The Relative Metabolism *in vitro* of Analogous Mammary Tumors*

I. Oxygen Uptake and Aerobic Glycolysis of Mammary Tumors Autogenous to dba and C_3H Strains of Mice†

ANNA GOLDFEDER, D.Sc., M.U.C.

WITH THE TECHNICAL ASSISTANCE OF ANNABELLE COHEN, M.S. AND MONTAGUE LANE, B.S.

(From the Cancer Research Laboratory, Department of Hospitals, City of New York, and from the Department of Biology, Graduate School of Arts and Science, Washington Square College, New York University, New York)

During the course of investigations on the relative radiosensitivities and rates of growth of two analogous mammary tumors autogenous to two inbred strains of mice (dba and C_3H), it became apparent that an investigation of their relative metabolic properties would be of value.

Observations on the relative values of oxygen uptake, respiratory quotient, and aerobic glycolysis of tissues of these two analogous mammary tumors are reported in this paper. To the author's knowledge, no such data are available in the literature on these tumors.

The author has demonstrated in previous reports (1, 2) that the two analogous mammary tumors, both diagnosed as adenocarcinomas, differed widely in their rates of growth and radiosensitivities. Since the results obtained in these previous experiments have some bearing on the observations made in the present study, a résumé of the results will be given. For the sake of brevity, the mammary tumors of the dba and C_3H strains of mice will hereafter be referred to as the dbrB and C_3H tumors, respectively.

Summary of previous experiments.—The mammary tumor of the C_3H strain of mice, which had been transplanted from host to host of the same strain, had a latent period of about 14–16 days. (The latent period is the time elapsing between the implantation of a tumor graft and the appearance of a tumor of measurable size.) This tumor killed the animal host within 3 months. The mammary tumor of the dba strain, designated dbrB tumor, which had also been transplanted from host to host, proved to have a latent period of only 4–6 days. It resulted in the death of the host within 3 weeks.

Implants of the C_3H tumor, approximately 2×2 mm. in size, required a dose of about 2,800 r to prevent their growth upon implantation to hosts of the same strain; while implants of the dbrB tumor of similar size required a dose of about 5,000 r to prevent their growth in hosts of the dba strain (1). Explants of the C_3H tumor, also about 2×2 mm. in size, required a dose of about 130,000 r to prevent their growth *in vitro;* while for dbrB tumor particles of similar size, a dose of about 80,000 r was sufficient to produce the same effect (2).

That a larger dose of irradiation is needed to prevent cellular growth *in vitro* than *in vivo* has been observed by several other investigators as well as by the author (3–9).

An observation of particular interest was that the C_3H tumor was more radiosensitive *in vivo* than the dbrB tumor, while the dbrB tumor was more radiosensitive *in vitro* than the C_3H tumor. The significance of this observation will be discussed later.

METHODS

In the present study on the relative values of the oxygen uptake, respiratory quotient, and aerobic glycolysis of the analogous dbrB and C_3H tumor tissues, the experimental procedure was essentially the same as that described by the author in previous publications (10, 11). It is mainly based on the direct method of Dickens and Simer (13).

* Supported by grants obtained from the National Cancer Institute, U.S.P.H.S., and from the Damon Runyon Fund.

† This paper was read by title at the 40th annual meeting of the American Association for Cancer Research, held at Detroit, in April, 1949.

Received for publication, August 27, 1949.

Because of the high glycolysis of the tumors, the phosphate buffer solution,[1] which usually has a pH of 7.4. was adjusted to a pH of 7.5. Glucose 0.2 per cent was used as substrate. The lactic acid that had been produced during the experiment was determined by the method of Friedman and Greasser (12). The $Q_G^{O_2}$ in the tables is the value of the lactic acid produced by 1 mg. of tissue during 1 hour, expressed in micrograms. The water content of the tumors in each experiment was determined by drying the tissue at 110° C. to constant weight. The final calculations were made on a dry-weight basis. Sections of the tumors of each experiment were fixed for histological studies.

