I. Mouse Mammary Tumors of the dba and C3H Strains

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INTRODUCTION

In previous communications by one of the authors (5—9), it was shown that mammary tumors, histologically diagnosed as adenocarcinomas, although morphologically almost identical, differed in their rate of growth, over-all metabolic activity, and radiosensitivity. The mammary tumors investigated are the dbB tumor, autogenous to the dba strain of mice, and the CSH tumor autogenous to the C3H strain of mice. For the sake of brevity these two tumors will be referred to as the dbB and CSH tumors, respectively.

As was reported, the rate of growth was judged by the latent period, i.e., the time elapsing between the implantation of a tumor graft and the detection of tumor growth. This latent period was about 4—6 days for the dbB tumor and about 14—18 days for the CSH tumor.

Furthermore, the metabolic rates, as determined by the oxygen intake and aerobic glycolysis in vitro (Warburg manometric technic), were as follows: for the dbB tumor, the rate of oxygen intake (QO2) averaged 5.9 and the aerobic glycolysis (QAC) averaged 84.3. For the CSH tumor, the rate of oxygen intake (QO2) averaged 3.6 and the aerobic glycolysis (QAC) averaged 8.5, determinations being done in 100 per cent oxygen. Thus, as measured by QAC, the dbB tumor was found to have a rate of growth and rate of metabolic activity about 3 times that of the CSH tumor.

Attempts are being made to compare the properties of these tumors by more detailed studies, so as to account for the differences in their biological and physiological behavior. One of these studies will be reported here.

As previously mentioned, the mammary tumors, dbB and CSH, exhibited a significant difference in their respiratory and over-all glycolytic activities in vitro. It was deemed of interest to investigate the pathways of the whole glycolytic cycle of the actively growing tumors in vitro. This was accomplished by determining the various components encountered in this cycle by the use of the freezing technic and modern enzymatic and chemical methods. To the knowledge of the authors, no such data on these particular tumors are available in the literature.

EXPERIMENTAL

Young inbred mice of the dba and CSH strains received, by trocar, grafts of a small tumor particle, autogenous to the strain, which was placed subcutaneously between the groin and subaxillary region. When tumors of a measurable size appeared, the mice were anesthetized by an intraperitoneal injection of nembutal so as to immobilize the animal. To exclude necrotic portions usually present in the center of the tumor, only surface portions of the actively growing tumors were dissected and immediately placed in either liquid air or in an ether and dry ice mixture. The frozen tumor tissue was pulverized in a specially constructed brass chamber by a heavy piston and was immediately placed in a small ice-cold beaker containing the necessary amount of 10 per cent trichloroacetic acid. Since the weight of the beaker plus the trichloroacetic acid had been determined previously, the amount of the tumor sample taken for analysis was thereby calculated by the difference in the two weights. About 1—2 gm. of tumor tissue, taken from surface portions of 2—3 tumors,
proved to be adequate for the determinations of the components involved in the Embden-Meyerhof scheme of the glycolytic cycle.

The known amount of pulverized tumor suspended in trichloroacetic acid was immediately transferred quantitatively into a Pyrex glass homogenizer of the Potter-Elvehjem (16) type in order to obtain as complete an extract of the tissue as possible. Strict precautions were taken to keep the tumor material for analysis ice-cold during all procedures requiring low temperature. When the tumor tissue could not be analyzed immediately, it was stored in a deep freezer at about -20°C.

The compounds determined included total acid-soluble phosphorus, inorganic orthophosphate, lactic acid, glycogen, glucose-1-phosphate, fructose-6-phosphate, fructose-1,6-diphosphate, phosphoglyceric acid, coenzyme I, adenylic acid, adenosine diphosphate, adenosine triphosphate, and phosphocreatine. The last two compounds are of special interest, since they represent the chief storehouse of immediately utilizable energy for tissues.

The methods for the determination of the individual components are essentially those described by LePage in the monograph "Manometric Techniques and Related Methods for the Study of Tissue Metabolism" (18) and used by him in his studies on phosphorylated intermediates in tumor glycolysis (12, 18). Fractionation procedure B was used. A slight modification in the determination of phosphopyruvic acid was introduced by determining the phosphorus without removing the excess iodine from solution. Adenylic acid, adenosine diphosphate, adenosine triphosphate, and CoI were assayed enzymatically by the method described by Albaum and Lipshitz (1). This method involves converting the higher nucleotides to adenylic acid by a combined hexokinase, myokinase, and nucleotide pyrophosphatase system and assaying the adenylic acid formed by enzymatic conversion to inosinic acid with a muscle deaminase preparation. In later determinations of CoI, the method of Racker (17) was used. In this procedure, the CoI is reduced by alcohol dehydrogenase and its concentration determined from the absorption at 340 μ.

