Oxidation of C\(^4\)-labeled Carbohydrate Intermediates
in Tumor and Normal Tissue*

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Attempts to characterize the metabolic pattern in neoplastic tissue have occupied the attention of biochemists for over 4 decades. The search for a single distinguishing biochemical characteristic which is inclusive of all tumors and exclusive of all normal tissues has not been fruitful, although most tumors, regardless of histogenesis (17), tend to demonstrate a somewhat characteristic pattern in metabolizing glucose when studied in vitro. A high anaerobic glycolysis, a moderately high aerobic glycolysis, a moderately good Pasteur Effect, a moderately high respiration, and a respiratory quotient which is intermediate between 0.7 and 1.0 are some of the biochemical properties which are noted quite constantly in a high proportion of malignant tumors (6).

In his classical studies of tumor metabolism Warburg (53) placed great emphasis upon the relatively high rates of aerobic glycolysis shown by tumor slices as evidence for a defect in respiration which distinguished them from normal tissues. Dickens and Simer (10) then pointed out that tumors, in contradistinction to normal tissues (brain, retina, embryo) having a high anaerobic glycolysis, showed respiratory quotients of only 0.85 instead of 1.0 when incubated aerobically with glucose. Next it was shown that the concentration of a number of oxidative enzymes was reduced in various tumors. Schneider and Potter (47) reported that the succinic acid dehydrogenase, cytochrome c, and cytochrome oxidase activities of the rat hepatoma were only 22–30 per cent of those found in normal liver. Catalase was reported by Greenstein et al. (18) to be absent in transplanted hepatoma, and a number of flavin enzymes (d-amino acid oxidase, cytochrome c reductase, and xanthine dehydrogenase) have been found to be markedly reduced by other investigators (48, 40, 17). In addition to this, Pollack, Taylor, and Williams (37), Shack (48), and Kessler, Sugiura, and Rhoads (20) have shown that the level of certain vitamins of the B-complex and their associated coenzymes are greatly reduced in tumors. The data on this subject for the primary rat hepatoma are shown in Table 1.

<table>
<thead>
<tr>
<th>Vitamin*</th>
<th>Per cent Coenzyme Per cent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thiamine</td>
<td>28</td>
</tr>
<tr>
<td>Riboflavin</td>
<td>18</td>
</tr>
<tr>
<td>Nicotinic acid</td>
<td>17</td>
</tr>
<tr>
<td>Pyridoxine</td>
<td>11</td>
</tr>
<tr>
<td>Pantothenic acid</td>
<td>10</td>
</tr>
<tr>
<td>Biotin</td>
<td>10</td>
</tr>
</tbody>
</table>

References:
* (87)  † (48)  ‡ (84)

§ Cohen et al. (8) have shown that transaminase in hepatoma is reduced to 50 per cent of that found in normal liver.

It was this peculiarity of tumor tissue which first attracted our attention to the problem of tumor metabolism 2 years ago, at which time we were concerned experimentally with the effects of vitamin deficiency and depressed tissue coenzyme levels upon the metabolism of surviving tissues (28–30, 33). As seen in Table 1, six vitamins of the B-complex occur in hepatoma to the extent of only 10–28 per cent of their concentration in normal liver. This amount of depletion of a vitamin in a normal tissue almost invariably leads to changes

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in tissue metabolism, and it seemed not improbable that if a tissue undergoing carcinogenesis lost up to 90 per cent of its vitamin and coenzyme content, some of the alterations in metabolism found in the neoplastic tissue might be explicable on this basis. On the other hand, it seemed unwarranted to draw conclusions about the efficacy of any given reaction in tumor tissue without exhaustive study, since the malignant tumor, despite its apparent deficiencies, appears to metabolize and grow in a highly effective manner.

