Effect of Endotoxin on Liver Carbohydrate of Mice Bearing Transplantable Tumors Sarcoma 37 and Krebs-2 Carcinoma*

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SUMMARY

A comparison is made between the stability of carbohydrate in the liver of normal mice and of mice bearing the transplantable tumors, Sarcoma 37 or Krebs-2 carcinoma. Although the presence of the tumor does not alter the level of liver carbohydrate it sensitizes the animal to endotoxin. Thus, liver carbohydrate is depleted in tumor-bearers by one-tenth as much endotoxin as is required for a similar change in normal animals.

Surgical removal of Sarcoma 37 restores normal stability in liver carbohydrate within a period of 48 hours.

Cortisone "protects" the carbohydrate in animals of each type (tumor-bearers and controls) from depletion by endotoxin, whereas Proferrin (colloidal saccharated iron oxide) greatly sensitizes the tumor-bearing mice to this effect of endotoxin.

Assays for liver tryptophan pyrrolose showed higher activities in tumor-bearing mice than in controls. A small dose of endotoxin raised the enzyme level in control mice but depressed it in tumor-bearing animals. Ten times as much endotoxin was required to produce a comparable depression of the enzyme in control mice.

The LD₅₀ of endotoxin was less in tumor-bearing mice than in controls, but it was elevated in both groups by cortisone, nicotinamide, and diphosphopyridine nucleotide and lowered by Proferrin.

The biochemical effects of tumors on host tissues and their relationship to other conditions of stress are discussed.

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Experimental conditions which alter the susceptibility of mice to bacterial endotoxins have been studied by a number of investigators. Lethal effects are enhanced by reticuloendothelial (RES) "blockage" (2), by prior infection with the Calmette-Guerin strain of the tubercle bacillus (BCG) (21), by exposure to simulated high altitude (about 20,000 ft.) (4), and by extremes of environmental temperature (37°C or 5°C) (19). Mice are also made more susceptible to bacterial endotoxin when bearing the transplantable tumors Sarcoma 37 and Krebs-2 carcinoma (14, 16). The animals may be protected against endotoxin by concurrent or prior injection of the appropriate adrenocortical hormone (7, 8) and of nicotinamide or diphosphopyridine nucleotide (DPN) (5).

In previous studies on the metabolic basis of endotoxemia, carbohydrate levels of liver, muscle, and total carcass have been compared in mice made more susceptible to endotoxin with those protected against it and with controls (6, 7). Without exception, mice which lose carbohydrates more rapidly than controls also succumb to smaller doses of endotoxin, whereas normal and protected mice maintain their carbohydrate reserves more effectively.

The purpose of the collaborative studies reported here was to extend investigations on metabolic effects of endotoxins to tumor-bearing mice. Previous experiments have established that mice bearing Sarcoma 37 and Krebs-2 carcinoma are 20–30 times more sensitive to endotoxin than normal mice (14, 16). Tumor removal restores these animals to essentially normal levels of resistance to endotoxin within 24 hours (15) and to normal enzymatic activity (13). Carbohydrate changes in liver, muscle,
and total carcass induced by endotoxin were determined in mice bearing two different transplantable tumors, Sarcoma 37 and Krebs-2 carcinoma, in mice following tumor excision, and in control animals. The extent to which cortisone, DPN, nicotinamide, and an RES "blocking" agent were able to modify the susceptibility of tumor-bearing mice to endotoxin was also evaluated.

MATERIALS AND METHODS

Mice.—Male ICR albino mice 8–10 weeks old and weighing 31–41 gm. (average, 36 gm.) were used in these studies. They were housed eight per cage, with pine shavings as bedding, and were given water and commercial pellets (Old Guilford, Emory Morse Co., Guilford, Conn.) ad libitum unless otherwise specified.

Transplantable mouse tumors.—Ascites Sarcoma 37 or Krebs-2 carcinoma cells were inoculated into the interscapular region of mice as described in detail elsewhere (14, 16). Seven days later animals with solid tumors of uniform size measuring 1.5–2.5 sq. cm. at the base were used in the experiments.

Tumor removal.—Removal of the 7-day-old tumors was performed under nembutal anesthesia as previously described (15). Care was taken to leave no obvious residual tumor.

