STUDIES ON MOUSE LEUKEMIA

XI. Metabolic Effects of Host Constitution

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The preceding study in this series, on the metabolism of cells of transmissible lymphatic leukemia (9), showed greater consistency in repeated observations than is reported by others for neoplastic tissue (1, 2, 5, 10). Since no other account has included a statement as to genetic uniformity of hosts, it seemed possible that the high variability of other results on tissue metabolism might be related to variability of hosts. The experiments herein recorded test directly the effects of genetically different host constitutions upon the metabolism of given lines of leukemic cells.

Material

The highly inbred strains of mice reported in earlier papers of this series by MacDowell and Richter (3, 4, 6) and two of the transmission lines of cells of lymphatic leukemia proved especially well suited for this study.

The experiments have been carried out with mice, 5 to 8 weeks old, from the 26th to 32nd generations of brother by sister matings of Strain C58 and from the 29th to 31st generations of brother by sister matings of Strain Storrs-Little. More than half of the mice from each strain came from the 30th generation. For observations on the normal metabolism of the lymph nodes, 48 mice from each strain were studied. Leukemic cells were used of Line I, Transfers 264–275, and of Line M-liver, Transfers 55–67. The metabolism of these cell lines growing in mice of Strain C58 has already been reported (8, 9).

The same doses of the same saline suspensions of cells were inoculated into mice of Strains Storrs-Little and C58. With cells of Line M-liver 100 per cent of 107 mice in Strain C58 died 3.5–6 days after inoculation, and 47 per cent of 59 mice in Strain Storrs-Little in 4–9 days. With Line I, 100 per cent of 52 mice died in 3.5–5 days in Strain C58, and 48 per cent of 60 mice in Strain Storrs-Little in 4–7 days.

Line M-liver was carried through Strain Storrs-Little for two and three transfers and then successfully transferred back to Strain C58 in 100 per cent of 16 mice. Line I, after one transfer through Storrs-Little mice was successfully returned to C58 hosts in 52 per cent of 25 mice.

Method

The method of observing metabolic rates used in this study is identical with that of the previous experiments (9). The oxygen consumption and aerobic and anaerobic glycolysis were measured with the Fenn modification of the Thunberg differential volumeter. Infiltrated nodes were taken at the time after inoculation when the infiltration of leukemic cells had become so extensive that the normal architecture of the gland had been completely obliterated. To determine the time required for this change, special histological studies were made with independent material with each of the lines in both strains during the course of these experiments. Judged by the degree of infiltration and number of mitotic figures in the involved areas, the distribution of lesions and the rate of growth of the inoculated cells are the same in Strain Storrs-Little as in Strain C58 for the first 3–5 days. In Strain Storrs-Little at this period no evidence was found of the necrosis and resorption of leukemic cells that subsequently appears in many cases in this strain in connection with recovery. This point is confirmed by cytological observations of tissues actually used in these experiments. From study of 50 sets of tissue taken from respirometers, no difference could be detected in staining reactions, occurrence of pycnotic nuclei, and degenerating cells from tissue fixed at death; and leukemic tissues from Strains C58 and Storrs-Little do not differ. Thus strain differences in the amount of living infiltrations due to unexpectedly early retrogression in Storrs-Little hosts are not involved in the general results.

The cervical, abdominal, and inguinal lymph nodes were studied. To obtain sufficient material for determination of the normal oxygen consumption, aerobic and anaerobic glycolysis, lymph nodes of four mice of the same sex and almost invariably from the same litter were combined. Each leukemic mouse usually gave sufficient material for a determination, although it was occasionally necessary to combine tissues from two or three mice. The nodes were immersed in glucose-free Ringer's solution and cut into slices 0.2–0.3 mm. thick and then weighed on a torsion balance accurate to 0.2 mg. 30–100 mg. of tissue, moist weight, were placed in each respirometer. The moisture content was estimated as 80 per cent and corrected by this factor (7).