Previous studies in our laboratory had shown that the effect of depletion of a given coenzyme in heart muscle by dietary restriction of the appropriate vitamin in rats and ducks gave unpredictable effects upon the metabolism of surviving tissue preparations. In thiamine (30) and biotin (29) deficiencies, the oxidation of pyruvate was markedly reduced in slices, whereas in pantothenic acid deficiency (33), even with reduction of cardiac coenzyme A to 20 per cent of normal, there was only a minimal effect upon pyruvate oxidation when studied with C14-labeled pyruvate. When pyruvate oxidation was studied in homogenates of pantothenic acid-deficient heart muscle, however, a marked defect, particularly in the oxidation of the C4-fragment derived from pyruvate, was observed. It was shown, furthermore, in homogenates of deficient ventricle, that citrate formation from pyruvate and fumarate was markedly reduced and could be restored by addition of coenzyme A (27). It appeared, therefore, that the enzymic architecture of the slice (and presumably the whole organ) was such that depletion of one factor required in the oxidative process might occur without serious effect, while the depletion of another co-factor required in a different step might seriously inhibit the same oxidative process. Comparative study of both slices and homogenates in deficient tissues where respiration was maintained in slices also seemed indicated. A study of hepatoma from this point of view seemed particularly attractive because of the moderately good respiration observed in slices of this tumor (6), despite the general poverty of respiratory catalysts and coenzymes.

In studying the metabolism of oxalacetate and pyruvate in fortified homogenates, Potter, LePage, and Klug (42) noted that while preparations of brain, liver, kidney, and heart tissue respired well in the presence of these acids, those of the primary hepatoma and two other tumors did not. Even with an actively glycolyzing system to maintain ATP levels in the tumor homogenate, the oxidation of oxalacetate was found to be negligible (41). Since the oxygen uptake reported for these experiments with tumor homogenates was much lower than that observed in slices of these tumors, it occurred to us that these discrepant results might be analogous to those obtained by us with homogenates and slices of pantothenic acid-deficient cardiac muscle slices. In this situation it was apparent that, although the depression of tissue coenzyme A levels was not sufficient to depress the oxidation of pyruvate in the slice, upon homogenization of the tissue with further dilution, the concentration of coenzyme A became limiting. Since the hepatoma is depleted not only of many coenzymes but also of certain apoenzymes, it seemed not unreasonable that its respiration should be abolished by homogenization.

Studies of the comparative respiration of homogenates and slices of the primary rat hepatoma were thus undertaken. It also seemed important to carry out a series of studies of the rate of oxidation of glucose and a number of carbohydrate intermediates labeled with radioactive carbon by slices of tumor, since, to our knowledge, no such study had been described in the literature. The use of tagged substrate, furthermore, seemed to provide the perfect opportunity to determine the actual magnitude of the oxidative pathway in tumor tissue and attempt to define the nature of the "defect," if any, which has been purported to exist in tumor respiration. The fate of labeled glucose, pyruvate, natural and unnatural lactate, acetate, and succinate have been studied in order to determine the disposition of substrate at various stages in the scheme of carbohydrate breakdown. Varying initial concentrations of substrate were used in order to evaluate mass action effects.

PROCEDURE

The tumor used in this study was the primary hepatoma induced by feeding young rats of the Sprague-Dawley (Hisaw) strain m'-methyl-p-dimethylaminoazobenzene at a level of 0.06 per cent in a diet moderately low in protein (12 per cent casein) and riboflavin (2 mg/kg) (15). The rats were kept on the carcinogenic diet for 5 months and then fed Purina Chow until hepatic tumors were palpable, at which time the animals were sacrificed for in vitro metabolic studies. Control animals received the experimental diet less azo dye for 3 months and then Purina Chow until used. Liver slices from fasted control rats were used as the control normal tissue in all experiments, and heart, brain, and liver slices from fed control rats were used in a few experiments. Only white, firm, grossly non-necrotic hepatoma nodules were selected for experiments with tumor tissue, and upon microscopic examination these pre-
sented the pathological picture of mixed hepatoma-cholangioma, with the ratio of hepatoma to cholangioma cells 7:1 or higher. Figure 1 shows a portion of a section of azo dye liver tumor which is almost exclusively hepatoma, and Figure 2 shows a portion of another section showing mainly the cholangioma formation.