Determination of LD₅₀ for endotoxin.—Tests of lethality of endotoxin in normal and in tumor-bearing animals were carried out over sufficiently wide dosage ranges to result in 0–100 per cent mortality. From these data the LD₅₀ was calculated according to the method of Reed and Muench (20).

Preparation of endotoxin.—Serratia marcescens (Havas Temple strain) was grown for 2 days on a synthetic medium (14), heat-killed at 68° C. for 1½ hours, the cells centrifuged at 5° C., and the supernatant dialyzed against distilled water. After exhaustive dialysis the material was lyophilized and stored over Drierite at 5° C. in a desiccator.

Glycogen determinations.—Liver glycogen was measured according to the procedure of Kemp and Kils van Heijnningen (17). Approximately 100 mg. of tissue was used in the assay, and the per cent carbohydrate was calculated on the basis of the total liver weight (5). The assay depends upon the development of 5-hydroxymethyl furfural from glucose in the presence of hot sulfuric acid. Sufficient color is developed from 10 µg of glucose to give a reliable measurement in the Coleman Model 14 spectrophotometer. The data are presented in the tables as the mean of the number of separate determinations shown in parentheses.

Tryptophan pyrrolyase assay.—The method of Knox and Auerbach (18) for determining tryptophan pyrrolyase in rat liver was modified for mouse liver as previously described (5). Data are expressed as µmoles kynurenine/gm dry weight of liver/hour.

Cortisone.—A suspension of 5 mg. of cortisone acetate (Nutritional Biochemicals Corp., Cleveland) in 0.5 ml. of saline was administered subcutaneously. The suspension, stabilized with a drop of Tween 80, was prepared in a glass homogenizer with Teflon pestle and injected immediately thereafter.

Nicotinamide, DPN.—Nicotinamide and diphosphopyridine nucleotide (DPN) (Sigma Chemical Co., St. Louis) were each dissolved in saline and injected intraperitoneally. Ten mg. of each substance per mouse was injected intraperitoneally in a volume of 0.5 ml. Nicotinamide, DPN, and cortisone were each given immediately preceding the endotoxin injection.

Proferrin (saccharated iron oxide).—Proferrin (Merck, Sharp and Dohme, West Point, Pa.) was injected into the tail vein of mice 2 hours prior to endotoxin administration. Each animal received 0.2 ml.

Injection schedules.—In the experimental groups receiving endotoxin, mice were given the injections at 8:00 a.m., and injected and control mice were sacrificed 5 hours later for glycogen determinations. For the tryptophan pyrrolyase assay the animals were given injections at 5:00 p.m. and sacrificed the next morning 17 hours post-injection. The same injection schedules were maintained throughout all experiments reported here to avoid possible variation due to the circadian rhythm.

RESULTS

Effects of tumor and tumor removal on liver carbohydrate levels of mice treated with endotoxin.—The total amount of liver carbohydrate in mice bearing a 7-day-old Sarcoma 37 tumor was not significantly different from that in normal mice (38.6 mg. vs. 41.6 mg.), but their sensitivity to endotoxin was greater. These results are presented in Table 1. A dosage of 150 µg. of endotoxin drastically lowered liver carbohydrate in tumor-bearing animals from 38.6 to 6.9 mg. (lines 2 and 5, Table 1), whereas in normal mice the drop was from 41.6 to 27 mg. (lines 1 and 3, Table 1). Even a dose of 1500 µg. of endotoxin reduced liver carbohydrate of normal mice to only 15 mg. (line 4, Table 1). Tumor removal alone did not appreciably alter the level of liver carbohydrate, but the mice were restored to normal susceptibility and showed a response to 150 µg. of endotoxin similar to that of control mice (compare lines 3 and 7 of Table 1).

Determinations carried out on control and Sarcoma 37-bearing mice included assays for carbohydrate in muscle and in the eviscerated carcass. These values were not tabulated, because muscle carbohydrate was uniformly low in the different groups of mice and underwent no significant change following endotoxin injection. Changes in the carbohydrate content of total carcass (minus skin, feet, tail, and digestive tract) could be accounted for almost entirely by the changes that occurred in liver carbohydrate levels. The data as presented express, therefore, the major changes in carbohydrate that resulted from the experimental conditions employed.