Adenylic deaminase (Schmidt's deaminase) was prepared according to the method of Kaledkar (11); myokinase, according to that of Colowick and Kaledkar (8). Hexokinase was prepared in partially pure form, according to the method of Berger, Stein, Colowick, and Cori (2) through step 3 (fractionation with ethanol at 0°C.) of the isolation procedure. The nucleotide pyrophospha-

tase was a gift from Dr. Kornberg1 of the National Institutes of Health, and the alcohol dehydrogenase was received from Dr. Racker2 of the New York University College of Medicine. The enzymes were kept in a deep freezer and were found to be stable over long periods of time.

RESULTS

The mean values of components considered in phosphorylative glycolysis obtained from 27 analyses carried out on dbB tumors and from 22 analyses carried out on CSH tumors, along with the standard errors for each mean, are recorded in Table 1. The values of each component represent mg. in 100 gm. wet weight of tumor tissue.

TABLE 1

<table>
<thead>
<tr>
<th>Component</th>
<th>CSH Mean S.E.M.</th>
<th>dbB Mean S.E.M.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total phosphorus</td>
<td>22 55.8±3.29</td>
<td>27 55.9±3.34</td>
</tr>
<tr>
<td>Phosphocreatine</td>
<td>17 51.6±4.50</td>
<td>17 27.6±4.65</td>
</tr>
<tr>
<td>Lactic acid</td>
<td>20 96.8±4.65</td>
<td>15 100.7±4.61</td>
</tr>
<tr>
<td>Phosphoglyceric acid</td>
<td>32 92.9±3.45</td>
<td>25 25.2±1.40</td>
</tr>
<tr>
<td>Glycogen</td>
<td>15 14.1±1.66</td>
<td>11 18.8±2.27</td>
</tr>
<tr>
<td>Hexose diphosphate</td>
<td>22 93±0.90</td>
<td>25 81.1±1.00</td>
</tr>
<tr>
<td>Fructose-6-P</td>
<td>22 4.9±0.95</td>
<td>22 5.7±0.31</td>
</tr>
<tr>
<td>Glucose-1-P</td>
<td>20 6.2±0.57</td>
<td>17 10.8±1.68</td>
</tr>
<tr>
<td>Adenylic acid</td>
<td>19 10.6±1.77</td>
<td>24 15.5±1.16</td>
</tr>
<tr>
<td>Adenosine diphosphate</td>
<td>15 24±1.50</td>
<td>18 21.7±4.91</td>
</tr>
<tr>
<td>Adenosine triphosphate</td>
<td>12 18±0.72</td>
<td>18 45±4.30</td>
</tr>
<tr>
<td>Coenzyme I</td>
<td>14 15.3±3.02</td>
<td>25 10.4±1.10</td>
</tr>
</tbody>
</table>

It should be mentioned that there is a difference in the water content of these tumors. As previously reported (8), the water content of the dbB tumor averaged 85.5 per cent and that of the CSH tumor averaged 81.5 per cent. The difference in the water content of the tumors is in accord with the difference in their rate of growth, for it is known that faster proliferating tissues possess a greater water content than slower proliferating ones.

To determine whether the difference between the means of the values included in the table were statistically significant, diff/S.E. values were calculated using the formula for standard error for a difference: S.E. diff. = √[S.E.1 + S.E.2]. Ratios for diff/S.E. of greater than 2 were considered significant. An analysis of the data recorded in the table revealed the following: the over-all pattern of components of both tumors resembles that of

1 The authors wish to express their appreciation to Dr. Kornberg and Dr. Racker for their generous assistance in supplying the above material.
normal differentiated tissues according to the Embden-Meyerhof phosphorylative glycolysis system, with the exception of the lactic acid values, which are relatively higher than those of normal differentiated tissues. This may be explained by the high rate of glycolysis of rapidly proliferating tissues.