The standard Warburg technic was employed in these experiments. Thin slices or homogenates of hepatoma and other normal tissues were incubated aerobically with radioactive substrates in a phosphate-saline medium (29) at 37° for 1 hour. The oxygen consumption was determined manometrically, the rate of oxidation of labeled substrate was determined by counting the activity of the C\textsubscript{14}O\textsubscript{2} trapped in the center well with KOH, and the rate of substrate disappearance by applying either chemical (29), physical (36), or enzymatic technics (52) to the contents of the Warburg flask at the end of the period of incubation. Several radioactive substrates were used at concentrations ranging from 2.5 to 40 mM/l. Uniformly labeled glucose-C\textsubscript{14} was prepared photosynthetically from C\textsubscript{14}O\textsubscript{2} as plant starch (23) and recrystallized with carrier after hydrolysis of the starch. Samples of glucose prepared by this method show only a very small percentile impurity when chromatographed on paper.\textsuperscript{1} Uniformly labeled \( l(+)-lactate\)-C\textsubscript{14} and \( d(-)-lactate\)-C\textsubscript{14} were prepared by microbiological fermentation of evenly labeled glucose using \textit{L. delbruckii} in the case of the natural isomer and \textit{L. leishmanii} in the case of the unnatural isomer (3). These compounds were isolated as their zinc salts and characterized by rotation, water of crystallization, and carbon analysis. Acetate-1-C\textsubscript{14} (carboxylic-labeled) and \( -2\)-C\textsubscript{14} (methyl-labeled) were prepared from C\textsubscript{14}O\textsubscript{2} and C\textsubscript{14}H\textsubscript{2}O\textsubscript{14}, respectively (45). Pyruvate-2-C\textsubscript{14} (carboxyl-labeled) and \( -3\)-C\textsubscript{14} (methyl-labeled) were prepared from the acetate-1-C\textsubscript{14} and \( -2\)-C\textsubscript{14}, respectively, via acetyl halide and acetyl cyanide, with subsequent hydrolysis to yield pyruvic acid, which was then distilled, and the sodium salt recrystallized from dilute ethanol (33). Carboxyl-labeled succinate-C\textsubscript{14} was prepared from unlabeled lithium acetylide by carbonation with C\textsubscript{14}O\textsubscript{2} and subsequent catalytic reduction of the acetylenedicarboxylic acid (51). The final product melted at 190° and showed no depression when mixed with authentic succinic acid. The radioactivity of these compounds was determined by the wet combustion method of Van Slyke and Folch (57), and oxidation rates in vitro were determined by precipitating the C\textsubscript{14}O\textsubscript{2} trapped in the center well as BaC\textsubscript{14}O\textsubscript{4} which was counted on planchets with an end-window Geiger-Müller counter (29). All metabolic data including gas exchange, C\textsubscript{14}O\textsubscript{2} production, and substrate utilization are expressed in terms of metabolic quotients (Q) which are defined as the number of \( \mu l \) of gas or substrate (1 \( \mu l \) = 22.4 \( \mu l \)) used or formed per milligram dry weight of tissue per hour. The final dry weight of the tissue was used as the basis for these calculations. The calculation of Net-\( \text{Q}_{\text{Pyr}} \) and of \( \text{Q}_{\text{C\textsubscript{14}O\textsubscript{2}}} \) was carried out as previously described (29, 30).