Effect of cortisone and proferrin on liver carbohydrate levels in endotoxin-treated Sarcoma 37-bearing mice.—A single injection of 5 mg. cortisone into tumor-bearing mice raised liver carbohydrate within 5 hours to 3 times the amount found in tumor-bearing controls (lines 2 and 8, Table 1). Cortisone and 150 µg. endotoxin given to Sarcoma 37-bearing mice resulted in a level of carbohydrate similar to that obtained in normal mice with an identical amount of endotoxin (lines 3 and 9, Table 1). When the dosage of endotoxin was increased to 1500
TABLE 1

LIVER CARBOHYDRATE LEVELS IN NORMAL, IN SARCOMA 37-BEARING, AND TUMOR-EXCISED MICE

| EXPERIMENTAL TREATMENT* | DOSE OF ENDOTOXIN (µg.) | LIVER CARBOHYDRATES | TOTAL/mouse (mg.) PERCENT BY
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<tbody>
<tr>
<td>Controls</td>
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<tr>
<td>Tumor-bearing controls</td>
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<td></td>
</tr>
<tr>
<td>Controls + endotoxin</td>
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<td></td>
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<tr>
<td>Controls + endotoxin</td>
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</tr>
<tr>
<td>Tumor-bearing + endotoxin</td>
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<tr>
<td>48 hr. after tumor excision</td>
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<tr>
<td>48 hr. after tumor excision + endotoxin</td>
<td></td>
<td></td>
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<tr>
<td>Tumor-bearing + 5 mg. cortisone</td>
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<td></td>
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<tr>
<td>Tumor-bearing + 5 mg. cortisone + endotoxin</td>
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<td></td>
</tr>
<tr>
<td>150 µg. cortisone + endotoxin</td>
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<td></td>
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<tr>
<td>0.2 ml. Proferrin + endotoxin</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.2 ml. Proferrin + endotoxin</td>
<td></td>
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</tbody>
</table>

* S. marcescens endotoxin and cortisone were injected 5 hours prior to the assays, and animals were fasted throughout this period. Proferrin was injected 2 hours prior to endotoxin administration. Each value is the mean of the number of determinations shown in parentheses.

The effect of endotoxin on tryptophan pyrrolase activity.—Table 2 presents the values of liver tryptophan pyrrolase activity (expressed in µmoles kynurenine/gm dry wt of liver/hr) obtained from normal and Sarcoma 37-bearing mice 17 hours after fasting and after an injection of endotoxin. Enzyme activity was higher in tumor-bearing mice than in the controls (19.3 µg vs. 14.5 µg). However, a comparatively small dose of endotoxin (150 µg) raised the enzymic level in control mice to that of the tumor-bearing controls (lines 2 and 3, Table 2). The same amount lowered the activity of the enzyme in tumor-bearing animals, whereas 10 times as much (1500 µg) was required in normal animals to produce a similar depression (lines 4 and 5, Table 2).

Liver carbohydrate levels in normal and in Krebs-2 carcinoma-bearing mice.—Mice bearing Sarcoma 37 lost a greater amount of liver carbohydrate than normal animals after an injection of endotoxin. It was of interest, therefore, to extend these observations to another tumor, the Krebs-2 carcinoma, which is also known to make mice more susceptible to the lethal effects of bacterial endotoxin (14). Similar data were obtained with Krebs-2 carcinoma, except that carbohydrate values were higher than those in Sarcoma 37-bearing mice (Table 3). These differences may reflect seasonal variations in carbohydrates, since the Sarcoma 37 tests were carried out in late fall and winter, whereas the Krebs-2 carcinoma experiments were done in late spring and early summer.

