# LIVER WEIGHT CHANGES ACCOMPANYING TUMOR TRANSPLANTATION IN RATS AND MICE

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When cells of a given transplantable tumor are injected subcutaneously in a recipient animal, the lesion grows at the site of administration. The proliferation of the tumor is not a local or isolated phenomenon but definite systemic changes occur, such as a decrease in the liver enzyme catalase, among others. One or more blood-borne principles originating in the tumor are thus able to affect the liver, an organ which is the major site of metabolism and detoxification. A study of other hepatic alterations in tumorous animals, such as size and solids and water contents would be of great importance in the understanding of tumor growth characteristics and, although such work is reviewed below, the results have often been equivocal.

In an early study, Schlottman and Rubenow (1932) reported that the water content of blood and liver, and to a lesser degree, kidney, from rats with Jensen and Flexner tumors ranged higher than in the corresponding controls; the reverse was noted for brain and muscle. Although no details were given, McEwen and Haven (1941) stated that the transplantation of carcinosarcoma 256 directly into the liver of rats caused an increase in hepatic water content. Similar results were obtained with the tumor transplanted subcutaneously provided that the lesion had reached a certain weight level (10 gm). The elevation in hepatic water content was independent of diet, underwent no further rise with more advanced tumor growth and did not stem from a decrease in dry liver weight. Yeakel and Farris (1947) noted liver enlargement in old female rats with spontaneous tumors as compared to old males or younger females. The study was extended to include a fibrosarcoma and a primary tumor arising from injection of methylcholanthrene (Yeakel, 1948). No unequivocal relationship existed between tumor size and hepatic enlargement, but the findings were more consistent with females. Actually, the augmentation was significant where the lesion weighed over 30 gm and the body length exceeded 175 mm and it was attributed to protein anabolism accompanying tumor proliferation. In the presence of large tumors, the hepatic nitrogen mounted, the accumulation paralleling the total body weight (Yeakel and Tobias, 1950; 1951). Previously, it had been shown that during Walker tumor growth, the nitrogen content of the lesion was higher than that stored by the host (Mider *et al.*, 1948; Sherman *et al.*, 1950).

Liver size and catalase depression have also been studied. Appleman, Skavinski and Stein (1950) reported an increase in liver weight accompanying hepatic catalase diminution in rats with a fibrosarcoma and the Jensen sarcoma. Liver wet-weight and nitrogen were also increased with chorioallantoic implants of Brown-Pierce carcinoma as well as the Jensen tumor in the chick embryo (Skavinski and Stein, 1951). Dounce and Shanewise (1950) pointed out that in view of the well known reversion to normal catalase levels on tumor extirpation, it is difficult to account for the lower enzyme concentration in the tumorous animals on the basis of liver enlargement. Marked increases in liver size have also been demonstrated with a transplantable rat lymphosarcoma, a $C_3H$ mammary carcinoma and several DBA mouse tumors as well as in the presence of egg-grown tumors (Kynette *et al.*, 1945; Klatt and Taylor, 1951; Knox *et al.*, 1952). A perusal of such data reveals that although wet liver enlargement due to tumor could be discerned in several cases, no definite increases are evident in the dry liver weights (Knox *et al.*, 1952). In rats with a transplantable fibroadenoma, a mixed fibroadenoma - fibrosarcoma undergoing malignant alteration and a fibrosarcoma, hepatic enlargement could be observed only on the basis of carcass weight; the depression in catalase was quite sharp when the tumor underwent malignant transformation (Begg *et al.*, 1953). According to Annau, Manginelli and Roth (1951a), rats with a reticuloendothelioma displayed increases in liver:total body weight ratios as the tumor approached an optimal weight; the results were more divergent with mice bearing a fibrosarcoma or various mammary tumors. Recently, an increase in the ratio has been reported for rats injected with Walker and Flexner-Jobling tumor extracts as well as in animals with these lesions amounting to 4% of the total body weight; the findings were negative with Jensen and Novikoff tumors and extracts (Kampschmidt *et al.*, 1960).

The present study was undertaken with the view of following liver weight changes in mice injected with four different tumors and in rats, with Jensen and Walker tumors. The latter was also introduced into the caudal lobe of the liver. Emphasis was directed toward the determination of any possible increase in dry liver weight over the controls. Also, to resolve difficulties relative to calculation of percentage liver weight on the basis of either total body or carcass weights, older or heavy animals were used in some of the series.

## Materials and Methods

*Animals.*— The male rats were of the Sprague-Dawley strain and Swiss and $BDF_1$ strains were employed in the mouse experiments. The rats were housed individually and the mice up to six per cage. Laboratory Chow and water were administered *ad libitum*.