The O_2 uptake, the respiratory quotient, and the aerobic glycolysis were then determined on the same portion of tissue. This procedure is particularly essential for experiments with tumor tissue in order to obtain reproducible results.

RESULTS

In Table 1 the values for the O_2 uptake, the R.Q., and lactic acid produced by the C_3H tumor tissue in 1 hour are recorded. Each figure represents a mean of the results obtained from six pairs of Warburg flasks. All values were calculated on a dry-weight basis. The CO_2 values obtained in seven experiments averaged −3.6, the lowest value being −3 and the highest, −4.4. The R.Q. values averaged 0.86, the lowest being 0.80 and the highest, 0.98. The $Q_G^{O_2}$ values averaged 8.5, the lowest being 7.0 and the highest, 9.9.

TABLE 1

OXYGEN CONSUMPTION, AEROBIC GLYCOLYSIS, AND RESPIRATORY QUOTIENT OF THE C_3H MAMMARY TUMOR*

Exp. no.	Q_{O_2}	$Q_G^{O_2}$	R.Q.
1	−3.7	9.9	0.89
2	−3.4	9.2	0.85
3	−3.1	8.4	0.86
4	−3.9	7.8	0.84
5	−4.4	7.0	0.83
6	−3.4	9.9	0.98
7	−3.0	7.7	0.80
Av.	−3.6	8.5	0.86

* The medium contained 0.2 per cent glucose Ringer phosphate solution, buffered to pH 7.4–7.5 at 37.5° C. Each value represents a mean of six paired Warburg flasks.

Table 2 contains the values for Q_{O_2}, R.Q. and $Q_G^{O_2}$ obtained from the dbrB tumor in six experiments. Here again each figure represents the mean of the results obtained from determinations from six pairs of vessel-manometers. The $Q_G^{O_2}$ values averaged −5.5, the lowest being −4.6 and the highest, −6.6. The R.Q. averaged 0.80, the lowest being 0.71 and the highest, 0.90. The $Q_G^{O_2}$ values averaged 24.3, the lowest being 19.1 and the highest 27.3. A comparison of the Q_{O_2} and the $Q_G^{O_2}$ for the two tumors is presented in Figure 1.

The Q_{O_2}, R.Q., and $Q_G^{O_2}$ values obtained in individual experiments, as recorded in Tables 1 and 2, differed within a rather narrow range. This is noteworthy, particularly concerning tumors, since they usually show a wide range of values for these three factors. Crabtree (20), in his experiments on tumor metabolism, found a great divergence in values of respiratory exchange even among tumors of the same strain. For example, for sarcoma 180 he reported Q_{O_2} ranging from −10.6 to −30.9. That the results in the present study differed within a narrow range may be due to the fact that the transplanted tumors used were of the same age; the dbrB tumors were about 8 days old, and the C_3H tumors about 3 weeks old.

TABLE 2

OXYGEN CONSUMPTION, AEROBI GLYCOLYSIS AND RESPIRATORY QUOTIENT OF THE DBRB MAMMARY TUMOR*

Exp. no.	Q_{O_2}	$Q_G^{O_2}$	R.Q.
1	−5.9	22.3	0.71
2	−4.6	25.6	0.80
3	−6.6	27.3	0.75
4	−5.1	26.0	0.82
5	−6.2	25.6	0.90
6	−4.8	19.1	0.79
Av.	−5.5	24.3	0.80

* The medium contained 0.2 per cent glucose Ringer phosphate solution buffered to pH 7.4–7.5, at 37.5° C. Each value represents a mean of six paired Warburg flasks.

Histological examination of sections of the tumors showed them to consist of about 90–95 per cent intact cancer cells, many of which were in active mitosis.

Comparison of the data in Tables 1 and 2 shows that for the dbrB tumor, the values for O_2 uptake and aerobic glycolysis are consistently higher than the corresponding values for the C_3H tumor. The difference in rate of metabolic activities of the two mouse mammary tumors can be noted at a glance in the accompanying graph.

