Respiration and Glycolysis of Earle's Strain L Cells.* (22517)

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In 1943 Earle's Strain L cells(6) after 2 years *in vitro* gave rise to 68% tumors when injected intramuscularly into C3H mice. The incidence of tumors produced in 1946, when cultures were injected, dropped to 1%. Recently it was reported that Strain L cells after 10 years *in vitro* (1950) produced 15% tumors in normal C3H mice and 64% tumors in mice that had been irradiated(13). A metabolic study made in 1942 on the *in vivo* tumors showed that their metabolism was similar to that which had been reported to be characteristic of malignant tissues(2). The purpose of the present work was to determine if the metabolism of Strain L cells today (1954-55) meets the metabolic criteria described by Burk for malignant tissues(2,3,17). No attempt will be made to compare metabolism of *in vitro* Strain L cells to that reported for *in vivo* Strain L tumors(3).

Materials and methods. Initial experiments indicated that about 2 million cells per 7 ml Warburg reaction vessel were necessary to get an accurate record of respiration. For this reason large cultures had to be maintained. Cells were grown on the glass floor of T-60 flasks(4) in culture medium composed of 40% horse serum, 40% Earle's solution and 20% chick embryo extract (50:50). Culture media were changed 3 times weekly. To prepare cells for a series of runs they were carefully washed with Krebs-Ringer-Phosphate solution(16) while still attached to the floor of T-60 flasks. The wash was discarded. A stainless steel nylon brush† was used to dislodge the cells from the flask and cell clusters were dispersed in Krebs solution. The number of cells in a sample was obtained by counting on a hemacytometer(15). Cell number was converted to dry weight in order to conform to terminology of most workers. Dry weight was determined for suspensions of the cells in Earle's solution in the following manner: the number of cells was determined, they were collected by slow centrifugation (500-1000 rpm), dried at 110°C and weighed. The mean weight of one million cells for 3 determinations was 0.46 mg. This value agrees with a calculated dry weight of 0.49 mg if one assumes a dry weight of 11.7%(8) and a mean cell diameter of 20 μ(12). The direct method of Warburg was employed to determine oxygen consumption and carbon dioxide production(16). For the latter determination, potassium hydroxide was omitted from the center well of the reaction vessel, and correction for carbon dioxide retention by the suspending solution was made. Lactic acid was determined by the chemical procedure of Barker and Summerson(1). Samples were run in pairs in an atmosphere of either oxygen or nitrogen. One sample was analyzed immediately after equilibrating for temperature on the Warburg respirometer; the other sample was analyzed one hour later. The difference between samples was taken as lactic acid formation or glycolysis. P-phenylenediamine measures the responsiveness of the cytochrome system in increasing its effectiveness above the natural endogenous rate of oxidation(11). This effect (P-PD), expressed as percent increase in Q_{O_2}, was obtained by adding p-phenylenediamine from the side arm of the reaction vessel so that the final concentration in the reaction vessel was 0.02 M.

Results. In previous experiments on 2-day-old cultures of Strain L cells(10) it had been noted that respiration was fairly high in neutralized horse serum(18), but at the beginning of the present study little or no oxygen uptake occurred in Krebs solution which contained no glucose (Fig. 1). It was desired to make determinations in a physiological saline solution, so that our work would be done under conditions similar to the earlier work on Strain L tumors(3). An isotonic solution of

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† Brush #61, nylon bristles with stainless steel handle, J. I. Holcomb Mfg. Co., Indianapolis.

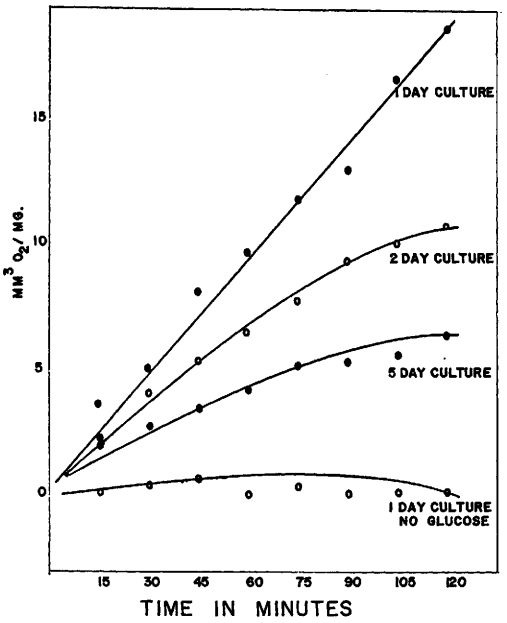

FIG. 1. Effect of media change and glucose on respiration of Strain L cells.

glucose was added to Krebs solution to bring the glucose concentration to 0.1%. Determinations were made in this solution, and respiration was found to be markedly increased by glucose (Fig. 1).

