Regulation of Lactate Production and Utilization in Rat Tumors in Vivo*

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These experiments were performed to determine the factor(s) that regulate lactic acid production and utilization by rat tumors in vivo. Arteriovenous differences for glucose and lactic, pyruvic, 3-OH-butyric, and acetacetic acids were measured across "tissue-isolated" Walker 256 sarcocarcinomas and Morris 5123C hepatomas in fasted rats anesthetized with sodium pentobarbital. Twenty-six per cent of the sarcocarcinomas (n = 53) and 48% of the hepatomas (n = 29) utilized blood lactic acid. The remainder released lactic acid into the venous blood. The steady-state rate of glucose consumption was similar in both lactate-producing and lactate-utilizing tumors. The range of lactate concentrations in the blood leaving the tumors was narrower than the range of lactate concentrations in the blood entering the tumors. This difference was caused by tumor lactic acid production at low arterial lactate concentrations and tumor lactic acid utilization at high arterial lactate concentrations. Individual tumors changed from lactic acid production to lactic acid utilization in a matter of minutes in response to an increased arterial lactic acid concentration. Mean lactic plus pyruvic acid concentrations and lactic/pyruvic acid ratios in the tumor venous blood were 2.15 ± 0.22 and 23.4 ± 3.7 mM, respectively, for Walker sarcocarcinoma 256 (n = 18) and 1.28 ± 0.13 and 48.1 ± 5.1 mM, respectively, for hepatoma 5123C (n = 11). The results suggest: 1) that a steady-state lactic plus pyruvic acid concentration and lactic/pyruvic acid ratio are maintained in the tumor cell cytoplasm by the active glycolytic pathway and by lactic acid dehydrogenase; 2) that the tumor intracellular concentrations equilibrate with the arterial blood and that the tumor steady state is expressed in the tumor venous blood; and 3) that tumor lactate acid production or utilization results from the equilibration between the variable arterial lactate acid concentration and the more constant tumor intracellular steady-state lactate acid concentration. Since the arterial lactate concentration may be less than, greater than, or equal to the intracellular steady-state concentration, an individual tumor may produce, utilize or neither produce nor utilize lactic acid.

In previous experiments performed with "tissue-isolated" rat tumors in vivo (1, 2) we found that some tumors released while others utilized blood lactic acid. A few other tumors did neither. The rates of glucose utilization were similar in all tumors and, presumably, so were the rates of intracellular pyruvic acid and NADH production. The data indicated that tumors in vivo have a large capacity for lactate utilization as well as for production. The experiments also suggested that specific host-tumor interactions were in some way regulating which process occurred in vivo. This was a surprising finding in view of the long-held idea that tumors are high lactate producers. In this study we have attempted to define the host-tumor interactions. The results show that movement of lactate from either arterial blood to tumor or from tumor to venous blood is down a lactic acid concentration gradient; release and uptake of lactic acid by tumors in vivo follows the principle of mass action.

EXPERIMENTAL PROCEDURES

Materials—Adult male and female Buffalo and Harlan Sprague-Dawley rats were obtained from colonies established here. The rats were fed a standard laboratory chow (Charles River Rat, Mouse, Hamster Formula, Agway, Inc, Syracuse, NY), had water ad libitum, and were subjected to alternate 12-h periods of dark and light. All animals were fasted for 48 h before death. Tumor implantation, growth of "tissue-isolated" tumors, preparation of the animal for tumor harvest, and collection of arterial and venous blood samples were as described previously (1, 2). At the time of tumor harvest the animals were about 90 days old and weighed about 200 g (Buffalo) or about 250 g (Sprague-Dawley). In a few experiments arterial blood was collected by heart puncture from nonanesthetized rats, both with and without tumors. The Morris hepatoma 5123C was originally obtained from the late Dr. Harold P. Morris, Morris Hepatoma Program, Howard University Cancer Center, Washington, D.C. The Walker sarcocarcinoma 256 was obtained from the E. G. and G. Mason Research Institute, Worcester, MA. These tumors have been carried in this laboratory for about 6 years. The mean tumor blood flow rate was 112 ± 2.2 μl/min (n = 74).