\textbf{TABLE 2}

\textbf{METABOLISM OF PYRUVATE IN SLICES AND HOMOGENATES OF HEPATOMA AND NORMAL LIVER*}

<table>
<thead>
<tr>
<th>TISSUE</th>
<th>No. of EXPERI-</th>
<th>PREPARATION</th>
<th>( Q_{\text{Pyr}} ) total</th>
<th>( \Delta ) lactate</th>
<th>Net ( C\textsubscript{14}O\textsubscript{2} )</th>
<th>( C\textsubscript{14}O\textsubscript{2} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hepatoma</td>
<td>15</td>
<td>Slice</td>
<td>7.0</td>
<td>-4.1</td>
<td>+1.8</td>
<td>-2.3</td>
</tr>
<tr>
<td>Liver</td>
<td>4</td>
<td>Homogenate</td>
<td>1.5</td>
<td>-2.5</td>
<td>+2.3</td>
<td>-0.2</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>Slice</td>
<td>7.4</td>
<td>-5.0</td>
<td>+1.0</td>
<td>-4.0</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>Homogenate</td>
<td>6.8</td>
<td>-4.0</td>
<td>+0.7</td>
<td>-3.3</td>
</tr>
</tbody>
</table>

\* 0.5 M of 10 percent homogenate in KCl was added to prepared Warburg flasks containing 5.5 ml. phosphate-saline with pyruvate-1-C\textsubscript{14} (5 \( \times 10^{-3} \) M) or pyruvate-8-C\textsubscript{14} (5 \( \times 10^{-3} \) M), ATP (10^{-3} M) and cytochrome c (10^{-3} M). The gas phase for homogenate experiments was air; for slices, O\textsubscript{2}.

\textsuperscript{1} Private communication from Dr. Paul Zamecnik of the Massachusetts General Hospital.
indicated by the greater recovery of C\textsuperscript{14}O\textsubscript{2} from pyruvate-2-C\textsuperscript{14} than the liver slice, even though the total O\textsubscript{2} consumption was approximately the same. As previously noted by Potter, LePage, and Klug (42), homogenates of hepatoma fortified with ATP and cytochrome c did not respire appreciably or oxidatively catabolize pyruvate, the small amount of pyruvate disappearance being accounted for by reduction to lactate. Besides blocking anabolic reactions of pyruvate, homogenization of liver resulted in an increased rate of decarboxylation of pyruvate and a decreased rate of oxidation of the C\textsubscript{2} fragment with about the same oxygen consumption as in the slice.

Attempts to restore the oxidative capacity of homogenates of hepatoma by addition of liver boiled juice, of co-factors (in the following amounts per flask: cocarboxylase, 8 \mu g.; DPN, 50 \mu g.; TPN, 10 \mu g.; and coenzyme A, 20 acetylation units), or of fluoride (39) with or without co-factors were unsuccessful. It is possible that the absence of unknown co-factors, insufficient amounts of known co-factors, a deficiency in oxidative apoenzymes, or an excess of enzymes catabolic to ATP and co-factors are responsible for this failure. It is of great interest, however, that despite the impotence of even the fortified homogenate in oxidizing pyruvate to CO\textsubscript{2}, the intact hepatoma cell is fully able to carry out this oxidation, which involves the co-ordination of many oxidative enzymes present in low amounts in this tumor.

Metabolism of pyruvate-1-C\textsuperscript{14}, -2-C\textsuperscript{14}, and -3-C\textsuperscript{14} in slices of hepatoma and normal liver.—The terminal reactions in the oxidation of carbohydrate in hepatoma slices, as studied with 2-, 3- and 4-carbon acids shall be considered first. Certain data on the metabolism of pyruvate-1-C\textsuperscript{14} and -2-C\textsuperscript{14} in hepatoma slices shown in Table 2 have already been discussed in connection with the parallel studies on homogenates. In Chart 1 more complete information about the metabolism of all three radioisomers of pyruvate in hepatoma and liver at initial concentrations of substrate ranging from 2.5—40 mM/l is presented. Data on Q_{O2} and Q_{CO2} are presented in the lower graphs, and data on disposition of substrate are shown in the upper graphs. The top curve in the upper graph represents total pyruvate disappearance as a function of initial concentration, the bottom curve represents the rate of conversion of pyruvate to lactate, and the intermediate cross-hatched area signifies the amount of pyruvate undergoing oxidation, as determined from the rate of decarboxylation of carboxyl-labeled pyruvate. The area bounded by the top curve and the next one below it, therefore, represent the conversion of pyruvate to nonlactate products without loss of CO\textsubscript{2}. This fraction is regarded as the anabolic fraction and probably represents conversion of pyruvate to hexose, triose, or triose precursors via reactions of the glycolytic chain (33).