*Tumor Transplantation.*—The Jensen and Walker tumor-saline suspensions (1:20) were injected into the rats in amounts of 0.30 ml, the controls receiving the same volume of saline. The Ehrlich ascites tumor was administered intraperitoneally

and as in all cases, extreme care was taken to deliver the identical amount to each animal. For leukemia, Tumor L1210, a total of 100,000 cells was inoculated intraperitoneally per mouse. Generally, these animals succumbed by the ninth day so that the test period comprised seven days. Tumor S-180 fragments of 0.1 ml in volume were injected into the leg by trocar and Tumor 755 (0.2 ml of 'standard' suspension) was administered subcutaneously. In one series, rats were anesthetized (ether), the abdomen incised and 0.025 ml of stock Walker suspension injected into the caudal lobe (Gershbein and Elias, 1954); the respective controls were treated in the same manner except that

TABLE 1.—Wet and Dry Liver-Total Body Weight Ratios for Mice with Transplanted Tumors.[a]

| Treatment | No. of mice | Average body weight at necropsy, gm | Wet liver per 100 gm body weight, gm | t | Dry liver per 100 gm body weight, gm | t |
|---|---|---|---|---|---|---|
| *Group 1-4* (BDF$_1$ males; duration: 12 days) | | | | | | |
| Controls | 50 | 19.6 ± 0.39 | 5.665 ± 0.088 | ..... | 1.673 ± 0.826 | ..... |
| Tumor 755 | 43 | 21.3 ± 0.46 | 5.708 ± 0.233 | 0.18 | 1.596 ± 0.044 | 1.57 |
| *Group 3-10* (BDF$_1$ males; duration: 12 days) | | | | | | |
| Controls | 43 | 19.6 ± 0.37 | 5.056 ± 0.101 | ..... | ..... | ..... |
| Tumor 755 | 36 | 20.7 ± 0.30 | 5.119 ± 0.083 | 0.74 | ..... | ..... |
| *Group 4-18* (BDF$_1$ males; duration: 15 days) | | | | | | |
| Controls | 38 | 20.5 ± 0.24 | 5.174 ± 0.071 | ..... | 1.403 ± 0.019 | ..... |
| Tumor 755 Based on total body weight | 27 | 21.7 ± 0.36 | 5.138 ± 0.078 | 0.33 | 1.392 ± 0.023 | 0.38 |
| Calculated on basis of carcass weights | ..... | 19.4 ± 0.32 | 5.756 ± 0.130 | 4.01[b] | 1.550 ± 0.028 | 4.45[b] |
| *Group 5-21* (Swiss females; duration: 8 days) | | | | | | |
| Controls | 30 | 21.5 ± 0.41 | 5.889 ± 0.167 | ..... | 1.741 ± 0.035 | ..... |
| Tumor S-180 | 28 | 18.5 ± 0.43 | 6.372 ± 0.158 | 2.10[b] | 1.728 ± 0.039 | 0.24 |
| *Group 6-33* (BDF$_1$ males; duration: 7 days) | | | | | | |
| Controls | 30 | 20.1 ± 0.31 | 4.611 ± 0.064 | ..... | 1.364 ± 0.019 | ..... |
| Leukemia | 40 | 22.6 ± 0.61 | 5.594 ± 0.150 | 5.40[b] | 1.424 ± 0.030 | 1.54 |
| *Group 7-50* (Swiss males and females; duration: 8 days) | | | | | | |
| Controls | 20 | 24.0 ± 0.36 | 5.559 ± 0.121 | ..... | 1.596 ± 0.036 | ..... |
| Ehrlich Ascites Tumor | 23 | 20.9 ± 0.67 | 7.211 ± 0.340 | 4.33[b] | 1.882 ± 0.089 | 2.73[b] |

[a] The standard error follows each ± sign in all cases.
[b] $P < 0.05$.

saline only was introduced. Where spillage was apparent at surgery or necropsy, such animals were excluded from consideration.

*Liver Removal.* — The animals were sacrificed (ether), the body weights recorded and the livers removed, drained in gauze and weighed. They were dried to constant weight in an oven at 100° C and the percentage water content determined.