Significant differences between the mean values for the two mammary adenocarcinomas analyzed can be noted for inorganic phosphorus, glucose-1-phosphate, and adenosine triphosphate. The difference in glycogen is almost statistically significant, diff/S.E. resulting in 1.94. There is no significant difference in the mean values of the lactic acid content of the two tumors. This is not in accord with the results obtained from the experiments carried out in vitro and reported in a previous publication (8), showing that the dbrB tumor produced about 8 times as much lactic acid as the C3H tumor.

DISCUSSION

In the study of the levels of the phosphorylated intermediates in glycolysis of the analogous mouse mammary tumors, dbrB and C3H, it was found that a significant difference exists between the mean values of inorganic phosphorus, glucose-1-phosphate, and adenosine triphosphate. As mentioned above, the difference in glycogen was almost statistically significant, diff/S.E. resulting in 1.94, particularly if the difference in water content between the C3H tumor (81.5 per cent) and the dbrB tumor (85.5 per cent) is taken into account.

The higher glucose-1-phosphate level, the critical ratio being +2.8, in the dbrB tumor suggests a more rapid rate of glycogen breakdown in this tumor. Further, it was noted that the dbrB tumor, as compared to the C3H tumor, had a relatively lower level of inorganic phosphorus, the critical ratio being about —3, and a relatively higher level of adenosine triphosphate, the critical ratio being about +5. These findings strongly suggest that a higher energy level is available for vital function of the dbrB tumor. The observations made in this study are in accord with those previously reported (5—9), showing that, although these mammary tumors are morphologically similar, the dbrB tumor grows more rapidly and has a higher rate of oxygen uptake than the C3H.

It is of interest to note in the table the almost similar mean values of lactic acid, which is 98.8 mg/100 gm ± 4.65 S.E.M. for the C3H tumor and 100.7 mg/100 gm ± 4.51 S.E.M. for the dbrB tumor. This relatively small difference in the amount of lactic acid present in the tumors in situ is not in agreement with in vitro determinations. As mentioned in the introduction, the dbrB tumor produces about 8 times as much lactic acid in vitro as the C3H tumor (8). How can this discrepancy be accounted for? The following explanation may be offered:

In the dbrB tumor, with its inherent greater rate of growth and richer vascularity, the removal of metabolites takes place at a faster rate. Therefore, the lactic acid produced diffuses into the blood stream at a faster rate than that of the C3H tumor. Reference is here made to the work of Cori and Cori (4).

Furthermore, it has been shown that tumors are rich in buffers and that the lactic acid is immediately neutralized to potassium lactate. The above accounts for the fact that the pH of tumor tissue is not lowered to the extent expected, owing to the high glycolysis—even after the glucose level is raised significantly by administering a high carbohydrate diet (10).

It follows, therefore, that a natural physiological mechanism, regulating the acid-base system and preserving the status quo in large part, exists in tumors. In fact, should it be otherwise, the tumor would be destroyed by its own glycolytic hyperactivity, and the whole organism would suffer from a pronounced hyperacidity. This is not the case. The relatively small increase in lactic acid in the blood of tumor-bearing individuals, animal and human, occurs only when the tumor is in a far advanced stage of growth and disintegration simultaneously sets in.

That the levels of the phosphorylated intermediates occurring in glycolysis of tumors are qualitatively and quantitatively similar to those occurring in normal differentiated tissues may be explained on the same basis—i.e., on the preservation of the acid-base balance, to a great extent, in tumors in vivo, permitting the whole glycolytic cycle to proceed as in normal differentiated tissues.

SUMMARY

Data are presented on the levels of intermediates in the glycolytic cycle for two mouse mammary adenocarcinomas, one of which is normally rapidly growing (dbrB) and the other relatively slowly growing (C3H). The pattern of compounds present corresponds to that found in normal differentiated tissues, except that the lactic acid level is considerably higher in these tumors.

The more rapidly growing mammary tumor (dbrB) shows significantly higher levels of glucose-1-phosphate, adenosine triphosphate, and possibly glycogen, and a lower level of inorganic phosphorus than those of the C3H mammary tumor. These findings are in agreement with other studies.
carried out with this tumor and strongly suggest that a higher level of energy is available for vital function in the dbrB tumor.

The significance of the in vitro and in vivo differences in the lactic acid production of the two mammary tumors is discussed.

REFERENCES

Phosphorylated Intermediates in Glycolysis of Analogous Mouse Mammary Tumors: I. Mouse Mammary Tumors of the dba and C3H Strains

Anna Goldfeder and Harry G. Albaum

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