The oxygen consumption was measured directly with the tissue in Ringer's solution of 0.9 per cent NaCl, 0.022 per cent KCl, and 0.0236 per cent $CaCl_2$, containing 0.2 per cent glucose. In some cases a phosphate buffer of pH 7.4 was used. The presence of the buffer did not influence the rate or duration of a constant oxygen use. CO_2 was absorbed by a 0.1 N NaOH solution placed in an inset. A control respirometer contained tissue immersed in glucose-free Ringer's solution to which 0.025 M $NaHCO_3$ was added. The solution was equilibrated with a gas mixture

of 5 per cent CO_2 and 95 per cent O_2 by bubbling the mixture through it for ½ hour. Then the gas mixture was run through the experimental bottle, after the tissue had been placed in the Ringer bicarbonate solution, for another 10 minutes. Flushing of the bottles with the gases was insured by having an outlet through a stop-cock attached to them. The excess aerobic and anaerobic glycolysis was measured with 0.2 per cent glucose in the Ringer-bicarbonate solution and by equilibrating the tissue and solution with 5 per cent CO_2 and 95 per cent O_2 for the aerobic, and 5 per cent CO_2 and 95 per cent N_2 for the anaerobic determinations. The pH in this bicarbonate system, according to the Henderson-Hasselbalch equation, is 7.44. The respirometers were shaken 140–160 times a minute through an excursion of 5 cm. The temperature was 37.5°C. ±0.005°C. At the conclusion of a metabolic determination the tissue in the respirometer was fixed in Flemming's fixative for microscopical study.

The results are expressed as follows:

$$Q_{O_2} = \frac{\text{c.mm. oxygen consumed}}{\text{mg. dry weight per hour}}$$

$$Q_{CO_2}^{O_2} = \frac{\text{c.mm. excess aerobic glycolysis}}{\text{mg. dry weight per hour}}$$

$$Q_{CO_2}^{N_2} = \frac{\text{c.mm. excess anaerobic glycolysis}}{\text{mg. dry weight per hour}}$$

RESULTS

In Table I are the individual determinations of the rates of oxygen consumption and aerobic and anaerobic glycolysis of the normal lymph nodes of Strains C58 and Storrs-Little. In Table II are the dates, transfer number, interval after inoculation, and individual determinations of the metabolism of lymph nodes of mice of Strains C58 and Storrs-Little inoculated with cells of Line M-liver. In Part A of this table are the observations for the line in Strain C58; in Part B the observations in Storrs-Little (the second column giving the number of transfers in Storrs-Little); and in Part C the metabolism of the cells on return to Strain C58 from Storrs-Little. Table III is similar to Table II; the results are concerned with Line I instead of M-liver. In Table IV, the first part is a summary of the means, their probable errors, and the standard deviations for these different lines of cells and strains of mice. The second part is a statistical analysis of the differences observed. Differences that are 3 or more times their probable errors are considered statistically significant. These

have been set in bold faced type. Fig. 1 represents the arithmetic means and their probable errors of the metabolic rates of leukemic cells of Lines I and M-liver, in Strain C58 and in Strain Storrs-Little.

TABLE I

Rates of Oxygen Consumption and Aerobic and Anaerobic Glycolysis of Lymph Nodes of Uninoculated Mice of Strains C58 and Storrs-Little— 6–8 Weeks Old

Q_{O_2}	$Q_{CO_2}^{O_2}$	$Q_{CO_2}^{N_2}$
Strain C58 uninoculated—48 mice used		
5.0	2.3	4.4
7.0	3.0	3.2
4.7	2.0	3.4
6.5	2.0	4.7
5.5	1.9	4.4
4.4	2.1	4.4
5.4	2.6	6.3
5.1	1.1	7.2
5.4	1.1	8.1
5.1	2.3	8.0
5.9	2.4	6.8
5.4	2.7	8.4
Strain Storrs-Little uninoculated—48 mice used		
5.8	1.5	5.5
6.0	2.4	3.9
6.6	1.1	5.7
5.5	3.1	6.3
6.5	2.4	6.4
5.5	3.0	6.3
5.0	2.0	7.1
5.4	1.4	6.9
5.6	1.5	5.4
5.6	3.6	8.5
5.1	2.8	8.9
4.9	2.8	7.1

These data and summaries reveal the following:

The Genetic Constitution of the Host Affects the Metabolism of Transmitted Leukemic Cells.—The five differences in bold faced type in the

