Respiration and Glycolysis of Cells Transformed with 4-Nitroquinoline-1-Oxide and Its Derivative (34776)

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An increased aerobic glycolysis (1, 2, 4) and an inhibition of respiration by glucose addition [the Crabtree effect] (3) are the most commonly reported alterations in malignant cells. This increased aerobic glycolysis is observed not only in many established strains induced by chemical carcinogens *in vivo* but also in transformed cells induced by viruses *in vitro* (5–8) as well as *in vivo* (9–10).

Recently in our laboratory, a transformation system for hamster embryonic cells (HE cells) with 4-nitroquinoline-1-oxide and its derivatives was established (11, 12). Therefore, an attempt to compare the glucose metabolism of nontreated HE cells with that of these transformants was made to observe if an increase in aerobic glycolysis and appearance of the Crabtree effect would be accompanied with even *in vitro* chemical carcinogenesis.

Materials and Methods. Three transformants from HE cells and nontreated HE cells, as control cells, were used in this study. Histories and characteristics of the cells are summarized in Table I. The detailed methods and procedures of transformation were described elsewhere (12). The culture medium consisted of Eagle's minimum essential medium supplemented with 1 m*M* pyruvate, 0.2 m*M* serine, and 10% calf serum. The exponentially growing cells ($5–10 \times 10^7$ cells cultured in 6–8 Roux bottles), were harvested with 0.025% pronase in PBS, followed by washing twice with Krebs-Ringer-phosphate buffer (pH 7.4) (Ca^{2+}-free), packed by centrifugation at 600*g* for 2 min, and resuspended in the same buffer. Incubation was carried out in air according to the procedures described elsewhere (13). Oxygen consumption was measured manometrically. The production of $^{14}CO_2$ was measured as described by Tsuiki and Kikuchi (14). Lactate was determined by the method of Barker and Summerson (15).

Results and Discussion. The results of rep-

TABLE I. Summary of the Histories and Characteristics of the Cells Used.

Cells	Carcinogens[a]	Days and generations after treatment with carcinogen	Malignancy (stage)[b]
HE	None	12 days, 3 gen.	—
HA-7	$10^{-5}M$ 4HAQO	142 days, 7 gen.	+ (M2)
HA-2	$10^{-5}M$ 4HAQO	118 days, 12 gen.	+ (M3)
NQ-19/Cl.4	$10^{-6}M$ 4NQO	163 days, 17 gen.	+ (M3)

[a] 4HAQO: 4-hydroxyaminoquinoline-1-oxide HCl; 4NQO: 4-nitroquinoline-1-oxide.

[b] M1, M2, and M3 indicate the stage of malignancy of transformed cells. At stage M2, the tumor developed progressively after a long latent period after host transplantation, while at stage M3, the tumor growth began soon after the transplantation and never regressed. Details were reported elsewhere (12).

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TABLE II. Oxygen Consumption and Glucose Metabolism of HE Cells and the Transformants.[a]

		HE cells	HA-7	HA-2	NQ-19/Cl.4
O_2 consumed	None (1)	2.37	1.85	1.61	1.33
	Glucose (2)	2.35	1.92	2.03	1.18
	Pyruvate (3)	2.53	2.34	3.00	NE[b]
	Glucose, pyruvate (4)	2.62	2.18	2.24	NE
Crabtree effect	(2)/(1)	1.00	1.04	1.25	0.89
	(4)/(3)	1.03	0.93	0.74	/
Lactate formed	Glucose	1.30	3.16	8.77	8.35
$^{14}CO_2$ formed	Glucose-1-^{14}C	0.069	0.064	0.125	0.091
	Glucose-6-^{14}C	0.015	0.015	0.040	0.020
	6-C/1-C	0.22	0.23	0.32	0.22

[a] All the substrates were 10 m*M*; the values are expressed as μmoles/hr/10 mg dry weight (about 2×10^7 cells).

[b] Not estimated.

resentative experiments are presented in Table II. When no substrate was added, oxygen consumption (endogenous respiration) was lower in the transformants than in the control cells. In the transformants, the rate of the endogenous respiration declined with time, apparently owing to decrease of endogenous substrates. In the presence of pyruvate, a fuel substance of the TCA cycle, oxygen consumption continued to proceed at a constant rate, and was higher in transformants than in control cells, suggesting that respiratory capacity was not reduced in these transformants. In virus-transformed cells, the reduction of respiration was described in some reports (5, 10), but was not observed in another report (6). Accordingly, this phenomenon seems to be not so characteristic of malignant cells as compared with an increase in aerobic glycolysis described below. When exogenous substrates were absent, the transformants except for NQ-19/Clone 4 did not exhibit the Crabtree effect. This is probably due to the low endogenous respiration in these cells, since the typical Crabtree effect was observed in the presence of pyruvate. The control cells did not show this effect in the presence as well as absence of pyruvate. Of the data shown in Table II, the most remarkable change observed in the transformants was an increase in aerobic glycolysis: aerobic lactate production was 3–9 μmoles for the transformants as compared with 1.3 μmoles for the control cells under the same conditions. In addition, the rate seemed to be correlated with the grade of malignancy. The $^{14}CO_2$ ratio of C-6:C-1 has been considered as a useful index for the evaluation of the participation of the TCA cycle or the pentose phosphate shunt in total glucose oxidation. The ratio is known to decrease in many tumors [suggesting greater participation of the shunt] (16). In our experiments, the ratio found for HE cells was low, and no significant difference was observed between the control cells and the transformants. Under comparable conditions, Ehrlich ascites tumor (13) and Yoshida ascites hepatomas (17) gave values ranging from 0.05 to 0.01. The ratio found for the *in vitro* transformants are, thus, higher than those for the ascites hepatomas, and this could be related to the lower inhibition of respiration by glucose in these transformants as compared with the ascites tumors.

Although the increased aerobic glycolysis is reported to be a characteristic of many malignant cell strains, its significance has been in much dispute. However, there are some reports which suggest that the rate of glycolysis increases with the rate of cell multiplication in virus-transformed cells (7, 8) as well as in transplantable rat hepatomas (18–20). Our experiments show that the transformation and development of malignancy induced *in vitro* with the chemical carcinogens are accompanied by an increase in aerobic glycolysis. Furthermore, the increase appears to be correlated with the grade of malignancy. Thus, the results may support the view (5–8) that the increased aerobic glycolysis can be a simple biochemical indicator of neoplastic conversion.

In the present experiments, the need for relatively large amounts of the transformed cells made it unable to use them in the earliest stage. If aerobic glycolysis increases as a sole consequence of long-term culture, there still remains the possibility that the transformation by the chemical carcinogens is not a sole cause of increased aerobic glycolysis reported above. However, Sanford *et al.* (7) reported that the glycolytic activities of the untreated hamster embryo cells did not increase at least until the 240th culture day. As the cells used in our experiments were maintained under similar conditions, the increased aerobic glycolysis could be attributed to the treatment with chemical carcinogens.

Summary. Hamster embryonic cells transformed ***in vitro*** with 4-nitroquinoline-1-oxide and its derivative exhibited an increased aerobic glycolysis and an inhibition of respiration by glucose addition in the presence of pyruvate (the Crabtree effect). These changes, which are known to be characteristic of many malignant cells strains, appear to be correlated with the grade of malignancy.

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Received Dec. 30, 1969. P.S.E.B.M., 1970, Vol. 134.