DISCUSSION

As shown in the experiments herein reported, the rates of respiratory activity and aerobic glycolysis of the dbrB tumor are greater than those of the C_3H tumor. In the case of these two mammary tumors a relationship between metabolic activity and rate of growth is evident. This finding does not always hold true, however, since several investigators have noted the independence of metabolism and tumor growth. Murphy and Hawkins (14), for example, found that respiration and glycolysis of spontaneous tumors were independent of growth.

[1] The author found that the use of this medium yielded more reproducible results than the medium containing bicarbonate, although the latter permits the work to be carried out under more physiological conditions.

Moreover, other investigators (15, 16), as well as the author (10, 17), have noted that growth may be inhibited without exerting an effect on respiration and glycolysis. However, in the present experiments, it may be said that the rates of growth of the dbrB and C_3H tumors correspond proportionately to their metabolic activities. Thus, the faster-growing tumor, dbrB, having a greater rate of growth, also has a higher rate of glycolytic activity.

Since the latent period of the dbrB tumor is about 4–5 days, and since that of the C_3H tumor is about 15–16 days, the ratio of their latent periods is approximately 1 to 3. The average $Q_{G^2}^{O_2}$ value of the dbrB tumor is 24.3 and that of the C_3H tumor 8.5 (see Tables 1 and 2), and the same ratio also prevails, although in reverse. It is seen, therefore, that the rates of growth and aerobic glycolysis of these two analogous mammary tumors are in the ratio of 1 to 3.

The difference in the relative metabolic activities of these tumors appears to have some bearing on their radiosensitivities.

The results of previous studies (1, 2) indicated a relationship between the rate of growth and the radiosensitivities of the dbrB and C_3H tumors. It is of interest therefore to correlate the values of the metabolic rate as obtained in the present experiments, with the radiosensitivities. As previously stated, implants of the C_3H tumor (2–3 mm. in diameter) required a dose of about 2,800 r to prevent their growth upon implantation into hosts of the same strain, while implants of the dbrB tumor of the same size required about 5,000 r to obtain the same effect. In a report of subsequent studies on these analogous tumors (18), experiments are described in which intact tumors of both strains, about 16 mm. in diameter, were treated *in situ*. A dose of about 8,000 r was required to produce total regression of the C_3H tumors, while for the dbrB tumor a dose of about 24,000 r was required to produce the same effect. Thus, the radiosensitivities of the C_3H and dbrB tumors when grown *in vivo* were in the ratio of 1 to 3. From the results recorded in Tables 1 and 2, it is seen that the relative aerobic glycolyses of the two tumors are in the proportion of 1 to 3 (8.5–24.3).

The above observations on the relationship between growth rate, metabolic activity, and radiosensitivity may be coincidental. Nevertheless, if confirmed by experiments on other types of tumors, this relationship might be significant in the classification of tumors with regard to radiosensitivity and metabolic activity.

It has been stated by the author (18) that the metabolic activity of tumors may serve as a better basis in evaluating their radiosensitivity than their purely morphological characteristics. In support of this view the following explanation was postulated to explain the greater resistance to x-rays of the faster-growing dbrB tumor when grown *in situ* as compared to the slower-growing C_3H tumor: "The toxic substances produced by radiation are eliminated more quickly in the faster growing dbrB tumor, therefore recovery takes place sooner, and additional dosages of radiation are necessary, while the slower growing C_3H tumor permits the accumulation of toxic substances produced by radiation. The effect of radiation is then greater; therefore, less dosage of radiation is necessary." The relative metabolic rates of the two analogous mammary tumors (C_3H and dbrB) obtained in the present study, when considered with their relative rates of growth and relative radiosensitivities, seem to support the above view.

Since, to the author's knowledge, no data on the metabolic properties of the two mammary tumors (C_3H and dbrB) are available in the literature, it was of interest to compare the findings reported herein with those observations made by other investigators on other types of tumors.