TABLE II

Line M-Liver in Lymph Nodes of Hosts from Strains C58 and Storrs-Little

No. of successive transfers of Line M-liver in hosts of each strain at time of observation	Length of time after inoculation	Date	Metabolic rates of lymph nodes		
			Q_{O_2}	$Q_{CO_2}^{O_2}$	$Q_{CO_2}^{N_2}$
Part A. Hosts from Strain C58—24 mice used					
	days	*1933*			
C58 55	4	Jan. 19	7.5	7.9	19.4
" 55	4	" 19	4.9	7.2	18.4
" 56	4	" 23	6.9	6.8	23.7
" 56	4	" 23	6.0	9.8	19.3
" 57	4	" 27	5.2	8.8	21.2
" 57	4	" 27	5.7	9.9	19.1
" 57	4	" 27	5.5	7.7	17.5
" 58	4	" 30	5.4	9.4	20.1
" 58	4	" 30	5.6	10.6	21.5
" 58	4	" 30	6.7	6.0	16.1
" 63	4	Feb. 20	4.7	8.1	20.3
" 63	4	" 20	5.3	8.6	20.7
Part B. Hosts from Strain Storrs-Little—20 mice used					
C58 61 Storrs-Little 1	5	Feb. 12	6.1	4.0	11.0
" 61 " 2	5	" 16	6.4	4.7	13.5
" 61 " 1	5	" 18	6.3	5.2	16.1
" 61 " 1	5	" 18	6.1	5.4	14.9
" 61 " 3	5	" 22	5.4	4.5	10.1
" 66 " 2	5	Mar. 13	6.2	4.5	11.9
" 67 " 2	6	" 15	6.2	5.0	13.4
" 67 " 2	6	" 15	6.6	4.8	14.7
" 67 " 3	7	" 22	5.0	4.1	11.7
" 66 " 4	6	" 25	5.4	4.4	14.8
Part C. Hosts from Strain C58 following transfers in Storrs-Little—10 mice used					
C58 61 Storrs-Little 3 C58 1	6	Feb. 28	5.6	7.2	20.8
" 66 " 2 " 1	3	Mar. 16	5.5	7.8	19.5
" 67 " 2 " 1	5	" 20	5.6	10.1	20.7
" 66 " 3 " 1	4	" 23	5.0	7.8	20.0
" 67 " 2 " 1	5	" 27	5.2	10.9	20.7

second part of Table IV provide the experimental evidence supporting this proposition. Each of these differences is in the metabolism of a line of cells in hosts of different constitution.

TABLE III

Line I in Lymph Nodes of Hosts from Strains C58 and Storrs-Little

No. of successive transfers of Line I in hosts of each strain at time of observation	Length of time after inoculation	Date	Metabolic rates of lymph nodes		
			Q_{O_2}	$Q_{CO_2}^{O_2}$	$Q_{CO_2}^{N_2}$
Part A. Hosts from Strain C58—20 mice used					
	days	*1933*			
C58 264	3	Feb. 23	5.9	7.0	16.2
" 264	3	" 23	5.8	10.5	18.8
" 266	4	Mar. 3	4.9	8.4	20.2
" 268	4	" 10	5.9	7.6	20.4
" 272	4	" 24	5.0	8.4	16.0
" 272	4	" 24	5.6	7.4	15.0
" 273	3	" 27	5.4	8.6	19.0
" 273	4	" 28	5.1	6.2	18.2
" 275	3	Apr. 3	5.9	6.3	17.7
" 275	3	" 3	5.1	7.4	18.7
Part B. Hosts from Strain Storrs-Little—20 mice used					
C58 260 Storrs-Little 1	4	Feb. 10	6.2	2.7	7.5
" 260 " 1	4	" 10	6.1	1.9	10.5
" 260 " 1	5	" 11	7.1	2.5	7.9
" 264 " 1	5	" 24	6.3	3.0	9.7
" 264 " 1	5	" 24	6.6	3.2	11.2
" 264 " 1	4	" 25	6.4	5.0	11.3
" 269 " 2	5	Mar. 22	6.7	5.8	15.2
" 269 " 2	6	" 23	6.8	7.3	13.7
" 272 " 1	4	" 28	6.3	4.8	13.6
" 272 " 2	4	Apr. 1	6.6	8.2	
Part C. Hosts from Strain C58 following transfers in Storrs-Little—12 mice used					
C58 269 Storrs-Little 1 C58 1	4	Mar. 21	5.7	7.6	19.8
" 269 " 1 " 1	4	" 21	5.1	7.3	17.3
" 272 " 1 " 1	3	" 31		7.5	19.6
" 272 " 1 " 1	4	Apr. 1	5.5	7.3	25.2
" 273 " 1 " 1	4	" 5	6.0	6.5	19.0
" 273 " 1 " 1	5	" 6	5.7	6.0	19.6

