The Concentration of Lipids and Water in Skeletal Muscle of Albino Rats Bearing Walker Carcinoma 256*

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The level of water, neutral fat, total cholesterol, free cholesterol, and phospholipid, expressed as gm/100 gm dry weight, in the carcass (host rat minus tumor) of albino rats bearing Walker carcinoma 256 shifts toward the level of these elements in the tumor (5). These changes, which have been categorized collectively as the hydrolipotropic shift, appear in the host carcass after 2–4 weeks of tumor growth (4). In most organs and tissues of the host, hydrolipotropic shifts toward tumor levels are not gross changes demonstrable by single analyses. They can be demonstrated by statistical analysis of differences between a series of twin rats, one inoculated and one not inoculated with Walker carcinoma 256.

Of the various organs and tissues studied to date, the skeletal musculature appears to make a major contribution toward the hydrolipotropic shifts in host carcass (4, 5). A decrease in the concentration of neutral fat and an increase in the concentration of the remaining four elements have been reported in skeletal muscles of albino rats bearing Walker carcinoma 256 (4, 5). This hydrolipotropic shift is identical to that in host carcass. In no other organ or tissue of the host rat have all five such shifts been recorded.

Hydrolipotropic shifts have been studied to date in the following organs of albino rats bearing Walker carcinoma 256: brain (9), duodenum (9), heart (9), intestinal tract (8), liver (9), lung (9), skin (9), spleen (8), testicle (4, 5), and thymus gland (6). A decrease in concentration of neutral fat, which includes all fatty acids not estimated to be present in cholesterol esters or phospholipids, has been found in skin, duodenum, and intestinal tract, with no significant alteration in the other organs. An increase in the concentration of total cholesterol was noted in testicle and thymus gland, a decrease in the enlarged spleen, and no change in the other organs. An increase in the concentration of phospholipid has been encountered in duodenum, intestinal tract, and thymus gland, with no change in the other organs. An increase in the concentration of water was recorded in heart, lung, duodenum, intestinal tract, liver, thymus gland, and spleen, a decrease in testicle, and no change in brain and skin. Aoki (1) recorded a mean (P = ?) increase in concentration of phospholipid and decrease in concentration of cholesterol, cholesterol esters, and fatty acids, per unit wet weight of skeletal muscle, in hepatoma-bearing rats.

This survey emphasizes the extensive contribution of skeletal musculature to the hydrolipotropic shifts in host carcass. Since the experimental data upon which this conclusion is based were from only one aliquot of skeletal muscles, namely from the hind limb (4, 5), it seemed desirable to extend these studies to include another group of skeletal muscles. This has been done upon the ventro-lateral muscles of the abdominal wall. This group of muscles is composed of M. obliquus externus abdominis, obliquus internus abdominis, cremaster, transversus abdominis, rectus abdominis, and pyramidalis (12). The aliquot of muscles studied will be categorized in brief as abdominal wall muscle.

MATERIALS AND METHODS

Walker carcinoma 256 was inoculated into the right groin of one twin of each of 54 pairs of inbred litter-mate albino rats by methods previously described (7). There were 54 pairs of males and twenty of females. The animals were 5–7 weeks old at the time of inoculation and were fed Purina Fox Chow checkers and water ad libitum. This arrangement may be said to eliminate almost all variation due to genetic and environmental differences (14), except that due to tumor inoculation. The pairs of animals were sacrificed with chloroform 2–7 weeks after inoculation. The skin was retracted and the left half of the abdominal wall removed by incising along the linea alba, the costal border, vertebral column, and crest of the thymus gland, a decrease in spleen, and no change in the other organs. An increase in the concentration of phospholipid has been encountered in duodenum, intestinal tract, and thymus gland, with no change in the other organs. An increase in the concentration of water was recorded in heart, lung, duodenum, intestinal tract, liver, thymus gland, and spleen, a decrease in testicle, and no change in brain and skin. Aoki (1) recorded a mean (P = ?) increase in concentration of phospholipid and decrease in concentration of cholesterol, cholesterol esters, and fatty acids, per unit wet weight of skeletal muscle, in hepatoma-bearing rats.

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The right half of the abdominal wall was not used because of the possibility of invasion by the tumor. The weight of the excised abdominal wall was doubled, and aliquots were taken for determination of water and lipid content by methods previously noted (9). The values determined were total lipid, neutral fat, total fatty acids, total cholesterol, ester cholesterol, free cholesterol, phospholipid, water, and dry weight. The results obtained were expressed as gm/100 gm of non-lipid dry weight.

RESULTS

Each measurement upon each nontumor-bearing rat was subtracted from the same measurement upon its tumor-bearing twin to obtain a difference. The differences were then plotted against increasing tumor size and a t test applied to the probability that the mean difference was zero (10). There were no significant differences due to sex of the rats, except that the loss of absolute weight of the abdominal wall was greater in the males than in the females. Relative to body and host weight, the loss of weight of abdominal wall was the same in both sexes.

Loss of weight of abdominal wall appeared in animals with tumors weighing 20–40 per cent of host weight, with no further significant loss in animals bearing larger tumors. This is shown in Chart 1. The mean ± standard deviation wet weight of abdominal wall muscle in the nontumor-bearing albino rats was 7.01 ± 2.28 gm and the mean ± standard deviation loss of wet weight of abdominal wall muscle in the tumor-bearing twins was 2.65 ± 2.24 gm, with P < 0.001. The loss was greater relative to body weight than to host weight.

Changes in concentration of the five components of the hydrolipotropic shift are illustrated in Charts 1 and 2. There were increases in the levels of water, free cholesterol, and total cholesterol, a decrease in the concentration of neutral fat, and no apparent change in the concentration of phospholipid in abdominal wall muscle of the tumor-bearing albino rats. The shifts in concentration were present in animals bearing tumors weighing 20–40 per cent and more of host weight. In each of the four instances in which changes in concentration occurred, the shift was toward the concentration of the same element in Walker carcinoma 256. A brief statistical analysis of the data is presented in Table 1.