Acetocetate, 3-OH-butyrate, lactate, pyruvate, and glucose and nucleotides and enzymes were purchased from Sigma.

Treatment of Experimental Animals—In the first group of experiments metabolite arteriovenous differences were calculated from single arterial and venous samples. In a second group of experiments the arterial lactate acid concentration was increased by intravenous infusion of glucose into insulin-treated rats (3). Two sets of arterial and venous samples were collected. A rat, lightly anesthetized with ether, was pretreated with insulin (25 units intravenously in the external jugular vein) 90 min before collection of the first sample set. Immediately following collection of the first sample set the animal was made hyperglycemic (and usually hyperlactemic) by injection of 200 mg of glucose (in 1 ml of saline) into the external jugular vein. The second sample set was collected 10 min after injection of the glucose. In another type of experiment in this second group, the first set of blood samples was collected, and the lactic acid concentration

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1 The term "tissue-isolated" tumor means a growing tumor isolated from the surrounding tissues but connected with the host by a single artery and vein.
of the host arterial blood was then altered by injection into the external jugular vein of 0.5 ml of a 60 mM solution of one of the following: sodium acetoacetate, sodium 3-OH-butyrate, sodium pyruvate, or sodium lactate. The second sample set was collected 5 min after this treatment. Sodium acetoacetate was prepared from the lithium salt by two consecutive passages through a 1.5 x 1.5-cm column of AG 50W-X2 (Na+ form). All injected substrate solutions were adjusted to pH 7.4.

Blood Sample Preparation and Assay—Neutralized perchloric acid extracts of arterial and venous blood were prepared as described earlier (1, 2). The pyruvic acid content of these extracts was low (or zero), and the results of duplicate pyruvate assays of the same extract often did not agree. We found that incomplete precipitation and denaturation of red blood cell lactic dehydrogenase by the cold 7% perchloric acid had occurred and that lactic dehydrogenase activity was carried over to the neutralized extract. The lactic dehydrogenase contaminating the sample removed the pyruvate during the temperature-equilibration period before the start of the fluorometric assay. Accurate assay of pyruvate in whole blood was obtained if the collection time for tumor venous blood was reduced to 2 min, and the blood was immediately deproteinized with an equal volume of ice-cold 20% perchloric acid. The samples were chilled in ice for 30 min and centrifuged; the clear supernatant fluid was removed and assayed immediately without neutralization. No residual lactic dehydrogenase activity was found in these extracts, and added pyruvate was completely recovered. Acetoacetate, 3-OH-butyrate, lactate, pyruvate, and glucose were measured either fluorometrically or spectrophotometrically by enzymatic methods (2).

Expression and Evaluation of Results—For interpretation of the arteriovenous difference measurements, we assumed that the tumor was in a steady state with regard to nutrient supply and metabolism and that this steady state continued during the 2- or 5-min period of sample collection. Utilization or production of a nutrient by the tumor occurred when the arteriovenous difference was positive or negative, respectively. Utilization and production rates are given in nanomoles of substrate utilized or produced per min per g of tumor, wet weight, and were calculated from the arteriovenous differences and the tumor blood flow rates (μl/min/g). Supply rates are expressed as nmol/min/g tumor, wet weight, and were calculated from the arterial whole blood concentrations and the tumor blood flow rates. Data are presented as mean ± S.E. Groups of results were compared by linear regression and analysis of variance (4).

RESULTS

Effects of Pentobarbital-induced Surgical Anesthesia on Host Arterial Whole Blood Metabolite Concentrations—Glucose metabolism in rats in vivo is influenced by anesthesia (5, 6); however, the response depends on the physiological state of the rat (7). Pentobarbital is preferable to ether because the arterial blood lactate concentration is less affected (8). We tested the effects of pentobarbital-induced surgical anesthesia on tumor-bearing host rats to determine if alterations in blood metabolite levels were produced that might be important to this study. Glucose, lactate, pyruvic, 3-OH-butyric, and acetoacetic acid concentrations were measured in host arterial blood of three rat groups: 1) nonanesthetized nontumor-bearing rats; 2) nonanesthetized tumor-bearing rats; and 3) anesthetized tumor-bearing rats. The results are shown in Table I. Data from the two rat strains are listed separately. Pentobarbital-induced anesthesia increased the mean blood glucose concentration in both rat strains, in agreement with previous studies (5, 6). The mean arterial lactic acid concentration was unaffected by either the presence of a tumor or anesthesia in Buffalo rats. The mean lactic acid concentration was increased by anesthesia in tumor-bearing Sprague-Dawley rats, but the increase was not significant. Lactic/pyruvic acid ratios were increased in the tumor-bearing anesthetized animals as compared to the nonanesthetized rats. In the three groups the concentrations of 3-OH-butyric acid were similar as were the 3-OH-butyric/acetoacetic acid ratios. Therefore, the ketone body response to fasting was normal in tumor-bearing rats.

