THE CARBOHYDRATE METABOLISM OF TUMORS

III. THE RATE OF GLYCOLYSIS OF TUMOR TISSUE IN THE LIVING ANIMAL *

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Experiments reported in the second paper of this series (1) have shown that tumor tissue glycolyzes, i.e. splits glucose into lactic acid, under in vivo conditions. This has been demonstrated in the following way. A chicken sarcoma was established on one wing and the venous blood leaving the tumor was compared with the venous blood from the normal wing. The blood that had passed through the tumor contained on an average 16 mg. per cent more lactic acid and 23 mg. per cent less glucose than the blood that had passed through the tissues of the normal wing. A similar result was obtained in experiments on a patient with a sarcoma of the forearm. Warburg and collaborators (2) repeated these experiments on tumor-bearing rats and confirmed them. In all cases Warburg found two to three times more lactic acid in the tumor vein than in arterial blood, while venous blood from normal tissues contained the same or even less lactic acid than arterial blood.

These experiments leave no doubt that tumor tissue, in contradistinction to normal tissue, glycolyzes in the living animal. However, the proof is of a qualitative nature. No attempt has been made to determine the rate of lactic acid formation of the tumor in the living animal in a quantitative way. A direct method, involving the measurement of the rate of blood flow through the tumor and lactic acid determinations in the blood entering and leaving the tumor, could not be applied. The smallness of the vessels and the large number of arteries entering a transplanted tumor made this technically impossible.
It occurred to the writers that an indirect method might be used. The principle of this method is as follows.

A certain amount of lactic acid leaves the tumor and passes into the blood stream. This lactic acid is disposed of in other tissues of the body, partly by conversion into glycogen, partly by oxidation. Obviously, there will be a limit to the capacity of the tissues to withdraw lactic acid from the blood. If this limit is exceeded, that is, if the rate of lactic acid entering the blood is greater than the rate of its disposal in the tissues, the lactic acid will accumulate in the blood and will be excreted in the urine. One can determine this tolerance limit by infusing lactic acid intravenously at varying rates and observing the maximum rate which just fails to cause an accumulation of lactic acid in the blood and an excretion of lactic acid in the urine. Having determined the tolerance limit for intravenously injected lactic acid on normal animals, one proceeds to do the same on tumor-bearing animals. Obviously, the capacity of the tumor-bearing animals to utilize lactic acid will be lower, because the tumor produces a certain amount of lactic acid which adds itself to the amount of lactic acid infused. The production of lactic acid by the tumor represents, so to speak, an internal infusion, on which is superimposed an external infusion. It might be expected that the tolerance of the tumor-bearing animals will be lowered to the same extent to which the tumor produces lactic acid. Hence, one can arrive at an estimate of the rate of lactic production of the tumor in the living animal by determining the difference in the intravenous lactic acid tolerance between normal and tumor-bearing animals.

Before the applicability of this method could be tested, dextrorotatory lactic acid had to be secured. The lactic acid produced in the body is of the d-form. It is well known that the body cells discriminate between two optical isomers, attacking the one and leaving the other unimpaired. An example in point, is the behaviour of the d- and l-forms of

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1 The older designation of the sarcolactic acid is used in this paper. From a chemical standpoint it should be designated as 1 (+)-lactic acid and the optical isomer as 1(−)-lactic acid.
various sugars in the body. Generally only one of the two optical isomers is utilized, and the same seems to be true of lactic acid. The l-form of lactic acid, which does not occur naturally in the body, seems to be excreted to a large extent in the urine when it is injected. Commercial lactic acid is racemic, that is, it consists of equal quantities of the d- and l-form. When racemic acid was infused intravenously into rats, a large amount of lactic acid, presumably of the l-form, appeared in the urine. This is shown in the following experiments. A male rat of 238 gm. of body weight received an intravenous infusion of racemic sodium lactate at a rate of 80 mg. per 100 gm. of body weight per hour. In the first hour 29 mg. and in the second hour 51 mg. of lactic acid was excreted in the urine. In a second experiment on a rat weighing 258 gm., the rate of infusion was 60 mg. per 100 gm. of body weight per hour, with an excretion of 27 mg. in the first and 34 mg. in the second hour of infusion. As will be shown later, only the amounts of lactic acid normally present are found in the urine, when sodium d-lactate is infused intravenously at the above rates. It was, therefore, necessary to use d-lactic acid for the experiments.