However different the constitution of the hosts from these two strains may be, the metabolism of their lymph nodes before inoculation is not significantly different; as shown by this same table, the

TABLE IV

Summary of Tables I to III; Means, Standard Deviations, and Differences

Line of leukemic cells	Strain of hosts		Q_{O_2}		$Q_{CO_2}^{O_2}$		$Q_{CO_2}^{N_2}$	
			Mean	Standard deviation	Mean	Standard deviation	Mean	Standard deviation
Uninoculated	C58	(1)	5.45±0.14	0.70	2.13±0.10	0.52	5.78±0.35	1.80
"	Storrs-Little	(2)	5.63±0.09	0.46	2.30±0.15	0.76	6.50±0.25	1.30
M-liver	C58	(3)	5.78±0.16	0.83	8.40±0.26	1.32	19.78±0.36	1.84
"	Storrs-Little	(4)	5.97±0.10	0.49	4.66±0.09	0.43	13.21±0.40	1.86
"	C58 from Storrs-Little	(5)	5.38±0.07	0.24	8.76±0.44	1.46	20.34±0.15	0.51
I	C58	(6)	5.46±0.08	0.39	7.78±0.26	1.20	18.02±0.36	1.70
I	Storrs-Little	(7)	6.51±0.06	0.29	4.44±0.43	2.04	11.18±0.56	2.47
I	C58 from Storrs-Little	(8)	5.60±0.09	0.30	7.03±0.17	0.62	20.08±0.68	2.46

Comparison with same line of cells continuously in hosts of Strain C58		Difference	Difference / *P.E.* difference	Difference	Difference / *P.E.* difference	Difference	Difference / *P.E.* difference
Uninoculated	Storrs-Little (2) minus (1)	+0.18±0.17	1.06	+0.17±0.18	0.94	+0.72±0.43	1.67
M-liver	Storrs-Little (4) minus (3)	+0.19±0.19	1.00	**−3.74**±0.28	**13.36**	**−6.57**±0.54	**12.17**
"	C58 from Storrs-Little (5) minus (3)	−0.40±0.17	2.35	+0.36±0.50	0.71	+0.56±0.39	1.44
I	Storrs-Little (7) minus (6)	**+1.05**±0.10	**10.50**	**−3.34**±0.50	**6.68**	**−6.84**±0.67	**10.21**
I	C58 from Storrs-Little (8) minus (6)	+0.14±0.12	1.17	−0.75±0.31	2.42	+2.06±0.77	2.68

greatest difference in means (anaerobic glycolysis) is less than twice its probable error, and the others are only about equal their probable errors.

In hosts of Strain Storrs-Little compared with Strain C58, Line I has a higher rate of oxygen consumption, but lower rates of aerobic and anaerobic glycolysis; Line M-liver has lower rates of aerobic and anaerobic glycolysis.

The statistical significance of these five differences is unquestionable; one is nearly 7 times its probable error and the others range from 10 to 13 times their respective probable errors. Among the ten other comparisons given in this table, the closest approach to statistical significance is 2.68 times the probable error of the difference. These results, however significant in themselves, can be interpreted only on the basis of the conditions of the experiment. Upon these depends the evidence that the differences observed are not concerned with changes in the cell lines themselves, and that the differences between hosts are genetic.

Changes in the characteristics of a line have been found according to all criteria, clinical, gross autopsy, interval before death, distribution of lesions, cytological traits, and metabolism. The metabolic rates of a line may be constant over long periods of successive transfers in the same hosts and then show a change. Several months before these experiments were started Line I and M-liver in hosts of Strain C58 gave different metabolic rates, whereas in these experiments the difference is shown to have disappeared. Accordingly, it was necessary to eliminate the possible influence of such a change by using the same transfer of the line of cells for both strains of mice, as indicated in Tables II and III. Since the results are not due to changes in the cell lines they must be due to differences between the host animals.