## Results

Average mouse body weights at necropsy, ratios of wet and dry liver to total body weight and the pertinent Fisher t values are listed in Table 1. Animals with Tumors S-180 and 755 were sacrificed after eight and twelve days, respectively, following transplantation and at necropsy the average wet tumor weights were 750 mg and 1100 mg in the order given. In one series employing Tumor 755 where the duration was fifteen days (Group 4C-18), the mean wet tumor weight was 2.29 ± 0.28 gm; the relevant liver percentages are calculated on the basis of both total body and carcass weights. No sex differences were noted in the ratios with the mice sacrificed eight days after transplantation of the Ehrlich ascites tumor, the sexes being about equally represented in both the controls and tumor-bearing animals. Similar liver data for male rats with Jensen and Walker tumors together with average wet tumor weights are shown in Table 2; the intervals following transplantation were eighteen and thirty days. The pertinent ratios obtained with rats in which Jensen tumor did not 'take' are also compared with the corresponding control values. The duration was 10.5 days for the series injected with Walker tumor directly in the liver (Group 11-86A,B). For the latter, tumor was carefully dissected prior to liver processing. In general, macroscopic hepatic findings were not remarkable except with the leukemic mice in which the livers were pale in color.

## Discussion

As calculated on the basis of total body weight, mice bearing Tumor 755 and sacrificed after a period of twelve or fifteen days displayed no significant increase in either wet or dry liver weights over the respective controls (Table 1). The dry liver contents were likewise not increased with the Tumor S-180 and leukemia series but the wet liver:total body weight ratios were definitely elevated. However, mice of either sex with the Ehrlich ascites tumor underwent an increase in percentage wet liver and that this was not due to water alone can be noted from the significant elevation in dry liver:total body weight ratio.

In marked contrast to the above findings with Tumor 755 (Group 4-18), when the wet and dry liver ratios were evaluated in terms of carcass weight (body weight excluding the mass of the lesion), both were definitely elevated. It will be recalled that the calculation of liver weight changes on the basis of either carcass or total body weights has posed a distinct problem. Thus, the opinion of McEwen and Haven (1941) is shared by the writer, name-

TABLE 2.—Liver-Total Body Weight Findings for Male Rats with Transplanted Jensen and Walker Tumors.[a]

| Treatment | No. of rats | Average body weight at necropsy, gm | Mean tumor weight, gm | Wet liver per 100 gm body weight, gm | t | Dry liver per 100 gm body weight, gm | t |
|---|---|---|---|---|---|---|---|
| *Group 8-40* (Duration: 18 days) | | | | | | | |
| Controls | 25 | 523 ± 10.3[b] | ..... | 2.964 ± 0.081 | ..... | 0.889 ± 0.018 | ..... |
| Jensen Tumor | 24 | 480 ± 13.1 | 26.5 ± 11.5 | 3.939 ± 0.117 | 7.02[c] | 1.074 ± 0.016 | 6.32[c] |
| Walker Tumor | 31 | 485 ± 5.5 | 23.7 ± 5.4 | 4.298 ± 0.095 | 10.31[c] | 1.052 ± 0.019 | 6.03[c] |
| *Group 9-32* (Duration: 18 days) | | | | | | | |
| Controls | 18 | 163 ± 5.9[d] | ..... | 4.848 ± 0.123 | ..... | 1.311 ± 0.029 | ..... |
| Jensen Tumor (Over-all) | 21 | 135 ± 6.9 | 6.80 ± 1.40 | 5.733 ± 0.214 | 3.43[c] | 1.413 ± 0.044 | 1.89 |
| Tumor | 11 | ..... | 2.30 ± 0.35 | 5.348 ± 0.268 | 1.92 | 1.372 ± 0.072 | 0.91 |
| Tumor | 10 | ..... | 12.0 ± 1.8 | 6.149 ± 0.300 | 4.71[c] | 1.450 ± 0.050 | 2.62[c] |
| Negatives[e] | 8 | 136 ± 14.5 | ..... | 5.163 ± 0.304 | 1.16 | 1.328 ± 0.060 | 0.29 |
| *Group 10-14* (Duration: 30 days) | | | | | | | |
| Controls | 25 | 471 ± 8.9 | ..... | 3.030 ± 0.085 | ..... | 0.888 ± 0.016 | ..... |
| Jensen Tumor | 22 | 513 ± 12.1 | 63.4 ± 8.1 | 3.770 ± 0.103 | 5.60[c] | 0.958 ± 0.019 | 2.80[c] |
| Negatives[e] | 10 | 456 ± 10.4 | ..... | 3.035 ± 0.106 | 0.03 | 0.885 ± 0.022 | 0.10 |
| *Group 11-86A, B* (Duration: 10.5 days) | | | | | | | |
| Controls | 18 | 296 ± 6.0 | ..... | 3.490 ± 0.054 | ..... | 1.013 ± 0.016 | ..... |
| Walker Tumor Injected into caudal lobe of liver | 19 | 278 ± 5.3 | 1.76 ± 0.15 | 3.667 ± 0.062 | 2.12[c] | 1.009 ± 0.014 | 0.20 |