Apart from the chemical resolution of the racemic acid into its optically active components, d-lactic acid can also be produced biologically. Pederson, Peterson and Fred (3) found that certain bacteria, when grown under special conditions, produce only the d-form of lactic acid from glucose. Through the kindness of Dr. Peterson of the College of Agriculture of the University of Wisconsin a sufficient supply of the d-lactic acid produced by bacteria was secured. The d-lactic acid, as prepared by Dr. Peterson, when converted into the zinc salt, gave a water of crystallization of 12.6 per cent, while the theoretical value for Zn(C₃H₅O₃).2H₂O is 12.9 per cent. Inactive zinc lactate contains 18.2 per cent water of crystallization. The specific rotation of the zinc salt for a 2.5 per cent solution of the water free salt was −8.2 per cent, while the value given by most investigators for the same concentration of the salt is −8.0 to −8.2 per cent. Since the rotation of the zinc salt is

*While this paper was in press, 1-lactic acid was found to be utilized 4 times more slowly in the rat than d-lactic acid.
opposite to that of the free acid, this proves that the material
used for the present experiments was dextrorotatory lactic acid.

EXPERIMENTAL

The experiments were carried out on normal rats and on rats
bearing the Jensen sarcoma. The animals were anesthetized by
an intraperitoneal injection of amytal (8 mg. per 100 gm. of body
weight). D-lactic acid in the form of its sodium salt, was infused
into a femoral vein. The technic of intravenous infusion in
rats has been described in detail elsewhere (4). By means of a
simple device the rate of infusion could be kept constant within
0.02 cc. Generally from 2 to 3 cc. of the lactic acid solution
were infused in 1 hour. The urine was collected in hourly
intervals by pressing out the bladder, while the blood was
collected at the end of the experiment, since 2 cc. were needed for
duplicate lactic acid determinations. In the case of the inocu-
lated rats, the tumor was dissected out and weighed after all
visible necrosis had been carefully removed. The calculations of
the rate of glycolysis are based on the weight of the tumor
without necrosis.

Lactic acid in the blood was determined by the Clausen (5)
H₂SO₄ procedure, with the precautions mentioned in a previous
paper (1). Lactic acid in the urine was extracted with ether and
then analyzed according to the Clausen method. The new
lactic acid method of Friedman, Cotonio and Shaffer (6) was not
available at the time the experiments were made, but in some
recent determinations in which the new method was used, the
same values were obtained as with the Clausen method. For
the determination of the blood and urinary sugar the Hagedorn
and Jensen (7) method was used.

First the lactic acid content of the blood of 6 normal rats was
determined. The following values, expressed in mg. per 100 cc.
of blood were obtained. 34.3; 29.0; 19.6; 25.8; 21.2; average:
26.8 ± 4.6.

In order to gain information as to the influence of the amytal
narcosis and the infusion on the lactic acid content of the blood
and urine, 5 normal rats were infused for 1 hour with glucose
The carbohydrate metabolism of tumors (200 mg. per 100 gm. of body weight per hour). The lactic acid values in blood and in the urine that was excreted during 1 hour of glucose infusion were as follows:

Blood 29.5; 24.0; 24.8; 23.5; 30.1; average: 26.4 = 2.7.
Urine 0.13; 0.21; 0.42; 0.3; 0.32; average: 0.28 mg.

The tolerance of normal rats for intravenously injected sodium d-lactate was determined on 15 animals (Table I).

In analyzing the data of Table I, one finds in 4 experiments (Nos. 2 to 5 incl.), at a rate of infusion of 110 to 119 mg. per 100 gm. of body weight per hour, an average lactic acid content of the blood of 71.2 mg. per cent and an average excretion of lactic acid in the urine of 4.7 mg. These figures indicate that the lactic acid tolerance of the rats is exceeded at the above rates of infusion. On the other hand, there are 7 experiments (Nos. 8 to 14 incl.), at a rate of infusion of 86 to 94 mg. per hour, with an average lactic acid content of the blood of 34.0 mg. per cent and of the urine of 1.4 mg. This is just below the tolerance of the rats for lactic

