Effect of Beef Spleen Extract on Respiration of Normal Liver and
dbrB Adenocarcinoma During Storage at 4° C.

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The data in the present report on the respiration of tumor tissue following storage at 4° C. in the presence of beef spleen extract were assembled during a series of experiments to establish a method of assay of the activity of beef spleen extract which has been reported as beneficial in the treatment of human basal cell epithelioma (1, 2). Amersbach, Walter, and Sperti (1) reviewed the earlier work on animals in this field. Extracts of mouse, horse, and beef spleen were found to enhance the respiration and depress the glycolysis of several mouse tumors (3) whereas extracts of other animal tissues similarly affected the metabolism of adenocarcinoma No. 63 and a methylcholanthrene-induced sarcoma (7). Extracts of beef spleen increased the respiration of normal rat liver and skin (4).

PROCEDURE

Female dba mice, 3 months old, were inoculated with dbrB tumor suspension into the groin. Two weeks later when the tumor was one-fourth the size of the animal, it was removed under light ether anesthesia and aseptic conditions, and macerated by passing through a fine mesh screen with a porcelain pestle. The pulp was suspended in twice its volume of Tyrode's solution and the suspension divided into 2 portions. To 1 was added one-third its volume of Tyrode's solution and to the other a similar volume of beef spleen extract (8), containing 142 mgm. of solids per ml. Due to dilution factors during preparation of the stock suspension the final concentration of beef spleen extract was approximately 35 mgm. per ml. and during the respiratory period it was 12 mgm. per ml. Both the tumor suspension in Tyrode's solution and the tumor suspension in beef spleen extract were stored at 4° C. for 12 days. At 48 hour intervals portions were removed and allowed to stand for 1 hour at room temperature prior to determination of the respiratory rate by the Warburg technic (5).

As a comparison for the study of the respiration of the dbrB mammary adenocarcinoma, liver was obtained from normal dba mice of the same age, prepared and stored in a manner similar to that employed for the tumor tissue. Prior to storage, in all except the first series of experiments, the respiratory rate of the tumor and liver tissues was determined within 30 minutes of removal from the animal and is designated in the tables as "0" day. Four series of experiments were performed, using a total of 77 tumor-bearing animals and 31 normal mice for the liver tissue. The determination of the respiratory rate of the tumor suspension and normal tissue was made at 37.5° C. in the Warburg respirometers. In each determination 4 of the flasks were used for tumor tissue, 4 for normal liver tissue, and 2 additional flasks served as barometric controls.

In the inner well of each flask was 0.2 ml. of 1 N potassium hydroxide and the outer chamber contained 2 ml. of Ringer-phosphate-glucose solution, pH 7.2. Of the 4 experimental flasks for tumor tissue, 2 contained 1 ml. each of tumor suspension that had been stored in Tyrode's solution and the other 2, suspension that had been stored in beef spleen extract. Similarly, of the 4 experimental flasks for normal tissue, 2 contained 1 ml. each of liver suspension stored in Tyrode's solution and 2 contained 1 ml. each of suspension stored in beef spleen extract. Readings were taken at 60, 120 and 180 minutes. The suspensions were then dried overnight in weighed crucibles in a drying oven at 70° C. The calculated weight of the salt content of the Ringer-phosphate-glucose solution was subtracted from the dry weight of the tissue. The cubic mm. of oxygen per hour per mgm. of dry weight (QO2) for the first and second 60-minute intervals and the total cu. mm. of oxygen per 3 hours per mgm. of dry weight were calculated. The values in the tables represent the average of duplicate determinations. When the experimental error was greater than 10 per cent the data were discarded.