The effects of pentobarbital on the metabolism of tumorbearing host rats appeared to be minor. On the average, the arterial glucose concentrations and the lactic/pyruvic acid ratios were increased, but no increase in the arterial lactic acid concentration was seen. We were unable to determine if pentobarbital influences the way the tumor metabolizes lactic acid and other substrates. Unfortunately, it is not yet technically possible to measure arteriovenous differences across a "tissue-isolated" tumor in vivo in the absence of surgical anesthesia.

Comparison of Lactic Acid Concentrations in Whole Blood from the Carotid Artery, the Femoral Vein, and the Tumor Vein—Table II shows the values observed for lactic acid concentrations of whole blood sampled from 3 sites in fasted tumor-bearing rats. The lactate content of blood draining the rat hind limb (femoral vein) was different from that draining the tumor in all animals tested. Lactic acid was most often produced by the hind limb, but utilization of arterial lactic acid occurred as well. In the five tumor-bearing Buffalo rats examined the mean lactic acid content was 3.5 ± 1.2 mM in the arterial blood, 5.6 ± 0.8 mM in femoral vein blood, and 2.6 ± 0.5 mM in tumor vein blood.

Host Arterial and Tumor Vein Lactic Acid Concentrations and Lactic Acid Production and Utilization in Rat Tumors in Vivo—Fig. 1 shows the relationship between the host arterial and tumor venous whole blood lactic acid concentrations in Walker sarcomatoma 256 (A) and Morris hepatoma 5123C (B). Each point represents a measurement made across an individual tumor in vivo. Pyruvate concentrations, which were less than 10% of the lactate concentrations, were not measured in all tumors and are not included. The dashed line with a slope of 1 separates lactate-producing tumors (above the line) from lactate-utilizing tumors. Twenty-six per cent of the Walker 256 tumors and 48% of the hepatomas utilized lactic acid from the arterial blood. Except for one high lactate-producing and one low lactate-utilizing tumor, the venous whole blood lactic acid levels ranged from about 1 to 4 mM for the Walker 256 tumors. The venous whole blood concentration range was 0.5 to about 3 mM lactic acid for hepatoma 5123C. These venous ranges were maintained despite an arterial blood lactic acid range from about 0.5 to about 7 mM for both Sprague-Dawley and Buffalo rats. Fig. 1 shows that tumors preferentially produce lactic acid at low arterial lactate concentrations and utilize lactic acid at high arterial lactic acid concentrations. In Fig. 1A, 90% of the tumors perfused by less than 2.5 mM arterial whole blood lactate produced lactic acid, and 82% of the tumors perfused by greater than 2.5 mM arterial lactate utilized lactic acid. A similar distribution was noted for hepatoma 5123C except that most lactate-utilizing tumors occurred above and most lactate-producing tumors occurred below 1.5 mM arterial lactate.