There are both similarities and significant dif-
ferences in the response of hepatoma and liver to increasing concentrations of pyruvate. In both cases the total utilization of pyruvate and the production of C\textsuperscript{14}O\textsubscript{2} are positive functions of initial concentration of substrate. The total utilization of pyruvate in hepatoma was 50 per cent greater than in liver at 2.5 mm/l and 35 per cent less at 40 mm/l. The families of curves describing C\textsuperscript{14}O\textsubscript{2} production from each of the differently labeled isomers of pyruvate show interesting differences. In liver the three curves are widely separated, suggesting significant diversion of substrate carbon from the main oxidative pathway at several steps in the reaction sequence. Diversion of C\textsubscript{1}-fragments from pyruvate into ketone body synthesis, lipogenesis, and other acylations (1) undoubtedly accounts for part of the difference in the recovery of C\textsuperscript{14}O\textsubscript{2} from pyruvate-2-C\textsuperscript{14} and -3-C\textsuperscript{14}. Another factor operating to reduce the recovery of C\textsuperscript{14}O\textsubscript{2} from pyruvate-2-C\textsuperscript{14} and -3-C\textsuperscript{14} is extensive exchange between the acids of the Krebs tricarboxylic acid cycle and nonisotopic compounds in equilibrium with them. In hepatoma slices the curves for recovery of C\textsuperscript{14}O\textsubscript{2} from the differently labeled pyruvates are much closer together, suggesting that diversion or exchange of the oxidative intermediates with nonisotopic endogenous compounds is considerably less in tumor tissue than in liver. It is also of interest that, although the rate of oxidation of the C\textsubscript{1}-fragment derived from pyruvate is more rapid in tumor at 5 mm/l (see also Table 2), the rates of its oxidation in the two tissues at 40 mm/l are identical. The total C\textsuperscript{14}O\textsubscript{2} produced from added pyruvate was greater in liver than in tumor at high concentrations of substrate; at 5 mm/l the combustion of added substrate accounted for 50 per cent of the observed oxygen consumption in both tissues, and at 40 mm/l for about 88 per cent of the total oxygen consumption in tumor and 97 per cent of the observed oxygen consumption in liver. Respiratory quotients based upon total CO\textsubscript{2} production were not determined, but values based upon Q\textsubscript{CO\textsubscript{2}} at 40 mm/l of pyruvate were 0.97 in tumor and 1.17 in liver slices. It is of interest that, although liver slices demonstrated anabolic transformations of pyruvate at all concentrations of substrate, such transformations were not evident in tumor slices until the pyruvate level was raised to 10 mm/l, and even at 40 mm/l reached only 40 per cent of that observed in liver. Another interesting effect of increasing pyruvate concentrations in hepatoma slices is the slight but definite lowering of the oxygen consumption which, in a tissue handicapped by lowered concentrations of hydrogen transport enzymes, might be the result of competition between pyruvate or anabolic products of pyruvate and oxygen for available hydrogen or electrons.

Many years ago Elliot, Benoy, and Baker (12) found that pyruvate at 20 mm/l resulted in a disappearance of pyruvate and stimulation of the R.Q. above unity in slices of Philadelphia No. 1 sarcoma and Walker carcinoma 256. Although they interpreted their data to indicate a limited conversion of pyruvate to succinate, these data, taken in conjunction with our own, strongly suggest that some tumors (perhaps all) have a sizeable aerobic pathway for the terminal oxidation of carbohydrate.