Repeat observations were not the same, and it was then discovered that degree of respiration was related to culture conditions. For example, respiration was found to be highest ($Q_{O_2} = 9.3$) one day following renewal of culture medium (Fig. 1). Respiration was considerably lower on the second ($Q_{O_2} = 6.6$) and fifth day ($Q_{O_2} = 4.3$) following change of culture medium. It was desired to use active viable cells and so only cultures which had been fed the previous day were used for subsequent determinations.

Results of 6 series of determinations are indicated in Table I. A series represents pooled one-day cultures on which all determinations were made at the same time. It was found that oxygen uptake was moderate ($Q_{O_2} = 9.4$) and the respiratory quotient unity. When p-phenylenediamine was added to a suspension of Strain L cells, oxygen uptake increased 251% ($Q_{O_2} = 33.7$). Both aerobic and anaerobic glycolysis was found to be low ($Q_{LA}^{O_2+CO_2} = 1.5$) ($Q_{LA}^{N_2} = 3.5$). Pasteur Effect was calculated by subtracting aerobic from anaerobic glycolysis and was found to be 2.0. Lactic acid formation was not greater than respiration and consequently fermentation excess ($Q_{LA}^{N_2} - 2Q_{O_2}$) was negative (−15.3).

Determinations of aerobic lactic acid formation were made under two conditions: one in which potassium hydroxide was placed in the center well of the reaction vessel and carbon dioxide absorbed, and another in which alkali was omitted and carbon dioxide allowed to accumulate. When carbon dioxide was absorbed, lactic acid formed aerobically ($Q_{LA}^{O_2} = 3.0$), was approximately equal to anaerobic formation ($Q_{LA}^{N_2} = 3.5$). In the presence of carbon dioxide produced by the cells, aerobic lactic acid formation was lower ($Q_{LA}^{O_2+CO_2} = 1.5$). As checked by the test for observations involving non-paired groups(7) there was a significant lowering of aerobic glycolysis due to carbon dioxide ($P<0.01$).

Discussion. The metabolic pattern which Burk(2) described as characteristic of most malignant tissues emphasized a moderate respiration, a respiratory quotient below unity, a very high anaerobic glycolysis and a high

TABLE I. Respiration and Glycolysis of Earle's Strain L Cells.

Series	Q_{O_2}	R.Q.	P-PD	$Q_{LA}^{N_2}$	$Q_{LA}^{O_2+CO_2}$	$Q_{LA}^{O_2}$
1	8.8 (4)	1.2 (4)		.7 (2)	1.2 (4)	.9 (4)
2	8.6 (2)	.9 (2)		3.4 (2)	.1 (3)	3.7 (4)
3	9.7 (3)	.9 (3)			3.3 (3)	5.1 (3)
4				5.2 (6)		
5	9.7 (3)					
6	9.6 (3)		251 (3)	1.9 (3)	1.5 (2)	2.9 (4)
Mean	9.4	1.0	251	3.5	1.5	3.0

All Q values are expressed as mm³/mg dry wt/hr.
Numbers in parenthesis indicate No. of determinations in each series.
P-PD is % increase in Q_{O_2} on addition of p-phenylenediamine.

aerobic glycolysis. Emphasis was placed also on the slight stimulation of respiration in tumor slices by addition of p-phenylenediamine. Respiration of normal tissues increases greatly (about 200%) when p-phenylenediamine is added.

Our findings indicate that the metabolism of Strain L cells at the present time does not fit Burk's pattern for malignancy. On the contrary, the over-all metabolism of Strain L cells now is more like that of normal tissues(9).

From studies on transplantation(5.6,13) it would appear that malignancy of Strain L cells has changed. They produced 15% tumors in 1950 in non-irradiated mice which was less than it was in 1942(13). Burk reported(17) that the metabolism of 2 lines of cancer cells grown *in vitro* by Sanford(14) paralleled differences in malignancy observed *in vivo*. In view of this evidence, the malignancy of Strain L cells transplanted to C3H mice should be very low if their metabolism *in vitro* parallels their malignancy *in vivo*. If this relation does not occur, then, Strain L cells may be an exception to the statement by Warburg that the respiration of cancer cells never returns to normal(17).

Under aerobic conditions, Strain L cells formed significantly less lactic acid when carbon dioxide was present. Probably the best explanation for this is that pyruvate in the presence of carbon dioxide can be converted to oxaloacetate.

Summary. Earle's Strain L cells were cultured in quantity, so that respiration and glycolysis could be adequately determined. Oxygen uptake was highest the day following renewal of culture medium. Little respiration occurred in the absence of glucose, respiration was moderate ($Q_{O_2} = 9.4$), respiratory quotient was unity (R.Q. = 1.0), anaerobic glycolysis was low ($Q_{LA}^{N_2} = 3.5$), aerobic glycolysis was low ($Q_{LA}^{O_2+CO_2} = 1.5$) and respiration was stimulated 251% when p-phenylenediamine was added. The metabolism of Strain L cells is like that of most normal tissue.

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