Metabolic Properties of Mouse Transplantable Adenocarcinoma

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SUMMARY

Some metabolic properties of a new, spontaneous, transplantable adenocarcinoma originating from a C3H mouse submaxillary gland have been analytically compared to the normal gland. The major findings may be summarized as follows: (a) The oxygen consumption of tumor cells is 38% higher than that of the normal gland. (b) In anaerobic conditions the normal submaxillary gland shows a higher degree of glycolysis, while in the presence of air it is unable to glycolyze. (c) The presence of pyruvate and fumarate greatly increases the oxygen consumption of tumor homogenate, while for the normal gland homogenate their utilization is very low. The diphosphopyridine nucleotide requirement for oxidation of pyruvate and fumarate is practically similar for the tumor and the normal gland.

The data obtained are evaluated in terms of their comparative significance and a tentative interpretation of the findings is suggested.

INTRODUCTION

Three years ago a tumor mass spontaneously originating in the submaxillary gland was found in a mouse of strain C3H belonging to the colony raised in this laboratory. Histologic examination revealed this mass to be an adenocarcinoma. The tumor was transplanted subcutaneously using suspensions of whole intact cells. A tumor cell line was thus established which for 3 years did not lose its original histomorphologic character. Upon "stabilization" of this tumor a series of investigations concerned with the morphologic, ultrastructural, virologic, and biochemical aspects of this adenocarcinoma have been carried out. In this paper an experimental investigation has been pursued in an endeavor to determine the metabolic differences between normal submaxillary gland cells and the cells of this tumor.

MATERIALS AND METHODS

Salivary gland tumors were excised 12 days after their transplantation. The tissues, freed from necrotic parts, were cut into thin slices according to the usual technics (2). The Warburg direct method was employed for measurements of respiration and glycolysis. Tissue slices were placed in the main compartment of the manometric flask with 2.8 ml of Ringer bicarbonate buffer, while 0.20 ml of 25% NaOH was added to the center well. NaOH was omitted and Ringer bicarbonate buffer with 73 μmoles of glucose/3 ml was employed for studies concerned with the measurement of glycolysis. The experiments were performed at a temperature of 37°C, and the gas phase was represented by air for the aerobic and by a mixture of argon + 5% CO₂ for the anaerobic measurements. After 10 minutes for thermal equilibration, the stopcocks were closed and the zero time period was recorded. At the end of the experiment, all the slices were collected and dried for 3 hours at 110°C for dry weight determination.

Other experiments were performed with tissue homogenates and the homogenization was carried out using 0.25 m sucrose for the measurements of the oxygen consumption and 0.154 m KCl for glycolysis. The final concentration of the homogenate was 10%. The reaction system for Krebs cycle oxidation (1) was composed as follows: 3.0 ml contained 30 μmoles of KH₂PO₄, pH 7.3; 6 μmoles of sodium fumarate; 6 μmoles of sodium pyruvate; 12 μmoles of MgCl₂; 3 μmoles of sodium ATP; 485 μmoles of sucrose. In the case of glycolysis measurements, the reaction mixture (3) was: 7.2 μmoles potassium phosphate, pH 7.5; 75 μmoles of NaHCO₃; 120 μmoles of nicotinamide; 1 μmole of sodium ATP; 6 μmoles of hexose diphosphate; 0.6 μmoles of diphosphopyridine nucleotide (DPN); 30 μmoles of glucose; 20 μmoles of MgCl₂; 15 μmoles of sodium pyruvate; 30 μmoles of NaF; a final volume of 3.0 ml.

RESULTS

Comparative values of the oxygen consumed by the tumor and the normal gland slices are reported in Table 1. It is of interest to note that the respiration of tumor slices is higher than 38%. Noteworthy is the total absence of aerobic glycolysis of the submaxillary gland. In regard to the data obtained in anaerobic experiments, it was found that normal submaxillary gland tissue showed a 42% increase in glycolysis.