Metabolism of uniformly labeled L(+)- and D(−)-lactate-C\textsuperscript{14} in slices of hepatoma and normal liver.—Data bearing on the metabolism of natural lactic acid by tumor and liver are presented in Chart 2. As in the case of pyruvate, increasing concentrations of L(+)-lactate resulted in an increased utilization of this substrate in both tissues. Although utilization was equal in tumor and liver at 5 mm/l, tumor lagged by 30 per cent at 40 mm/l. Keto acid appearance, which apparently accounted for some of the lactate which disappeared, Q\textsubscript{CHO\textsubscript{2}}, and C\textsuperscript{14}O\textsubscript{2} production increased slightly with increases in concentration of substrate in both tumor and liver. The absolute rates of oxidation of L(+)-lactate in tumor and liver were almost identical at all levels of substrate and accounted for about 80 per cent of the observed oxygen consumption. The amount of lactate undergoing oxidation beyond pyruvate was calculated from the Q\textsubscript{CO\textsubscript{2}}, values in Chart 2 by referral to the curves for Q\textsubscript{CO\textsubscript{2}} (total) and the Q\textsubscript{CO\textsubscript{2}} (carboxyl) in Chart 1, and plotted on the upper diagram in Chart 2 as the cross-hatched area. It was of some interest that, although lactate was oxidized only \( \frac{1}{3} \) as fast as pyruvate at comparable concentrations, it was anabolized 3–4 times as well as pyruvate in both liver and tumor. Although the depressing effect of substrate load upon Q\textsubscript{CO\textsubscript{2}} in tumor was not noted with lactate as substrate, it is doubtful that the better anabolism of lactate is due only to the presence of two extra hydrogen atoms, since a more active anabolism of lactate was also noted in liver. The level of keto acid in these experiments in no way approaches the levels obtained when pyruvate was added as substrate, and yet the rate of anabolic transformation is greater with lactate. These data and certain others obtained by Miller (24) and Brin (3) suggest that a pathway of lactate toward phosphoglyceric acid which circumvents the pyruvate pool may be present in both tumors and normal tissues. Elliot, Benoy, and Baker (12) observed negligible effects of DL-lactate on R.Q. and Q\textsubscript{CO\textsubscript{2}} of Philadelphia sarcoma and Walker 256.
tumor, although small amounts of substrate disappeared.

From the historical point of view the data on oxidation of lactate-C\textsuperscript{14} are of interest in connection with the calculation of the Meyerhof Oxidation Quotient (5) as a measure of the Pasteur Effect. This formulation attempted to relate the decrease in glycolysis occurring when a tissue was changed from an anaerobic condition to an aerobic one to the rate of lactic acid oxidation under aerobic conditions, assuming that lactic acid oxidation accounted for the entire observed oxygen consumption, viz: 

\[ \text{M.O.Q.} = \frac{Q_{\text{CO}_2} - Q_{\text{CO}_2}}{Q_{\text{CO}_2}} \]

Although the measurement of the Pasteur Effect in terms of resynthesis is now an outmoded procedure (7, 19, 22), it is of interest that on the basis of true lactate oxidation the M.O.Q. becomes 3-4 times as large. The M.O.Q. for the hepatoma is 2.9 calculated by the classical procedure (6) and 10.1 calculated on the basis of true lactate oxidation. This latter value is high enough to cast additional doubt (22) upon the hypothesis of resynthesis as an explanation of the Pasteur Effect.

The unnatural D(−)-lactate is utilized only about \( \frac{1}{2} \) as well as the natural isomer in both tumor and liver, as shown in Chart 3. The oxidation rate of this compound was also \( \frac{1}{2} \) as rapid as that of L(+)lactate. Less keto acid appeared. There was no effect upon oxygen consumption in the tumor and only a slight effect in liver. These effects may possibly be due to the relatively feeble action of a racemase (3) in these tissues.