In regard to age, food, care, and experience in life, all the host mice were highly uniform. All were bred at Cold Spring Harbor and carried to New York by hand *via* train and subway soon after they were a month old. Since all were used between 60 and 80 days after birth, they spent roughly the same amount of time in the New York mouse room. Thus non-genetic differences between the two strains of hosts appear to have been eliminated. Positive evidence of genetic

differences is provided by the pedigree record of every mouse born in these strains. They differ in three genes for hair color, namely, black *vs.* brown, intense pigment *vs.* dilute, and pink eyes *vs.* dark eyes. Mice of Strain C58 are all black, as have been all those born in this strain since 1922; mice of Strain Storrs-Little are called pink-eyed, dilute brown, and all mice born in this strain since 1923 have been of the same description. These strains differ further in an undetermined number of genes influencing susceptibility of different lines of leukemic cells; one of these, necessary for the continued growth of Line I in one long period of its history, has been identified (4).

Non-genetic differences do not exist; genetic differences do exist. Whatever the nature of the differences between strains involved in the modification of the metabolism of the leukemic cells, it seems necessary to conclude that they are under the control of heredity. Under these conditions the effect of differences between these strains is open to investigation. The only point that can be indicated at present is that the metabolism of the normal lymphoid tissue is not concerned, since in this respect, as already pointed out, these strains are essentially alike.

The Effect of Host Constitution Varies with the Line of Leukemic Cells.—In Strain C58 the metabolism of Line I is similar in all three criteria to Line M-liver. Yet in passing to hosts of Strain Storrs-Little the metabolism of these lines is altered in different ways. The rate of oxygen consumption of Line I was significantly increased (difference is 10 times its probable error) in Strain Storrs-Little, while that of Line M-liver was significantly the same as in Strain C58 (difference equals its probable error). Although both lines in Storrs-Little show a similar change in means for aerobic and anaerobic glycolysis, a further difference in response of lines is indicated by the greater variability in determinations of aerobic glycolysis of Line I. The coefficients of variability of the aerobic glycolysis for Line I is 46 per cent, for Line M-liver, 10 per cent.

Thus the metabolic response to changes in their environment may reveal differences between lines that by all other criteria are indistinguishable. This extends to a considerable degree of subtility the previous conclusion that there are inherent differences between lines of transmissible lymphatic leukemia that modify their metabolism, and indicates the care necessary before concluding that two lines are alike.