DISCUSSION

The results described above indicate that there occurred in the ventro-lateral muscles of the abdominal wall of albino rats bearing Walker carcinoma 256 a change toward tumor levels in four of the five components of the hydrolipotropic shift. A possible reason for the absence of a shift toward tumor levels in the concentration of phospholipid was encountered when concentrations in nontumor-bearing twins of the several measurements noted in Table 1 were plotted against the weight of the tumor, expressed as per cent of host weight, of their tumor-bearing twins. In these scatterplots no correlation was observed, except in the instance of concentration of phospholipid, which increased in the muscles of nontumor-bearing twins with increase in tumor size of the tumor-bearing twin. Increasing levels of muscle phospholipid in the control animals were probably related to increasing age of these young albino rats (11), which in turn may be related to increasing physiological activity of skeletal muscle (2). Assuming that these physiological factors were less pronounced in the tumor-bearing rats but that the presence of Walker carcinoma 256 produced a corresponding rise in the level of muscle phospholipid, it is possible to explain the failure to demonstrate significantly higher levels of phospholipid in the muscle of tumor-bearing twins. This postulate does not explain the difference between skeletal muscles of the hind limb and those of the ventro-lateral abdominal wall. It is possible that the physiological increase in phospholipid levels is less pronounced in muscles of the hind limb than in muscles of the ventro-lateral abdominal wall over the period of growth of these young rats. This would explain the previously demonstrated increase in phospholipid levels in skeletal muscles of the hind limb of albino rats bearing Walker carcinoma 256 over their nontumor-bearing litter-mates (4, 5).

While there were increases in concentration of the cholesterol fractions and water, as shown in Charts 1 and 2 and Table 1, the total amount of all measured elements in abdominal wall muscles declined as the tumor grew in size. The total amounts of the measured components were expressed as mg/kg body weight in the nontumor-bearing twins and mg/kg body (tumor plus host) and host weight in the tumor-bearing rats. The mean differences between tumor-bearing and nontumor-bearing twins were then expressed as a percentage of the mean total amount of the respective component in the nontumor-bearing twins to obtain the average losses, all of which had a P value of less than 0.05. These average losses in total amounts have been assembled in Table 2.

The differences in concentration of the measured elements between tumor-bearing and nontumor-bearing twins were plotted against the age of the tumor in the tumor-bearing twin. Loss of weight of abdominal wall muscles and shifts in
CHART 1.—Shifts in the wet weight, water level, and concentration of neutral fat in the muscular layer of the ventro-lateral abdominal wall of albino rats bearing Walker carcinoma 256. (The results are plotted as the value in each tumor-bearing twin minus the value in its nontumor-bearing control twin. The columns to the right indicate the mean level in Walker carcinoma 256 minus the mean level in the muscular layer of the ventro-lateral abdominal wall of nontumor-bearing albino rats.)
CHART 8.—Shifts in the levels of free cholesterol, total cholesterol, and phospholipid in the muscular layer of the ventro-lateral abdominal wall of albino rats bearing Walker carcinoma 256. (The results are plotted as the value in each tumor-bearing twin minus the value in its nontumor-bearing control twin. The columns to the right indicate the mean level in Walker carcinoma 256 minus the mean level in the muscular layer of the ventro-lateral abdominal wall of nontumor-bearing albino rats).
concentration appeared after 2–4 weeks of tumor growth. Differences in the various measurements were also plotted against the rate of increase in tumor weight of the tumor-bearing twin, calculated as per cent of host weight/day of tumor growth, with results essentially similar to the correlations depicted in Charts 1 and 2.

Finally, it should be emphasized that the standard of reference for concentration used in these studies was per 100 gm. nonlipid dry weight. Loss of neutral fat could not account mathematically for increases in the concentration of total cholesterol, free cholesterol, and water. There is a considerable loss of nitrogen in the skeletal muscles of albino rats bearing Walker carcinoma 256 (19). Since the bulk of nitrogenous substances in skeletal muscle is in the nonlipid dry weight fraction, it is apparent that the denominator used in calculating concentration of the elements measured in this project varies during growth of Walker carcinoma 256. Values for the concentration per unit nonlipid dry weight noted in Charts 1 and 2 and Table 1 may be considered as ratios of the measured element to 100 gm. of nonlipid dry weight.

**SUMMARY**

The muscular layer of the ventro-lateral abdominal wall of 54 albino rats bearing Walker carcinoma 256 and of 54 nontumor-bearing litter-mates was removed, weighted, and analyzed for water, dry weight, total lipid, neutral fat, total fatty acids, total cholesterol, ester cholesterol, free cholesterol, and phospholipid.

In the tumor-bearing twins there were statistically significant decreases in the wet weight of abdominal wall muscle and in its total content of water, dry weight, and all lipid fractions/kg body weight or host weight.

The concentration of water, total cholesterol, and free cholesterol per unit nonlipid dry weight of abdominal wall muscle was increased, while that of neutral fat was decreased in the tumor-bearing rats. The concentration of phospholipid rose in abdominal wall muscle of the tumor-bearing rats but did not differ significantly from the level in nontumor-bearing litter-mates—which also increased over the same time interval.

Correlation of these shifts in weight, water, and lipids with the weight of Walker carcinoma 256 revealed that they occurred in animals bearing tumors weighing 20 to 40 per cent or more of host weight. The shifts in concentration of water, neutral fat, total cholesterol, and free cholesterol were toward the concentration of each of these elements in Walker carcinoma 256.

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