Mean lactic acid concentrations and information on other metabolites measured in these tumor-host preparations are listed in Table III. Data for the sarcomatoma-Sprague-Dawley rats and hepatoma-Buffalo rats were separated because of quantitative differences in tumor metabolism. Each tumor-host preparation series was divided into three groups. Tumors in group I produced lactate at a rate greater than 20% of the rate of glucose utilization. Tumors in the second group either produced or utilized lactate at a rate less than 20% of the rate of glucose utilization. Finally, tumors in group III were high lactate utilizers; lactate utilization was greater than 20% of the rate of glucose utilization. For the sarcomatoma-Sprague-Dawley preparations the mean tumor vein lactic acid concentrations were the same in groups I, II, and III. On the other hand, the mean arterial lactic acid concen-
Table I

Glucose, lactic, and 3-OH-butyric acid concentrations, and lactic/pyruvic and 3-OH-butyric/acetoacetic acid ratios in arterial whole blood from Sprague-Dawley and Buffalo rats

Samples of whole blood were obtained from three different animal groups within each rat strain. In group A blood was obtained by heart puncture from nonanesthetized, non-tumor-bearing rats. In group B blood was obtained by heart puncture from nonanesthetized, tumor-bearing rats. In group C blood was obtained from the carotid artery of tumor-bearing rats under pentobarbital surgical anesthesia. All animals were fasted for two days.

Sample preparation and assay were as described under “Experimental Procedures.”

| Sprague-Dawley rats | Glucose mM | Lactic Acid mM | Lactic/Pyruvic | 3-OH-Butylic mM | 3-OH-Butyric/Acetoacetic |
|---------------------|------------|----------------|----------------|----------------|-------------------------|
| A                   | 3.95±0.32  | 1.09±0.28      | 10.4±1.2       | 1.81±0.61      | 2.76±0.49               |
| B                   | 3.69±0.1e  | 1.84±0.52      | 7.4±0.3b       | 1.65±0.29      | 2.34±0.29               |
| Walker-256          | n=8        | (0.47-2.82)    |               | (0.67-4.27)    |                         |
| C                   | 5.33±0.33e | 2.33±0.44      | 22.5±3.6b      | 2.59±0.33      | 6.79±1.6                |
| Walker-256          | n=18       | (0.83-7.30)    |               |               |                         |
| Buffalo rats        |            |                |                |                |                         |
| A                   | 1.94±0.09  | 1.40±0.34      | 7.6±2.1        | 1.95±0.07      | 3.67±0.22               |
| B                   | 2.30±0.24a | 1.20±0.26      | 13.1±1.5a      | 1.77±0.28      | 2.45±0.35               |
| hepatoma-5123C      | n=12       | (0.93-3.07)    |               |               |                         |
| C                   | 3.37±0.40a | 1.26±0.15      | 19.8±2.8a      | 2.40±0.30      | 3.51±0.48               |
| hepatoma-5123C      | n=11       | (0.69-2.13)    |               |               |                         |

*p is less than 0.05.
*The numbers in parentheses are the ranges.

Table II

Lactic acid content of whole blood from carotid artery, femoral vein, and tumor vein in “tissue-isolated” tumor-bearing rats

Samples were drawn simultaneously from the 3 sites. The rats were fasted for 2 days. Sample preparation and assay were performed as described under “Experimental Procedures.”

| Experiment no. | Carotid artery mM | Femoral vein mM | Tumor vein mM |
|----------------|-------------------|-----------------|--------------|
| Buffalo rats- |                   |                 |              |
| hepatoma-5123C| 117                | 4.66            | 4.91         |
|                | 118                | 0.96            | 7.70         |
|                | 119                | 0.81            | 3.24         |
|                | 122                | 6.89            | 6.71         |
|                | 125                | 6.38            | 5.20         |
|                | 126                | 0.99            | 1.33         |
| Sprague-Dawley |                   |                 |              |
| rat-Walker 256 | 126                | 0.99            | 1.33         |

Fig. 1. Relationship between the lactic acid concentration in whole blood from the tumor vein and the carotid artery in Sprague-Dawley rats bearing the Walker sarcomas carcinoma 256 (A) and Buffalo rats bearing Morris hepatoma 5123C (B). Each point represents the determination for a single “tissue-isolated” tumor and host rat. The dashed line has a slope of one. Points above this line represent tumors that released lactic acid into the venous blood and points below this line are tumors that utilized arterial blood lactic acid.