[a] Except for the last group, tumor was transplanted subcutaneously.
[b] The controls averaged higher in initial weight, the value being 518 gm as compared to the mean of 470 gm for the tumor animals.
[c] $P < 0.05$.
[d] Average initial weight for rats of this group: 94 ± 4.9 gm.
[e] Rats transplanted with Jensen cells but in which tumor was absent.

ly, that the tumor should be viewed as an integral part of the entire organism rather than a foreign or extraneous entity. Reference has already been made to similar calculations by Begg, Dickinson and Millar (1953). Although Yeakel (1948) could not correlate percentage liver based on carcass weight with tumor size, in her later study with Tobias (1951), the relative amount of hepatic nitrogen was greater in rats with large tumors only when computed in terms of carcass weights. Also, an elevated sensitivity noted with female tumorous animals in earlier work (Yeakel, 1948; Knox *et al.*, 1952), was not too apparent in the present study.

On the basis of total body weights, the wet liver percentages were increased in heavy rats bearing both large Walker and Jensen tumors (Groups 8-40 and 10-14; Table 2). It will also be noted that this stemmed in great measure from an increase in liver solids, the dry liver:body weights likewise being markedly elevated for both groups. In one series (Group 9-32), Jensen tumor implanted subcutaneously and averaging 6.80 ± 1.40 gm in weight caused an over-all increase in wet liver weight to the exclusion of any statistically significant change in the dry liver:body weight ratio. However, when the data from the treated animals were analyzed on the basis of tumor size, half of the rats which possessed lesions averaging 12.0 ± 1.8 gm in weight displayed both increases in wet and dry liver percentages; with the remainder (average tumor weight: 2.30 ± 0.35 gm), the wet liver:total body weight ratio was elevated over the controls but not to a statistically significant degree. As might be expected, no increase in wet liver weights ensued with animals injected with tumor cells but in which the lesion did not take. A rise in percentage wet weight occurred with rats bearing Walker tumor in the liver (average: 1.76 ± 0.15 gm; Group 11-86A,B) but the corresponding dry liver:body weight ratio was not affected. Presumably, a relatively small tumor present in the liver might predispose to a greater effect on the percentage wet liver as compared to the subcutaneously transplanted series discussed above (Group 9-32).

It should be pointed out that in the larger animals, body weight gains are reflected primarily in fat deposition whereas in the young growing rats, tissue nitrogen is still being laid down. Also, in those bearing rapidly growing tumors, an elevation in carcass water has been demonstrated (Recheigl *et al.*, 1961). In fact, the use of dry rather than wet carcass weights has been recommended by Haven, Mayer and Bloor (1961) in carrying out various comparisons between control and tumorous animals, a practice not explored in the current study.

The present findings further attest to a definite role of the liver in tumor growth and proliferation. Certainly, a variety of factors are involved in the increased liver size and must encompass a rise in the amount of hepatic water and possibly an increased content of solids as well as an elevated anabolism. A partial explanation has been advanced by Annau, Manginelli and Roth (1951b) based on an increased mitotic activity of the liver. In tu-

morous mice displaying especially high liver to body weight ratios, the mitochondria were enlarged and fine granular to the virtual absence of rod or filament shapes. Distinct mitochondrial changes would be expected on the basis of the increased water content of the tissue (Opie, 1947).

## Summary

The ratio of wet or dry liver to total body weights in $BDF_1$ male mice bearing the carcinoma, Tumor 755, did not vary markedly over the respective control values, the duration of the experiments being twelve and fifteen days. For the fifteen day series, increases in both ratios ensued when these were computed on the basis of carcass weights. The wet liver:total body weight ratios were significantly elevated in Swiss mice with Sarcoma 180 and $BDF_1$ animals injected with leukemia (L1210) but the corresponding dry liver ratios were in the range of the controls. Swiss mice of either sex bearing the Ehrlich ascites tumor displayed marked elevations in both the wet and dry liver percentages over a period of eight days as was also the case of very heavy rats with Jensen and Walker tumors (duration: up to 30 days). Liver enlargement did not occur in rats in which the tumor did not 'take' or in a series where the Jensen tumors were small (average weight: 2.3 gm). The hepatic water content was significantly higher in rats in which Walker tumor was transplanted directly into the liver (average weight: 1.8 gm) to the exclusion of any increase in liver solids or dry liver: total body weight ratio.

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