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RESULTS

The data on the oxygen consumption of the dbrB tumor from dba mice and of normal dba mouse liver after storage at 4° C. for 2 to 12 days are presented in Tables I, II and III. This information represents the average of 4 series of experiments performed, using tumor-bearing animals and normal mice for the liver tissue. Examination of these tables show that: (a) during storage at 4° C. the respiration of both tumor and normal liver progressively decreased; (b) the respiratory rate of the tumor tissue was higher than that of normal liver tissue; (c) the respiratory rate for the tumor tissue was maintained during the second 60 minutes of the experimental period of 3 hours, while that of the liver was not; (d) the QO₂ for the third experimental hour was inconsistent for both the tumor and normal tissue; (e) in the presence of the beef spleen extract the QO₂ as well as the total oxygen uptake of the tumor was lower than in the presence of Tyrode’s solution; (f) conversely, that of the liver was higher after storage in the beef spleen extract than after storage in Tyrode’s solution.

TABLE I: AVERAGE QO₂ FOR FIRST 60 MINUTES OF DDBR B TUMOR AND NORMAL DBA LIVER STORED AT 4° C. FOR 2 TO 12 DAYS IN TYRODE’S SOLUTION AND BEEF SPLEEN EXTRACT

<table>
<thead>
<tr>
<th>Series I to IV</th>
<th>DAYS IN STORAGE</th>
<th>DDBR TUMOR</th>
<th>Tyrode’s solution</th>
<th>2.55</th>
<th>2.26</th>
<th>1.74</th>
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<th>0.71</th>
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TABLE II: AVERAGE QO₂ FOR SECOND HOUR OF DDBR B TUMOR AND NORMAL DBA LIVER STORED AT 4° C. FOR 2 TO 12 DAYS IN TYRODE’S SOLUTION AND BEEF SPLEEN EXTRACT

<table>
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<th>Series I to IV</th>
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<th>Tyrode’s solution</th>
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TABLE III: AVERAGE OF TOTAL OXYGEN UPTAKE (CU. MM. PER MCM. OF TISSUE) OF STORED DDBR B TUMOR AND NORMAL MOUSE LIVER TISSUE IN TYRODE’S SOLUTION AND BEEF SPLEEN EXTRACT OVER A PERIOD OF 3 HOURS

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DISCUSSION

The data presented on the respiration of dbrB adenocarcinoma from dba mice, and of liver from normal dba mice, confirm the observations of other investigators that some tumor tissues may have a higher respiratory rate than certain normal tissues, as well as the data of Cook and Walter (4) who reported that the respiration of rat liver was increased in the presence of beef spleen extract. In contrast to the lowered respiratory rate of the dbrB adenocarcinoma in these experiments in the presence of spleen extract, Büngeler (3) reported an increased respiratory rate for Ehrlich’s adenocarcinoma, a transplantable sarcoma and a tumor carcinoma. Moreover, Schroeder and Cook (7) using heterozygous strains of mice and rats, found that the respiratory rate of adenocarcinoma No. 63 and methylcholanthrene-induced sarcomas was raised in the presence of extract of mouse embryos and of organs of the mouse exclusive of the spleen.

The effect of the spleen extract on respiration of dbrB adenocarcinoma in these experiments is of interest in view of the results obtained by Macfarlane, Schmock and Nadeau (6) with implantation of suspensions of the same tumor stored in spleen extract at 4 to 7° C. for as long as 8 days.

SUMMARY

The oxygen consumption of dbrB adenocarcinoma from dba mice and of liver from normal dba mice was measured in the Warburg respirometer at 48 hour intervals during a period of 12 days of storage at 4° C. in Tyrode’s solution and in beef spleen extract. The respiratory rate of both tumor and liver tissue progressively decreased during storage, that of the adenocarcinoma being consistently higher than that of the normal liver. Tumors stored in beef spleen extract had a lower respiratory rate than tumors stored in Tyrode’s solution, while normal liver stored in beef spleen extract had a higher rate of respiration than liver stored in Tyrode’s solution. The depression of respiration with the beef spleen extract of dbrB tumor from dba mice is in contrast with the stimulation reported by others using spleen extract and other types of tumors in heterozygous strains of rodents.

REFERENCES


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