Lactic/pyruvic acid ratios on the tumor venous blood. Although the blood leaving hepatoma 5123C contained a lower lactic acid concentration, the ratio of lactic/pyruvic acid was
The animals were divided into three groups based on a relationship between the rate of tumor glucose utilization and the rate of either tumor lactic acid production or utilization. Tumors in group I produced lactic acid at a rate that was greater than 20% of the rate of glucose utilization. Group II tumors either produced or utilized lactic acid at a rate less than 20% of the rate of glucose utilization. Tumor groups III utilized lactic acid at a rate greater than 20% of the rate of glucose utilization. All animals were fasted for 2 days. Sample preparation and assay were as described under "Experimental Procedures." The values in parentheses are the number of tumors and hosts examined.

### Table III

| Glucose | Lactic Acid | Ketone Bodies |
|---------|-------------|---------------|
| Arterial Glucose (mM) | Tumor Glucose Utilization (nmol/min/g) | Arterial Lactic Acid/pyruvic acid (mM) | Tumor lactic acid utilization (nmol/min/g) | Arterial 3-OH butyric acid (mM) | Tumor 3-OH butyric acid utilization (nmol/min/g) |
| Sprague-Dawley rats | Walker sarccarcinoma 256 | | | | |
| | | | | | |
| I | 6.0±0.30 | 41.0±29.1 | 1.2±0.17 | 11.7 | 2.2±0.33 | 48.3 | 21.2±1.4 | 3.2±0.9 | 9.5±4.3 |
| II | 5.1±0.39 | 64.3±10.9 | 1.3±0.16 | 15.1±1.3 | 1.3±0.12 | 52.2±17.1 | 3.3±3.7 | 3.8±0.3 | 3.8±0.4 |
| III | 4.3±0.12 | 34.9±13.5 | 2.3±0.52 | 28.9±4.6 | 1.2±0.22 | 41.0±19.1 | 19.9±4.9 | 7.6±3.8 | 6.5±2.1 |
| Buffalo rats | Morris hepatoma 5123C | | | | |
| | | | | | |
| I | 6.0±0.30 | 41.0±29.1 | 1.2±0.17 | 11.7 | 2.2±0.33 | 48.3 | 21.2±1.4 | 3.2±0.9 | 9.5±4.3 |
| II | 5.1±0.39 | 64.3±10.9 | 1.3±0.16 | 15.1±1.3 | 1.3±0.12 | 52.2±17.1 | 3.3±3.7 | 3.8±0.3 | 3.8±0.4 |
| III | 4.3±0.12 | 34.9±13.5 | 2.3±0.52 | 28.9±4.6 | 1.2±0.22 | 41.0±19.1 | 19.9±4.9 | 7.6±3.8 | 6.5±2.1 |

*p is less than 0.05.

Lactic acid production.

about 3 times greater than that observed in venous blood leaving the Walker sarccarcinomas 256.

Effect of an Acute Increase in the Arterial Lactic Acid Concentration on Tumor Lactic Acid Production and Utilization—The above results indicate that the lactate concentration in the tumor venous blood is relatively constant. Presumably, this concentration value represents the tumor intracellular concentration. The data also suggest that tumors produce lactic acid when the arterial concentration is lower than the tumor intracellular concentration and utilize lactate when the arterial concentration is higher than the tumor intracellular concentration. Therefore, an individual tumor should respond accordingly to an acute increase in the arterial whole blood lactic acid concentration. This reasoning was tested by the experiments described in this section. After control arterial and venous blood samples were collected the arterial lactate concentration was increased and a second sample set was collected. Arterial lactate was increased by the infusion of either glucose (3) or sodium 3-OH-butryate into hypoglycemic rats and by the infusion of either sodium lactate or sodium pyruvate into unpretreated rats. The results of 12 experiments are shown in Table IV. The host arterial lactic acid concentration was increased in 11 of the 12 animals. The tumors responded by shifting from lactate production to lactate utilization (3) (tumors 135, 141, 143, 146, 148, 158, 172, 174), from a low rate of lactate utilization to a higher rate of lactate utilization (142, 173), or from a low rate of production to a slightly higher rate of lactate production (150). Experiment 140 showed a small decrease in arterial lactate after glucose infusion, and the tumor shifted from a low rate of lactate utilization to a low rate of lactate production. Tumor 140 did not utilize arterial glucose, and the released lactic acid must have come from some other source. Tumor 150 is the only tumor that appeared to respond inappropriately to the elevated arterial lactate concentration. The arterial glucose concentration and tumor glucose utilization rate both increased to high values in this experiment. Conceivably, the tumor intracellular lactic acid concentration was increased over the unusually low arterial concentration, and lactic acid efflux occurred (compare experiment 143). We cannot explain the response of tumor 150 based on the data collected. Table IV also shows the results of four experiments in which the host animals were infused with sodium acetacetate. Infusion of this substrate decreased the arterial lactate concentration slightly in experiments 157 and 161. Both tumors showed an increase in lactic acid production. In experiments 167 and 168 there was no change in the arterial lactic acid concentrations, and tumor 167 showed no change in lactic acid production. Tumor 168, on the other hand, showed an increased rate of lactate production, as apparently inappropriate response. Only tumors 150 and 168 of the 16 tumors examined showed a shift in lactic acid production and utilization different from that predicted.
Tumor Lactic Acid Metabolism in Vivo