<table>
<thead>
<tr>
<th>Exp. No.</th>
<th>Body Weight Gm.</th>
<th>Lactic Acid Infused per 100 Gm. of Body Weight per Hour Mg.</th>
<th>Lactic Acid Excreted per 100 Gm. of Body Weight per Hour Mg.</th>
<th>Lactic Acid in Blood Mg. %</th>
<th>Blood Sugar Mg. %</th>
<th>Rate of Infusion Above or Below Tolerance</th>
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<td>158</td>
<td>15.8</td>
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</tr>
<tr>
<td>2</td>
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<td>119</td>
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<td>67.9</td>
<td>124</td>
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<td>3</td>
<td>213</td>
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<td>3.1</td>
<td>74.6</td>
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<td>194</td>
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<td>124</td>
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<td>194</td>
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</tr>
<tr>
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<td>94</td>
<td>1.4</td>
<td>30.2</td>
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</tr>
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<td>&quot;</td>
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<td>1.2</td>
<td>34.6</td>
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<td>208</td>
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<td>36.5</td>
<td>128</td>
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<tr>
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<td>94</td>
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<td>60.1</td>
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acid. There remain 2 experiments (Nos. 6 and 7) in which the rate of infusion is intermediate. In one of these the tolerance is exceeded at a rate of infusion of 105 mg. per hour; in the other the rate of infusion of 106 mg. per hour falls below the tolerance. The tolerance limit of normal rats for intravenously injected sodium d-lactate, under the experimental conditions here described, is therefore at a rate of infusion of 95 mg. per 100 gm. of body weight per hour, with a deviation from the mean of approximately = 5 mg. The mean value of 95 mg. is used in the calculation of the rate of glycolysis of the tumor in a manner to be described later.

The injection of sodium lactate, in the amounts used in the experiments in Table I, produces a marked increase in the NaHCO₃ content of the blood and in some cases an actual change in the pH of the blood to the alkaline side. This is due to the fact that the lactic acid is disposed of in the tissues, while the base with which it was combined is left behind and can only be eliminated by the rather slow process of kidney excretion. Alkalosis of the blood in itself tends to cause a rise in the lactic acid concentration of the blood. For this reason the lactic acid tolerance might be found to be higher if it were possible to inject free lactic acid instead of the sodium salt.

Before starting with the experiments on tumor-bearing rats it was necessary to carry out some lactic acid determinations in the blood of these animals. Previous determinations (1) showed that 6 rats in which the tumor constituted from 13.7 to 22 per cent of the body weight, the lactic acid content of the blood was higher than normal. Animals with tumors of this size are therefore unsuitable for the determination of the lactic acid tolerance. In order to find out which animals would be best suited, a new series of determinations was made (Table II). It will be noted that in experiments 1 to 5, with a tumor percentage of 4.9 to 7.9, the lactic acid content of the blood was within normal limits, while with a tumor weight of 14.2 to 21 per cent (exp. 6 to 11) the blood lactic acid was markedly increased. These results made it advisable to use only such animals in which the tumor corresponded to less than 10 per cent of the body weight.
The experiments on tumor-bearing rats are recorded in Table III. In the first experiment in which the tumor constituted 8.8 per cent of the body weight, a rate of infusion of 80 and 90 mg. of lactic acid per hour was markedly above the tolerance. In the
second experiment on a rat with an equally large tumor, a rate of infusion of only 70 mg. per hour was also above the tolerance. The intravenous lactic acid tolerance of tumor-bearing rats is therefore decidedly lower than that of normal rats. This is ascribed to the fact that the tumor produces a certain amount of lactic acid which passes into the blood stream and adds itself to the amount of lactic acid which is being infused. In the following experiments the aim was to infuse lactic acid as nearly as possible at the tolerance rate in order to arrive at an estimate of the rate of glycolysis of the tumor. Experiments 3 to 8 of Table III are regarded as successful in this respect. It will be noted that the extent to which the tolerance is lowered below the normal value of 95 mg. per 100 gm. of body weight per hour is fairly proportional to the size of the tumor. Rats with tumors ranging from 8.1 to 6.4 per cent of their body weight show a lactic acid tolerance of 40 to 50 mg. per hour, while in rats in which the tumor corresponds to 3.8 to 4.4 per cent of the body weight the tolerance for lactic acid is 66 to 68 mg. per hour.