**TABLE 3**

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Substrate</th>
<th>FA</th>
<th>Cholesterol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hepatoma</td>
<td>Glucose</td>
<td>224</td>
<td>28</td>
</tr>
<tr>
<td></td>
<td>Acetate</td>
<td>128</td>
<td>43</td>
</tr>
<tr>
<td>Liver (fed)</td>
<td>Glucose</td>
<td>15</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>Acetate</td>
<td>150</td>
<td>78</td>
</tr>
</tbody>
</table>

*ICPM = initial counts per minute of substrate concentration of glucose = 5 mm/l; concentration of acetate = 10 mm/l; time = 6 hrs; medium = Krebs-Ringer.

Succinate which disappeared. The appearance of C\textsubscript{4}O\textsubscript{2} under these conditions suggests, however, that the oxidation of a portion of this fumarate toward lipogenesis and other acylations (1). The degree of stimulation of oxygen consumption corresponds quite closely to that expected for a one-step oxidation of the succinate which disappeared. The appearance of C\textsubscript{4}O\textsubscript{2} under these conditions suggests, however, that the oxidation of a portion of this fumarate had proceeded to oxalacetate or beyond with a small inhibition of endogenous respiration. Small amounts of lactate and keto acid also appeared when hepatoma slices were incubated with succinate.

Walker sarcoma 256, in contrast to the rat hepatoma, is virtually unable to oxidize acetate-1-C\textsuperscript{14} to C\textsuperscript{14}O\textsubscript{2} (36). Part of the nonoxidative or anabolic utilization of acetate is undoubtedly directed toward lipogenesis and other acylations (1).

The data in Table 3 are taken from a study (26) in which acetate-1-C\textsuperscript{14} was incubated aerobically with hepatoma and liver slices for 3 hours and the cholesterol and fatty acids isolated from the slices and counted. The figures suggest that the incorporation of acetate carbon into the fatty acids and cholesterol of hepatoma, though somewhat less rapid than in liver from fed rats, is nonetheless a very active process.

**Metabolism of carboxyl-labeled succinate-C\textsuperscript{14} in slices of hepatoma and liver.**—Succinic acid was chosen for study because of its membership in the tricarboxylic acid cycle and because of the general interest in the succinoxidase system in tumor tissue. Studies of the succinoxidase system in tumor homogenates have shown low values, as previously indicated (47). Previous studies of succinic acid oxidation in slices of tumor tissue have shown some variability (12, 13, 46), although in general the data have shown only small effects of succinate upon \( Q_{\text{CO}_2} \) with correspondingly small values for substrate utilization. The metabolic data obtained with carboxyl-labeled succinate-C\textsuperscript{14} in slices of hepatoma and liver in the present study are shown in Chart 5. A double ordinate representing substrate disposition on the one hand and gas exchange on the other is so numbered that a change of 2 \( \mu l \) of substrate is equivalent to a change of 1 \( \mu l \) of \( O_2 \). This numbering is based upon the theoretical situation of the pure succinoxidase preparation catalyzing the following reaction:

\[ \text{Succinate} + \frac{1}{2} O_2 \rightarrow \text{Fumarate} + H_2O. \]

If the two lines for oxygen consumption and substrate utilization were to coincide in Chart 5, the slice would be functioning as a succinoxidase preparation with complete suppression of endogenous respiration.

In hepatoma there was a stimulation of oxygen consumption as succinate concentration was raised, although the effect was negligible after 20 mm/l was reached. The degree of stimulation of oxygen consumption corresponds quite closely to that expected for a one-step oxidation of the succinate which disappeared. The appearance of C\textsubscript{4}O\textsubscript{2} under these conditions suggests, however, that the oxidation of a portion of this fumarate had proceeded to oxalacetate or beyond with a small inhibition of endogenous respiration. Small amounts of lactate and keto acid also appeared when hepatoma slices were incubated with succinate.
CHART 2.—The metabolism of uniformly labeled \( \text{L}(+)\)-lactate-\( ^{14}\text{C} \) in slices of hepatoma and rat liver. Metabolic quotient is plotted against substrate concentration. Disposition of substrate is represented in the top graphs and gas exchange in the bottom ones. Each point represents at least four determinations.