**TABLE IV**
The effect of the intravenous infusion of either glucose, or Na pyruvate, or Na lactate, or Na 3-OH-butyrate, or Na acetooacetate on arterial blood lactic acid concentration and on tumor lactic acid utilization and production in vivo

Host rat pretreatment, infusion of substrates, collection of arterial and tumor venous sample sets, and sample preparation and assay were as described under "Experimental Procedures." All rats were fasted for 2 days. All tumors were Walker 256 sarcomas except experiments 140 and 142, which were Morris hepatomas 5123C.

| Glucose | Lactic Acid |
|---------|-------------|
| **Experiment** | **Host Arterial (mM)** | **Tumor Utilization (nmol/min/g)** | **Host Arterial (mM)** | **Tumor Vein (mM)** | **Utilization (nmol/min/g)** | **Production (nmol/min/g)** |
| Glucose | 125 | control | 1.21 | 19 | 1.47 | 2.08 | -- | 20 |
| | | after infusion | 5.76 | 34 | 6.61 | 3.86 | -- | 84 |
| | 140 | control | 2.21 | 7 | 5.15 | 4.72 | -- | 8 |
| | | after infusion | 3.83 | 0 | 4.84 | 5.50 | -- | 13 |
| | 141 | control | 3.94 | 64 | 4.08 | 4.83 | -- | 99 |
| | | after infusion | 9.92 | 194 | 5.08 | 3.66 | -- | 48 |
| | 142 | control | 1.18 | 5 | 3.26 | 2.75 | 9 | -- |
| | | after infusion | 3.88 | 19 | 7.21 | 4.07 | 46 | -- |
| | 143 | control | 3.28 | 37 | 4.14 | 5.27 | -- | 39 |
| | | after infusion | 13.41 | 287 | 5.51 | 4.95 | 21 | -- |
| | 150 | control | 1.80 | 32 | 1.83 | 2.40 | -- | 24 |
| | | after infusion | 12.91 | 252 | 2.66 | 3.58 | -- | 35 |
| Pyruvate | 158 | control | 5.23 | 34 | 0.72 | 2.22 | -- | 59 |
| | | after infusion | 7.80 | 125 | 4.12 | 3.11 | 43 | -- |
| | 172 | control | 7.17 | 84 | 2.20 | 2.80 | -- | 19 |
| | | after infusion | 5.41 | 72 | 7.44 | 4.87 | 83 | -- |
| Lactate | 173 | control | 7.01 | 76 | 1.56 | 1.38 | 9 | -- |
| | | after infusion | 6.54 | 126 | 2.68 | 2.05 | 36 | -- |
| | 174 | control | 6.57 | 57 | 1.11 | 2.62 | -- | 60 |
| | | after infusion | 8.10 | 116 | 4.24 | 4.10 | 5 | -- |
| 3-OH-butyrate | 146 | control | 2.36 | 50 | 2.99 | 3.85 | -- | 31 |
| | | after infusion | 1.75 | 30 | 4.77 | 3.87 | 30 | -- |
| | 148 | control | 2.44 | 14 | 0.48 | 4.56 | -- | 19 |
| | | after infusion | 1.30 | 7 | 6.36 | 5.28 | 23 | -- |
| Acetoacetate | 157 | control | 4.37 | 46 | 0.81 | 1.40 | -- | 34 |
| | | after infusion | 4.47 | 57 | 0.70 | 2.25 | -- | 98 |
| | 161 | control | 4.88 | 39 | 0.97 | 2.06 | -- | 39 |
| | | after infusion | 4.80 | 43 | 0.76 | 3.38 | -- | 87 |
| | 167 | control | 5.94 | 70 | 9.89 | 2.69 | -- | 81 |
| | | after infusion | 6.79 | 90 | 0.90 | 2.43 | -- | 77 |
| | 168 | control | 5.53 | 36 | 1.10 | 2.86 | -- | 78 |
| | | after infusion | 6.96 | 62 | 1.12 | 4.13 | -- | 134 |