The method of calculation of the rate of glycolysis may be illustrated in experiment 6 of Table III. In the first hour lactic acid was infused at a rate of 68 mg. per hour. This led to an excretion of 0.7 mg. of lactic acid in the urine. In the second hour in which the same rate of infusion was maintained, 2.4 mg. of lactic acid was excreted in the urine and the blood lactic acid rose to 42.5 mg. per cent. A rate of infusion of 68 mg. of lactic acid per hour is therefore as close to the tolerance limit of this particular rat as one is able to determine it. If this rat were normal, it should tolerate 95 mg. of lactic acid per hour without an appreciable rise in blood lactic acid. Hence, the tolerance is lowered by $95 - 68 = 27$ mg. From what has been said before, 27 mg. represents the lactic acid production of the tumor. By dividing this amount by the weight of the tumor one finds that $\frac{27 \times 100}{4.4} = 610$ mg. of lactic acid are produced per 100 gm. of tumor per hour. The values for the rate of glycolysis of the tumor obtained in the other experiments vary from 570 to 800 mg. per hour, with an average of 690 mg. These values must be
regarded as approximations only, because it is impossible to
calculate exactly the magnitude of the errors that might enter
into an indirect method of the type here used.

The method is based on the assumption that the non-cancerous
tissues of the tumor-bearing rats have the same capacity to
dispose of lactic acid as the tissues of the normal rats. There is
the possibility that the presence of a large tumor, by its general
effect on the health of the animals, lowers the capacity of the
non-cancerous tissues to dispose of lactic acid. In this case the
values calculated for the rate of glycolysis of the tumor would be
too high. In order to investigate this source of error, the
glucose tolerance of rats with large tumors was determined,
under the assumption that if the disposal of lactic acid is affected
by the presence of a large tumor the same might also be true for
glucose. It should be noted that the rats used for the experi-
ments in Table III showed no signs of cachexia, the average
weight of the tumors being 6.5 gm. per 100 gm. of body weight.
The rats used for the glucose experiments had larger tumors,
since the average tumor weight was 9.9 gm.; nevertheless, the
tolerance for intravenously injected glucose was not diminished.
Table IV indicates that a rate of infusion of 250 mg. of glucose
per 100 gm. of body weight per hour is tolerated without an
appreciable rise in blood sugar, while a rate of infusion of 300 mg.

<table>
<thead>
<tr>
<th>Body Weight</th>
<th>Tumor in Per Cent of Body Weight</th>
<th>Glucose Infused per 100 Gm. of Body Weight per Hour</th>
<th>Sugar Excreted per 100 Gm. of Body Weight per Hour</th>
<th>Blood Sugar</th>
<th>Rate of Infusion Above or Below Tolerance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gm.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>243</td>
<td>14.0</td>
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<td>223</td>
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<tr>
<td>184</td>
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<td>0.30</td>
<td>8.1</td>
<td>207</td>
<td>Below</td>
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<tr>
<td>185</td>
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<td>0.23</td>
<td>1.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>303</td>
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<td>1.4</td>
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<tr>
<td>337</td>
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<td>172</td>
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<tr>
<td>165</td>
<td>12.7</td>
<td>0.30</td>
<td>8.5</td>
<td>107</td>
<td>Above</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>14.4</td>
<td>368</td>
<td>&quot;</td>
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per hour is above the tolerance limit. This corresponds exactly to the values obtained previously on normal rats (4).

**DISCUSSION**

Warburg observed the following rates of aerobic glycolysis of the Jensen sarcoma *in vitro*, when the sections of the tumor were suspended in blood serum.

<table>
<thead>
<tr>
<th>Glucose Concentration of Blood Serum</th>
<th>Mg. Lactic Acid Produced per 100 Gm. Fresh Tumor per Hour</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.2</td>
<td>1,400</td>
</tr>
<tr>
<td>0.1</td>
<td>1,000</td>
</tr>
<tr>
<td>0.05</td>
<td>400</td>
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</tbody>
</table>