In Table 3 selected data are compiled from re-

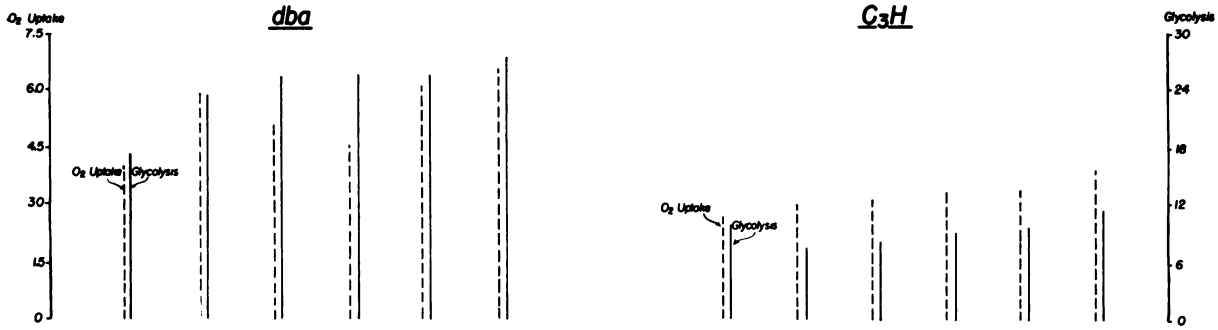

FIG. 1.—This graph demonstrates the difference in metabolic rate between the two mammary adenocarcinomas of the C_3H and dba strains of mice as measured by oxygen consumption and glycolysis of tissue slices in vitro. (Barcroft-Warburg manometric technic). The dotted lines represent the O_2-uptake, and the solid lines represent the glycolysis. Note that the metabolic rate of the mammary tumor of the dba strain is about three times as high as that of the mammary tumor of the C_3H strain.

ports published by some of these investigators. The values obtained in the present experiments are in general agreement with those obtained by other investigators, namely, the R.Q. is lower than 1.0 and the aerobic glycolysis is relatively high. Both of these factors are characteristic of tumors. With regard to oxygen uptake and aerobic glycolysis the dbrB tumor resembles the Flexner-Jobling rat carcinoma (19); the C_3H tumor most closely resembles the Yale mouse carcinoma I (21) in regard to the aerobic glycolysis, but it possesses a comparatively lower rate of respiration.

SUMMARY

The relative rates of respiration and aerobic glycolysis of mammary adenocarcinomas which arose in two inbred strains of mice, dba and C_3H, have been considered in the present study. The mammary tumor of the C_3H strain was found to have an average Q_{O_2} of -3.6 in 100 per cent oxygen, an average aerobic glycolysis ($Q_G^{O_2}$) of 8.5, and an average R.Q. of 0.86. The mammary tumor (dbrB) of the dba strain proved to have an average Q_{O_2} of -5.9, an average aerobic glycolysis of 24.3, and an average R.Q. of 0.80.

The relatively greater metabolic activity of the dbrB tumor corresponds to its relatively greater rate of growth as compared with that of the C_3H mammary tumor. The two mammary tumors, although histologically almost identical, proved to differ in their physiological characteristics: rate of growth, radiosensitivity, and metabolic activity. On the basis of observations made in previous and present studies on these mouse mammary tumors, it may be inferred that histological appearance alone is not a sufficient guide for the classification of tumors. Their physiological characteristics might be of greater value in such a classification.

The significance of the metabolic rate of these tumors in relation to their radiosensitivities is discussed.

Experiments to determine the metabolic properties of these tumors are now in progress.

TABLE 3

COMPARISON OF RESPIRATION AND GLYCOLYSIS OF THE DBRB AND C_3H MAMMARY TUMORS WITH THOSE OF OTHER TUMORS

Tumor	Species	Q_{O_2}	$Q_G^{O_2}$	Reference
dbrB	mouse	− 8	24.3	Present experiment
C_3H	mouse	− 3.6	8.5	Present experiment
Flexner-Jobling carcinoma	rat	− 7	25.0	Burk (19)
Jensen's sarcoma	rat	− 9	18.0	Crabtree (20)
Rous sarcoma	chicken	− 5	20	Burk (19)
Tar carcinoma 2146	mouse	−20	15	Crabtree (20)
Cracker sarcoma	mouse	−16	17	Crabtree (20)
Sarcoma 37	mouse	−14	12	Crabtree (20)
Yale carcinoma I	mouse	− 7	7	Belkin and Stern (21)