Krebs-2 carcinoma did not sensitize the animals to

TABLE 2

EFFECT OF S. MARCESCENS ENDOTOXIN ON LIVER TRYPTOPHAN PYRROLASE ACTIVITY IN NORMAL AND IN SARCOMA 37-BEARING MICE

<table>
<thead>
<tr>
<th>EXPERIMENTAL TREATMENT*</th>
<th>LIVER TRYPTOPHAN PYRROLASE ACTIVITY (µmoles kynurenine/gm dry wt/hr ± S.E.)</th>
<th>P value† (as indicated)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>14.5 ± 1.0 (9)</td>
<td>(1) vs. (2) P = 0.05</td>
</tr>
<tr>
<td>Tumor-bearing controls</td>
<td>19.3 ± 1.1 (9)</td>
<td>(1) vs. (2) P = 0.05</td>
</tr>
<tr>
<td>Controls 17 hr. after 150 µg. endotoxin</td>
<td>18.0 ± 1.1 (12)</td>
<td>(1) vs. (3) P = 0.05</td>
</tr>
<tr>
<td>Controls 17 hr. after 1500 µg. endotoxin</td>
<td>9.7 ± 0.6 (7)</td>
<td>(1) vs. (4) P = 0.01</td>
</tr>
<tr>
<td>Tumor-bearing mice 17 hr. after 150 µg. endotoxin</td>
<td>11.8 ± 1.0 (11)</td>
<td>(2) vs. (5) P = 0.001</td>
</tr>
</tbody>
</table>

* All animals were fasted for 17 hours prior to the assay. Each value is the mean of the number of determinations shown in parentheses.
† The P values were calculated by the rank order test of White (25).
endotoxin to the same degree as did Sarcoma 37, since about twice as much endotoxin was required to bring about a corresponding reduction in liver carbohydrate. This is apparent when lines 3 and 4 of Table 3 are compared. An injection of cortisone raised the liver glycogen in Krebs-2 tumor-bearing mice from 98.8 to 160 mg. When cortisone and 300 μg. of endotoxin were injected simultaneously, liver carbohydrate decreased to the same value as that observed in tumor-bearing mice given only 150 μg. of endotoxin (lines 3 and 6, Table 3).

In marked contrast to the action of cortisone, Proferrin "sensitized" mice with Krebs-2 carcinoma to endotoxin so that as little as 2 μg. produced a greater drop in liver glycogen than did 300 μg. in tumor-bearing mice given no Proferrin (lines 8 and 4, Table 3). Proferrin alone resulted in a slight decrease in liver carbohydrate compared with tumor-bearing controls (lines 7 and 2, Table 3).

Lethality tests.—The LD₅₀ of control mice was compared with those for Sarcoma 37 and Krebs-2 carcinoma-bearing animals under a variety of experimental conditions. The results are summarized in Table 4. Several points of interest emerge from these data. Sarcoma 37 and Krebs-2 carcinoma sensitized mice to endotoxin compared with control mice. The respective LD₅₀'s were one-fifth to one-half of the control value (compare lines 2 and 7 with line 1, Table 4). The marked protective effect of nicotinamide, DPN, and especially cortisone against endotoxin in Sarcoma 37-bearing mice was apparent (lines 3, 4, and 5, Table 4). By contrast, in Krebs-2 carcinoma-bearing mice neither nicotinamide nor DPN was particularly effective in protecting against endotoxin, whereas cortisone was even more protective with this tumor than with Sarcoma 37 (cf. lines 7-10, Table 4).

Proferrin "sensitized" mice with each type of tumor to endotoxin to about the same dosage level, but compared with the control groups differences between LD₅₀'s were greater in mice with Krebs-2 carcinoma than with those bearing Sarcoma 37 (cf. lines 6 and 2 with 11 and 7, Table 4).

**DISCUSSION**

The data presented in this report establish that tumor-bearing mice contain levels of liver glycogen similar to those found in normal animals. However, the quantity of endotoxin required to deplete the carbohydrate of tumor-bearing animals within 5 hours is less than one-tenth the dose required in control mice. Tumor removal

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**TABLE 3**

<table>
<thead>
<tr>
<th>Experimental treatment</th>
<th>Liver Carbohydrate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Dose of endotoxin (μg.)</td>
</tr>
<tr>
<td></td>
<td>Total/mouse (mg.)</td>
</tr>
<tr>
<td>(1) Controls</td>
<td>150</td>
</tr>
<tr>
<td>(2) Tumor-bearing controls</td>
<td>300</td>
</tr>
<tr>
<td>(3) Tumor-bearing mice + endotoxin</td>
<td>150</td>
</tr>
<tr>
<td>(4) Tumor-bearing mice + endotoxin</td>
<td>300</td>
</tr>
<tr>
<td>(5) Tumor-bearing mice + 5 mg. cortisone</td>
<td>160.0 (12)</td>
</tr>
<tr>
<td>(6) Tumor-bearing mice + 5 mg. cortisone + endotoxin</td>
<td>300</td>
</tr>
<tr>
<td>(7) Tumor-bearing mice + 0.2 ml. Proferrin</td>
<td>78.0 (12)</td>
</tr>
<tr>
<td>(8) Tumor-bearing mice + 0.2 ml. Proferrin + endotoxin</td>
<td>2</td>
</tr>
</tbody>
</table>