*Rats were pretreated with insulin (25 units intravenously) 90 min before collection of the first sample set.*

### DISCUSSION

In this report we have presented information on the utilization and production of lactic acid by rat tumors in vivo. Three new findings aid in interpretation of these and previous results. First, we found that each tumor type imposed on the venous blood a defined range of lactic acid concentrations and a nearly constant lactic/pyruvic acid ratio. The range of concentration values for lactic acid in tumor venous blood was narrow when compared to that of the arterial blood (Fig. 1, Table III). This resulted from tumor lactate production when the arterial lactate concentration was low and from tumor lactate utilization when the arterial concentration was high (Fig. 1). We also found that the mean 3-OH-butyric/acetoacetate acid ratios measured in the tumor venous blood were essentially constant under a range of different host conditions (Table III, see also Ref. 2). These findings suggest that the concentrations of lactic, pyruvic, 3-OH-butyric, and acetooacetate acids in the venous blood represent the steady-state intracellular concentrations and that their ratios represent the intracellular redox state of the substrate couples.

Presumably, the very active metabolism of the tumor dominates the arterial blood because of the slow rate of blood flow through the tumor mass and because of active carriers for glucose, the ketone bodies, and lactic and pyruvic acids in the tumor cell plasma membrane. These carriers must promote a rapid equilibration of the arterial blood lactic and pyruvic acid concentrations with the steady state intracellular concentrations generated in the tumor cells. As a result, the tumor intracellular steady state concentrations are imposed on the blood leaving the tumor. To our knowledge the V_max for entry and exit of these monocarboxylic acids has not been measured in vivo in these tumor cells. However, transport of lactate, pyruvate, and 3-OH-butyrate is rapid in mouse ascites tumor cells in vitro (9). It is of interest that lactate, pyruvate, 3-OH-butyrate, and possibly acetooacetate are transported over the same carrier (9) since the flux of these anions between blood and tumor is often in opposite directions. The 3-OH-butyric and acetooacetate acid concentrations are higher in arterial blood and net transport is into the tumor. Lactic acid is often higher in the tumor and, when it is, net transport is
into the blood. Clearly, sufficient time and carrier capacity are available for these equilibrations to occur. The computed regression line of data points of tumor vein versus arterial 3-OH-butyryl acid concentrations for the 82 tumors examined (data not shown) was found to pass through the origin. Unlike lactic acid (Fig. 1), 3-OH-butyryl acid is not generated in the tumor cell, and the rate of 3-OH-butyrate utilization is strictly dependent on the supply rate via the arterial blood (2).

The second finding of interest was that tumor lactate production and utilization were reversible in a matter of minutes. An individual tumor could change from production to utilization, from production to a higher rate of production, from utilization to a higher rate of utilization, and from utilization to production. Examples of each were found. The change from utilization to production required a large decrease in a high arterial lactate concentration; we could not devise a method to accomplish that in vivo. We found no evidence to indicate that the production and utilization of lactic acid by tumors were not completely reversible pending an appropriate change in the host arterial blood lactic acid concentration.