REFERENCES

1. GOLDFEDER, ANNA. Further Studies on the Relation between Radiation Effects, Cell Viability, and Induced Resistance to Malignant Growth. IV. Comparison of Effects of Roentgen Rays on Mammary Tumors Autogenous to Inbred Strains of Mice (dba and C_3H). Radiology, **49**: 724–32, 1947.
2. GOLDFEDER, ANNA, and CAMERON, GLADYS. Growth in Tissue Culture of Analogous Mouse Mammary Carcinomas and Their Response to Radiation. Cancer Research, **8**:465–71, 1948.
3. RUSS, S., and SCOTT, G. M. The Effects of X-rays upon Rous Chicken Tumor. Lancet, **2**:374–75, 1926.
4. SPEAR, F. G. The Delayed Lethal Effect of Radium on Tissue Culture *in vitro*. Proc. Royal Soc., London, s.B., **108**: 190–95, 1931.
5. MOPPETT, W. A Note of Radiosensitivities of Normal and Malignant Cells. J. Cancer Res., Comm. Univ., Sydney, **4**:95–97, 1932.
6. SUGIURA, K. Studies on Radiosensitivity of Sa-180 Irradiated *in vivo* and *in vitro*. Radiology, **29**:352–61, 1937.
7. GOLDFEDER, ANNA. The Lethal and Sub-lethal Dose of X-rays and Radium. Radiology, **31**:75–80, 1938.
8. GREGORI, A. Wirkung von Roentgenstrahlen auf das Knochenmark *in vivo* und *in vitro*. Strahlentherapie, **65**: 163–72, 1939.
9. DOLJANSKI, L., and GOLDHABER, G. Radiological Studies in Tissue Culture. Growth, **6**:235–49, 1942.
10. GOLDFEDER, ANNA, and FERSHING, JENNINGS L. The Effect of Radiation on Cell Respiration. Radiology, **31**:81–88, 1938.
11. GOLDFEDER, ANNA. Respiratory Changes *in vitro* in Normal and Malignant Tissue following Irradiation. Am. J. Cancer, **36**:603–8, 1939.
12. FRIEDMAN, T. E., and GRAESSER, J. B. The Determination of Lactic Acid. J. Biol. Chem., **100**:291–308, 1933.
13. DIXEN, MALCOLM. Manometric Methods. Cambridge: Cambridge University Press, 1934.
14. MURPHY, J. B., and HAWKINS, J. A. Comparative Studies on the Metabolism of Normal and Malignant Cells. J. Gen. Physiol., **8**:115–30, 1925.
15. BOYLAND, E., and BOYLAND, M. E. The Independence of Tissue Respiration and Glycolysis, and the Growth Rate of Tumors. Biochem. J., **33**:618–25, 1939.
16. SUMMERSON, W. H. Personal communication.
17. GOLDFEDER, ANNA. Experimental Serum Studies. II. Effect of Experimental Serum on Cell Respiration *in vitro* of Sarcoma 180. Am. J. Cancer, **29**:344–54, 1937.
18. ———. Anomalous Radiosensitivities of Analogous Mouse Mammary Carcinomas. Radiology, (in press).
19. BURK, D. On the Biochemical Significance of the Pasteur Reaction and Meyerhof Cycle in Intermediate Carbohydrate Metabolism. Cancer Problem Symposium, Science (suppl.), **85**:121–61, 1937.
20. CRABTREE, H. G. Observations on the Carbohydrate Metabolism of Tumors. Biochem. J., **23**:536–45, 1929.
21. BELKIN, M., and STERN, K. G. Metabolism of Yale Carcinoma I. Cancer Research, **3**:164–67, 1943.

The Relative Metabolism *in vitro* of Analogous Mammary Tumors: I. Oxygen Uptake and Aerobic Glycolysis of Mammary Tumors Autogenous to dba and C3H Strains of Mice

Anna Goldfeder, Annabelle Cohen and Montague Lane

Cancer Res 1950;10:89-92.