* Effect of *S. marcescens* endotoxin and/or cortisone or Proferrin 6 hours after injection in mice fasted throughout the period prior to the assays. Each value is the mean of the number of determinations shown in parentheses.
restores these mice to a normal level of resistance to endotoxins 48 hours after surgery and to normal stability of tumor-bearing animals to the lethal effects of endotoxin plantable tumors (16), infection with BCG (21), exposure to simulated high altitude (4), or cold (19). In all these, protection against carbohydrate depletion can be achieved by cortisone. Profferin, on the other hand, sensitizes the tumor-bearing animals to the lethal effects of endotoxin and to carbohydrate depletion.

A number of enzymatic changes in animals having spontaneous or transplantable tumors have been reported in the literature. Greenstein and Andervont (13) found that the presence of spontaneous, induced, or transplantable tumors (including Sarcoma 37) reduced liver catalase activity from one-half to one-twentieth of that found in control animals. Regression of the tumor or surgical removal resulted in the return of catalase activity to normal levels. Goranson (10) found that tumor-bearing mice and rats given glucose deposited less liver glycogen than did normal controls. This effect was believed to be related to production of excessive amounts of adrenocortical hormones.

Adams (1) found that in mice treated with Sarcoma 37 homogenates adrenal factors which normally influence hepatic catalase activity are prevented from operating and that liver catalase activity remained depressed in spite of cortisone administration. Other enzymes found to be decreased in livers of animals bearing spontaneous or transplantable tumors were carboxy peptidases, arginase, d-amino acid oxidases, lipases, and esterases (12).

A number of investigators have shown that the presence of spontaneous or transplantable tumors produces biochemical and metabolic changes in tissues that contain no malignant cells and are remote from the site of the tumor (3, 12). There is no question, therefore, that in some way the presence of a malignancy alters the biochemical balance of the animal.

The metabolic basis for these changes in normal and tumor-bearing animals remains obscure. It is also not clear at the present time, as previously stated (6), whether the rapid loss of liver glycogen in endotoxin-poisoned mice is the consequence of augmented utilization, depressed synthesis, or a combination of the two. There is some inferential evidence, however, pointing to an impairment in glycogenesis in the tumor-bearing mice.

Greengard et al. (11) and, more recently, Weber et al. (22) have shown that actinomycin, a potent inhibitor of protein synthesis, prevents the rise in liver glycogen induced in rats by hydrocortisone. From these findings the authors conclude that the cortisone-induced glycogenesis is dependent upon enzyme induction.

It has also been shown (5) that tryptophan pyrrolase, induced by certain adrenocortical hormones, is (a) elevated about 5 hours after the injection of an LD_{50} of endotoxin, (b) depressed to an activity about one-half normal 17 hours post-endotoxin, and (c) fails to increase when cortisone is given 4 hours after the endotoxin. If tryptophan pyrrolase synthesis serves as a measure of enzyme induction by cortisone, it is clear that endotoxin, after a few hours, acts as an inhibitor of enzyme synthesis. Assays for tryptophan pyrrolase in tumor-bearing mice (Table 2) show a higher level of activity than that seen in control mice, but this activity is depressed by a dose of endotoxin one-tenth that required by normal animals. A possible explanation may be found in the fact that tumor-bearing mice possess enlarged adrenal glands, low in ascorbic acid and cholesterol compatible with exhaustive hypofunction of the adrenal cortex (3). With inadequate adrenocortical reserves, these animals could be expected to be less able to cope with stress, including endotoxina, than normal animals. A complex interrelationship between hormonal balance, enzyme induction, and metabolic function begins to emerge, therefore, as a possible explanation for not only carbohydrate changes but some of the other biochemical effects of bacterial endotoxins.

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REFERENCES

14. HAVAS, H. F., AND DONNELLY, A. J. Mixed Bacterial Toxins in...


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