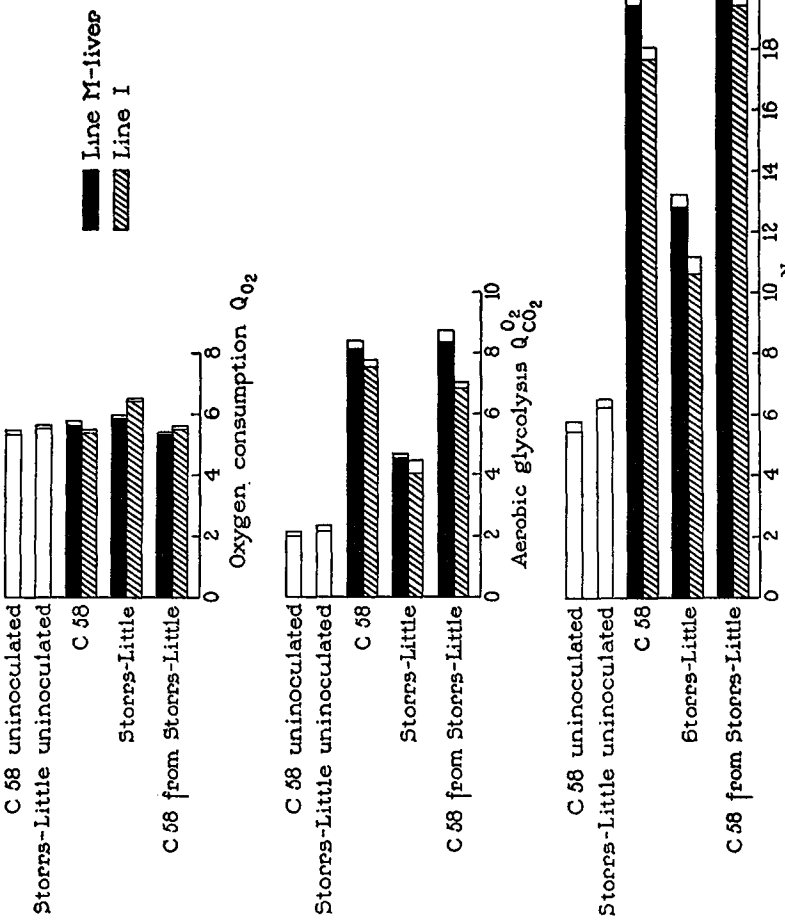

FIG. 1. Arithmetic means (large rectangles) and their probable errors of the metabolic rates of leukemic cells of Lines I and M-liver in Strains C58 and Storrs-Little. The small rectangle at the end of each large one equals 1 times the probable error of the mean. See text for explanation.

Host Constitution Does Not Modify Inherent Constitution of the Cell Line.—As soon as the cells are returned from Strain Storrs-Little to Strain C58, the metabolic rates return to their original level in C58 hosts. None of the differences between the metabolic rates before and after transfer through Storrs-Little hosts is statistically significant. In Line M-liver, after two and three transfers through Storrs-Little hosts, the differences for all three criteria have the opposite sign from the changes caused by the Storrs-Little hosts. In Line I, after one transfer through Storrs-Little hosts, the one difference that most closely approaches statistical significance (anaerobic glycolysis) varies in the opposite direction from the rate in Storrs-Little; in the other two criteria the deviation is in the same direction, but as noted, not significant.

These results indicate that the metabolism of these leukemic cells is modified by their immediate environment. Clearly enough a change in temperature will change metabolic rates; a change in hosts is on a higher level of complexity in its influence on metabolic rate. In both cases the changes are reversible. The metabolic rate observed for a given line is not a trait of the line as such; it is rather the reaction of a line of cells to a certain host. If the host can be defined the observation acquires significance, and can be repeated. In other hosts the reaction may be different, and yet the essential mechanism upon which the metabolic activity of the cell line depends may be constant throughout.

These results both provide an interpretation of difficulties previously met in the field of metabolic studies on neoplastic tissue, and establish the necessity for the use of host animals whose uniformity is controlled genetically and ontogenetically. To ignore temperature would be unthinkable; but the complicated specific differential relationships of cell line and host strain, demonstrated herein, indicate that the most strict control of host animals from which neoplastic tissue is taken can be considered of no less importance than the temperature under which the tissue is studied.

SUMMARY AND CONCLUSIONS

1. Two highly inbred strains of mice of different genetic constitution (Storrs-Little and C58) were used in a study of the influence of hosts on metabolism of cells of transmissible lymphatic leukemia.

The experiments were carried out with leukemic cells of transmission Line I as well as with Line M-liver. About 50 per cent of the Storrs-Little mice were killed by each line of cells at this time, while 100 per cent of the C58 mice were killed.

2. The normal lymphoid tissues of the two strains of mice were significantly the same in regard to rates of oxygen consumption and both aerobic and anaerobic glycolysis.

3. Leukemic cells of Line I, growing in hosts of Strain Storrs-Little, gave significantly lower rates of aerobic and anaerobic glycolysis than when growing in hosts of Strain C58. Oxygen consumption was significantly higher. Leukemic cells of Line M-liver, growing in hosts of Strain Storrs-Little, gave significantly lower rates of aerobic and anaerobic glycolysis than when growing in Strain C58. Oxygen consumption was not significantly different.

4. After one to three passages through hosts of Strain Storrs-Little, the cell lines were returned to hosts of Strain C58, with immediate return to significantly the same metabolic rates originally given by each line in hosts of Strain C58.

5. These results lead to the more general conclusions that: (*a*) The genetic constitution of the host modifies the metabolism of the cell line. (*b*) The same host constitution may modify the metabolism of different cell lines in different ways. (*c*) Host constitution does not appear to modify the inherent constitution of the leukemic cells, but acts as a determining environmental factor on their metabolism.

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