Tumor homogenates which underwent oxidations of the Krebs cycle indicated that the oxygen consumption of pyruvate and fumarate, used as substrates, was higher than that utilized by normal salivary gland homogenate.

Under the experimental conditions adopted, it is worthy to notice that the oxygen consumption of the homogenates, in cases both of tumor and normal glands, was lower than that previously reported for the slices. This finding is a function of many variables related to the composition of the medium which are capable of influencing respiration, such as the different ionic compositions and the increased concentration of

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phosphates. The rate of oxygen consumption of the tumor homogenate was very high in the presence of the substrates and appeared to be a function of time (Chart 1). When the substrates were not added, the oxygen consumption was low and it increased slowly with time. This behavior is very similar to that observed for the normal gland homogenate both in the presence and in the absence of substrates.

In a series of experiments, the effects of the addition of DPN to the homogenates was investigated in an effort to characterize further the oxidation of the substrates of the Krebs cycle. It was noted that the DPN requirement for oxidation of pyruvate and fumarate is the same for the tumor and for the normal gland. In this respect the experimental data indicated that the addition of DPN consistently increased the oxygen consumption both in the absence and in the presence of the substrates. In regard to glycolysis, normal gland homogenate is unable to glycolyze aerobically, while in anaerobic conditions the rate of glucose degradation is 300% higher than that of the tumor. In anaerobic conditions, the production rates of CO₂ by the tumor and submaxillary gland homogenates differed greatly (Chart 2). The analyses of the curves reported indicate different kinetics, the normal gland producing about two-thirds of CO₂ in the first 10 minutes. The CO₂ production by the tumor homogenate increased regularly as a function of time, although the value is one-third of that calculated for the normal gland homogenate, if considered at the end of the determination.

**DISCUSSION**

The metabolic activity of this new spontaneous submaxillary gland adenocarcinoma in terms of

\[ Q_{O_2}, Q_{CO_2}^{O_2}, \text{ and } Q_{CO_2}^{O_2} \]

appears to be of the same order as that described for other spontaneous mouse neoplasms with histologic characteristics similar to those of the tumor under investigation. The difference between the metabolism of the adenocarcinoma and that of the normal gland are relevant if it is considered that, in the course of its evolution, the tumor retains its histogenetic derivation from the normal gland parenchyma. The differences observed are both qualitative and quantitative, the former being related to the conspicuous aerobic glycolysis present in the tumor and absent in the normal gland. The quantitative differences refer to the greater value of CO₂ of the tumor, while anaerobic glycolysis is notably lower.

The validity of such an experimental model, consisting of a tumor and its original tissue, is also clearly indicated by the data obtained concerning the utilization of certain substrates of the Krebs cycle. In fact, the addition of pyruvate and fumarate to the normal gland homogenate decreases their utilization, a finding contrary to that observed for the tumor. This reduced utilization in the tumor may perhaps be related to the fact that in normal tissue a concentration of pyruvate and fumarate already exists, and it may be too high to saturate the greater part of the enzyme systems. On the other hand, in the case of the tumor, the addition of pyruvate and fumarate induces a considerable increase in the consumption of oxygen.
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It is worthy to note that the data reported for the utilization of pyruvate and fumarate by the tumor and by the normal gland are to be considered a particular case, when compared with certain data in the literature (4, 5). Emphasis should be placed not so much on the considerable power of utilization by the tumor as on the reduced capacity of the normal gland, which may be set apart from other glandular tissues; these, as is well-known, avidly utilize most substrates, including those present under our experimental conditions.

An important conclusion which may be drawn from the present study is that the tumor studied does not seem to possess a low oxidative reserve in the sense that its oxidative capacity, though already sufficiently high, is capable of responding to the addition of substrate with an increase in the consumption of oxygen.

It may be concluded that the normal mouse submaxillary gland and spontaneous mouse adenocarcinoma both represent metabolic entities of a particular interest, to which more detailed investigations should be devoted.

REFERENCES

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