (71.5 ± 0.3 per cent). The difference, 2.8 per

TABLE 1

NITROGEN DISTRIBUTION AS PER CENT TOTAL NITROGEN

	Liver (per cent)	Kidney (per cent)	Heart (per cent)	Nodes	Spleen (per cent)	Carcass
Addis *et al.* (4), 30 rats, pooled	4.3	0.6	0.3		94.8	
37 normal rats	4.3±0.5	0.67±0.08	0.28±0.03	0.41±0.10	0.53±0.17	93.8±0.10
32 tumor animals	5.78	0.81	0.27	0.20	0.73	92.3
32 pair-fed controls	3.56	0.66	0.27	0.34	0.42	94.8
10 pair-fed controls losing more than 10% of body weight	3.29	0.66	0.26	0.34	0.42	95.1

cent, is more than 6 times its standard error and therefore statistically significant.

The Walker tumor had a strikingly constant water content of 84.4 ± 0.1 per cent. The nitrogen content of the tumor was likewise quite constant regardless of size (14).

The distribution of the nitrogen in each organ as a proportion of total nitrogen in normal rats (i.e., rats not paired to tumor animals), gave values that agree closely with those derived by Addis and others (3, 4), who used large numbers of pooled rats and organs (Table 1). As would be expected from the observations above, the nitrogen distribution in tumor animals shows that: (*a*) the heart nitrogen remained the same as both controls and normal, (*b*) the node nitrogen was lower than both, (*c*) the spleen nitrogen was higher, and (*d*) the carcass nitrogen was slightly lower. The fact that the kidney nitrogen was slightly higher than both normals and controls is not easily explainable.

DISCUSSION

These results indicate that most of the tissues that relinquish protein (2) or lose weight (19) during the course of simple caloric starvation also yield up a fraction of their nitrogen during the period of tumor growth, with the exception of the liver, spleen, and kidneys. This strengthens the over-all concept of starvation as an important mechanism by which malignant neoplasms may kill their hosts and, as has been shown previously, the degree of starvation exceeds that imposed on the animal by anorexia alone (13). The splenic changes probably do not accurately reflect the metabolic picture in respect to over-all requirements for calories or building blocks. We have no explanation for the failure of the kidneys to lose nitrogen in rats bearing the Walker carcinoma.

Those organs and tissues that relinquished nitrogen during the course of tumor growth represent only *potential* sources of building blocks for the neoplasm. One must assume that the nitrogen was contributed to the metabolic pool, hence, became available for the general needs of the body as well as the tumor. Study of the nitrogen excretion of rats bearing Walker carcinomas and their pair-fed, nontumorous controls in this laboratory has failed to reveal any marked difference in their excretion of total nitrogen, urea, or ammonia until the *ante mortem* period of rapid weight loss. Then, most tumor-bearing rats excreted significantly more nitrogen as urea than did the pair-fed controls. Most of the nitrogen lost from tissues probably was used by the tumor under such circumstances.

The hypertrophy of the liver accompanying growth of transplanted tumors in rodents is well authenticated (5, 6, 11, 12, 18, 20), but apparently only McEwen and Haven (12) have shown previously that the liver tends to grow smaller late in the course of tumor development. The question immediately arises as to the nature of the hypertrophied liver. Greenstein (8), Lan (10), and others have shown that the activity of certain enzymes in the livers of tumor-bearing animals is decidedly different from that found in normal rats. The small difference in the water content cannot account for the increased weight which is due in part, at least, to nitrogen in some form or other, as these experiments show.

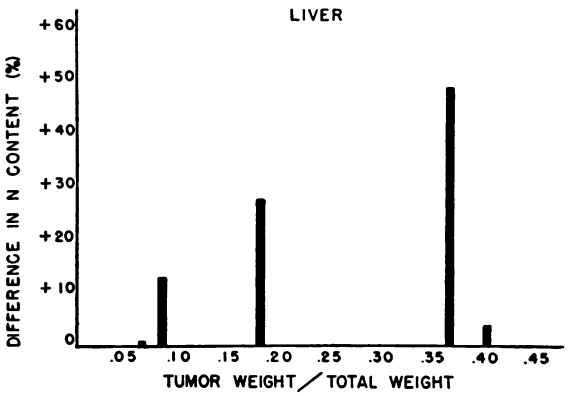

FIG. 4.—Difference in nitrogen content of livers of rats bearing Walker carcinoma 256 and pair-fed controls of same age and sex at various stages of tumor growth. Dietary intake identical in group.

A consideration of the evidence suggests that growth of the Walker tumor may be divided into three phases: (*a*) a short period during which the major part of the nitrogenous building blocks necessary for tumor formation are derived from the diet's contribution to the metabolic pool—in most cases this period ends when the tumor has attained approximately 10 per cent of the total body weight (rat plus tumor); (*b*) a period of tumor growth when the dietary intake is inadequate to furnish all of the building blocks that the neoplasm needs; dispensable stores of nitrogen in various tissues of the body are relinquished to the metabolic pool and potentially serve as precursors of tumor tissue; the onset of liver hypertrophy appears to coincide with the onset of this period; (*c*) a short *ante mortem* period of precipitous weight loss, when even the liver relinquishes the greater part of itself either to be used for tumor growth or for necessary metabolic function. During this last period most of the host's lipid stores have been depleted (13) and it may be that the nitrogenous substances lost by

the liver represent amino acids that are deaminated in order that their residues may furnish additional energy.

How far these findings apply to the generality of malignant neoplasms is conjectural. The phenomenon of liver hypertrophy in tumor-bearing rodents is adequately documented. It is well known that in certain cachectic patients dying of cancer small livers are revealed at autopsy. Abels *et al.* (1) describe liver hypertrophy as affecting 42 per cent of the patients with gastro-intestinal cancer whom they studied. Murphy and Sturm (15) found that the lymph nodes of rats bearing a transplanted lymphoma became smaller as tumor growth progressed. Homburger, on the other hand, observed an increase in the nitrogen content of the lymph nodes of mice in which either Sarcoma 180 or a transplantable mammary adenocarcinoma grew progressively (9, 17). This may represent a species difference. Less information is available with respect to the change in lymph nodes of clinical cancer. In 1906, Sampson (16) showed that the amount of lymphoid tissue in the pelves of women with uterine cancer was considerably increased, and Gilchrist and David (7) have described lymphoid hyperplasia in rectal carcinoma. Whether or not the entire lymphatic system of patients with clinical cancer is materially altered remains to be determined.

SUMMARY

The nitrogen content of the lymph nodes, livers, spleens, hearts, kidneys, and "residual carcasses" of rats bearing Walker carcinoma 256 and pair-fed noncancerous rats of the same age and sex was compared at intervals during tumor growth. The "residual carcasses" and lymph nodes of the tumor-bearing rats contained less nitrogen than did the same tissues of the pair-fed controls. The hearts lost nitrogen only among those rats with relatively huge tumors. No difference in renal nitrogen content was found between the two groups. The livers of the cancerous rats acquired nitrogen during a part of the period of tumor growth but, at death, some of them contained no more nitrogen than did the livers of the pair-fed controls.

The organs and tissues that lost nitrogen while the Walker tumor was growing represent potential sources of nitrogenous building blocks for the Walker carcinoma.

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