Finally, we found that the lactic acid content of the tumor venous blood changed with time even in the absence of an appreciable change in the arterial lactate concentration (Table IV, experiments 157, 161, and 168). We assume these changes are a response to alterations in intracellular lactate concentrations. The exact intracellular lactate concentration at any time would depend on the rate of glucose flux through glycolysis to pyruvate, on the rate of pyruvate generation from other sources, on the rate of pyruvate utilization, and on the NAD/NADH ratio. Table IV shows that the rate of glucose utilization may change. Also, the combined action of several dehydrogenase reactions must determine the NAD/NADH ratio (and thus the lactic/pyruvic acid ratio) in the extramitochondrial space in vivo. Therefore, the ranges in intracellular lactate concentrations of 1-4 mM for Walker 256 and 0.5-5 mM for hepatoma 5123C, as defined in Fig. 1, A and B, probably represent the low and high limits. Variation within this range of intracellular concentrations may explain why the Walker 256 sarcoma in experiment 168, Table IV, increased the concentration of lactic acid in the venous blood without change in the arterial lactic acid concentration. Assuming that the venous blood pyruvate and lactate concentrations are equal to the intracellular concentrations, we may predict that the mean intracellular pyruvic acid concentrations are about 0.1 mM in Walker 256 and 0.05 mM in hepatoma 5123C. The mean intracellular lactic acid concentrations are about 2.3 mM in Walker 256 and 1.6 mM in hepatoma 5123C. As judged by the mean lactic/pyruvic and 3-OH-butyric/acetocetic acid ratios (Table III), we calculate (10) that the NAD/NADH ratios in the cytoplasmic spaces of Walker sarcomas 256 and hepatoma 5123C in fasted rats are 518 and 190, respectively. The calculated NAD/NADH ratio in the mitochondrial matrix space is 5.7 for both tumors.

In an earlier report (2) we proposed that tumor lactic acid production and utilization were regulated by the arterial lactic acid concentration and/or by redox state differences between tumor and host. The results reported here indicate that the first consideration is the more important. Tumor-host redox state differences appear to exist primarily because the redox state of the host varies and the redox state of the tumor is relatively constant. We cannot rule out the possibility that tumor-host redox state differences play a subtle role that is undetectable in these experiments.

The results described here are not necessarily incompatible with the results of the historically important reports of Cori and Cori (11) and Warburg et al. (12). These early papers described in vivo glucose consumption and lactic acid production in Rous sarcoma and Jensen sarcoma, respectively. The procedure employed by Cori and Cori (11) was indirect in that the Rous sarcoma was implanted in one wing of a chicken. Glucose and lactic acid were measured in venous blood coming from the tumor-bearing wing and from the control, opposite wing. True arteriovenous differences were not measured. A lower glucose concentration and a higher lactic acid concentration were found in the venous blood coming from the tumor-bearing wing. In studies with the Jensen rat sarcoma, Warburg et al. (12) collected arterial blood from the aorta and venous blood from a vein emerging from the tumor. They observed that all tumors (n = 6) consumed glucose and that all tumors (n = 10) produced lactic acid. Mean arterial whole blood glucose and lactic acid concentrations were 5.9 and 3.5 mM, respectively. The range of lactic acid concentrations measured in the tumor venous blood was 4.4-25.6 mM (mean = 9.9 mM). These data and those of Cori and Cori (11) provided important in vivo support for the idea, derived primarily from in vitro experiments with tumor slices (13), that tumors are high lactate producers. The tumor vein lactic acid concentrations observed by Warburg et al. (12) are higher than the values observed by us in either Walker sarcoma 256 or hepatoma 5123C. At this time we are unable to explain this discrepancy. It is unlikely that the Jensen sarcoma pumps lactic acid out of the cell against a concentration gradient. If the above interpretations of our data are correct, we may conclude that the Jensen rat sarcoma maintains a higher intracellular lactic acid concentration than either the Walker sarcoma 256 or hepatoma 5123C and that this higher level is imposed on the tumor venous blood. Spencer and Lehninger (9) mentioned that intracellular lactic acid was about 20 mM in mouse ascites tumors cells in vitro. An intracellular concentration in that range in the Jensen sarcoma in vivo would be expected to yield an equivalent concentration in the tumor venous blood. An understanding of the discrepancy will not be in hand until experiments with the Jensen sarcoma are repeated in the better defined "tissue-isolated" tumor system employed here.

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