It is seen that the rate of aerobic glycolysis of the tumor *in vitro* is influenced in a marked way by the glucose concentration. The rate of glycolysis changes considerably between the concentrations of 0.05 and 0.1 per cent and attains a maximum at a concentration of 0.2 per cent. The glycolysis of the tumor in the living animal, as determined in the experiments in Table III, varied between 570 and 800 mg. lactic acid per 100 gm. tumor per hour, with an average of 690 mg. In order to compare the rates of glycolysis under *in vivo* and *in vitro* conditions, one must know how high is the glucose concentration in the tumor in the living animal. In determinations made in the first paper of this series (8), the tumor tissue contained on an average 51 mg. per cent sugar, when the average blood sugar of the tumor-bearing animals was 135 mg. per cent. In the present experiments the average blood sugar was 138 mg. per cent and the average sugar concentration in the tumor tissue was 70 mg. per cent. At this sugar concentration in the tumor the glycolysis should amount to 640 mg. lactic acid per 100 gm. tumor per hour, when calculated on the basis of the rate of glycolysis at the same sugar concentration *in vitro*. The value of 690 mg. lactic acid which was found in the experiments in Table III is only slightly higher. Warburg found that the arterial blood of the Jensen sarcoma contained on an average 124 and the venous blood 54 mg. per cent sugar. He assumes, on the basis of his determinations of the rate of glycolysis *in vitro*, that the tumor cells glycolyze at a
different rate in different parts of the tumor. The tumor cells adjacent to the arterial part of the blood capillaries glycolyze at a sugar concentration of 124 mg. per cent and their rate of glycolysis should therefore be 1000 mg. lactic acid per hour. On the other hand, the tumor cells adjacent to the venous end of the capillaries are surrounded by a sugar concentration of only 54 mg. per cent and this should reduce their rate of glycolysis to 400 mg. lactic acid per hour. The rate of glycolysis of the tumor as a whole would then be 700 mg. lactic acid per hour. The value actually determined in the present experiments is almost identical with Warburg's estimate. This indicates that the lactic acid production of the tumor proceeds at the same rate under in vivo and in vitro conditions, provided the glucose concentration is equal.

The present experiments emphasize the importance of the sugar concentration for the glycolytic activity of the tumor in the living animal. At a normal blood sugar level the glycolysis corresponds to one half the maximal possible glycolysis of the tumor. Under these conditions the tumor derives approximately 15 per cent of its total energy from glycolysis, the remaining 85 per cent being supplied by oxidation. At higher blood sugar levels the sugar concentration in the tumor is increased and this results in a greater rate of glycolysis. Previous experiments (8) have shown that the tumor is very permeable to glucose at higher blood sugar levels. Thus, when the blood sugar was raised to 360 mg. per cent by an injection of glucose, the sugar concentration in the tumor rose to 250 mg. per cent, which is five times higher than at a normal blood sugar level. In this case the tumor glycolyzes at its maximum rate and derives approximately 30 per cent of its energy from glycolysis.

The influence of the blood sugar concentration on the activity of the tumor raises the question as to whether any benefit would be derived from a low carbohydrate diet in the treatment of patients suffering from malignancy. The fact, that aerobic glycolysis is one of the fundamental characteristics of the malignant cell, should be sufficient reason to keep the glycolytic activity of the tumor at a low level by preventing an undue rise
in the blood sugar concentration. Since the hyperglycemia following an ordinary meal containing a full share of carbohydrates rarely exceeds 150 mg. per cent, a severe restriction of carbohydrates seems unnecessary, but food which tends to increase the blood sugar above this level should be avoided.

**SUMMARY**

1. The tolerance limit of normal rats for intravenously injected sodium d-lactate, using the lactic acid content of blood and urine as an index, was at a rate of infusion of $95 \pm 5$ mg. of lactic acid per 100 gm. of body weight per hour.

2. Rats with tumors weighing from 14.2 to 21 per cent of their body weight showed a marked increase in the lactic acid content of the blood. When the tumors corresponded to less than 10 per cent of the body weight, the lactic acid content of the blood was within normal limits.

3. The intravenous lactic acid tolerance of tumor-bearing rats was decidedly lower than that of normal rats and the decrease in tolerance was fairly proportional to the size of the tumor. The lower tolerance is ascribed to the fact that the tumor produces a certain amount of lactic acid which adds itself to the amount of lactic acid which is being infused.

4. By determining the difference in the lactic acid tolerance between normal and tumor-bearing rats it was possible to arrive at an estimate of the rate of lactic acid production of the tumor tissue in the living animal. The values obtained varied between 570 and 800 mg. lactic acid per 100 gm. of fresh tumor per hour with an average of 690 mg.

5. The rate of glycolysis of the tumor *in vivo* depends on the blood sugar concentration. At a normal blood sugar level of the tumor-bearing animals the glycolysis corresponds to one half the maximal possible glycolysis of the tumor.

6. The intravenous glucose tolerance of tumor-bearing rats is the same as that of normal rats.

The authors wish to thank the Cancer Institute of Columbia University for supplying us with the Jensen rat sarcoma which was used for the transplantations.
REFERENCES


* Presented in part before the twentieth annual meeting of the American Association for Cancer Research in Rochester, New York, April, 1927.

* Determinations of the oxygen consumption of the tumor in the living animal will be reported in a subsequent paper.