CHART 3.—The metabolism of uniformly labeled \( \text{D}(–)\)-lactate-\( ^{14}\text{C} \) in slices of hepatoma and rat liver. Metabolic quotient is plotted against substrate concentration. Disposition of substrate is represented in the top graphs and gas exchange in the bottom ones. Each point represents at least four determinations.
As the succinate concentration was raised in experiments with liver slices, there was a steady rise in the $Q_{O_2}$ from the endogenous value of 8 to 28, accompanied by a biphasic change in C$^{14}O_2$ production and in the accumulation of lactic and keto acids. In view of the close approximation of the lines for $-Q_{O_2}$ and $-Q_{succinate}$ at 40 mM/l, it seemed probable that at this concentration of succinate the liver slice was approaching the status of a succinoxidase preparation and pouring so much succinate hydrogen into the hydrogen transport system that other oxidations, including those of fumarate, were inhibited. Studies of the behavior of liver slices at higher concentrations of succinate-C$^{14}$ are in progress in an attempt to delineate the relationship between succinic dehydrogenase and the cytochrome system in the intact liver cell.

By use of the data on succinic acid disappearance at 40 mM/l in Chart 5 as an index of succinoxidase activity, the ratio of activities obtained for hepatoma and liver slices is 10:52 (which is probably high, since no plateau was obtained in liver), compared to a ratio of 36:88 obtained in homogenates of these tissues by Schneider and Potter (47).

The metabolism of uniformly labeled glucose-C$^{14}$ in slices of brain, heart, liver, and hepatoma.—An investigation of the oxidative metabolism of glucose-C$^{14}$ in hepatoma slices seemed particularly desirable because of the extensive literature presenting evidence interpreted as consistent with the existence of a metabolic defect in the oxidation of glucose by tumor tissue (17). In a preliminary report we had noted, paradoxically, that the oxidation of radioactive glucose to C$^{14}O_2$ appeared to proceed faster in hepatoma than in liver from which it was derived. The data shown in Chart 6 confirm and extend these observations. In the upper graphs of Chart 6, the glucose uptake is plotted as triose in order to express the transformations of substrate in terms of C$^3$-fragments. The amount of C$^3$-compound undergoing decarboxylation was obtained from the C$^{14}O_2$ production in the same way as for lactate (vide supra) and plotted as the cross-hatched area. Aerobic glycolysis is represented by the area below the curve marked +Lac in the upper graphs.

As in the case of other substrates tested in this study, the rates of removal and oxidation of glucose increased with increasing concentrations of substrate, although in tumor and liver the rates were markedly different. In hepatoma slices about 10 per cent of the glucose which disappeared was oxidized to CO$_2$, and about 80 per cent appeared as lactate. In liver slices (from fasting rats), the uptake of glucose was lower and the oxidation and aerobic glycolysis almost negligible. In tumor, the combustion of glucose at a concentration of 10 mM/l accounted for about 80 per cent of the observed oxygen consumption, while in liver it accounted for about 2 per cent. At 10 mM/l, further-
Chart 5.—The metabolism of carboxyl-labeled succinate-C\(^14\) in slices of hepatoma and rat liver. Metabolic quotient is plotted against substrate concentration. Substrate disposition and gas exchange are plotted on different scales on the ordinate so arranged that 1 µl. change in \(O_2/C\(^14\)O\(_2\) exchange is equivalent to 2 µl. change in substrate utilization (see text). Each point represents at least four determinations.

Chart 6.—The metabolism of uniformly labeled glucose-C\(^14\) in slices of hepatoma and rat liver. Metabolic quotient is plotted against substrate concentration. Disposition of substrate is presented in the top graphs and gas exchange in the bottom ones. Each point represents at least six determinations.
more, the rate of glucose combustion in tumor was 25 per cent of that of pyruvate and 75 per cent of that of L(+)-lactate at comparable substrate concentrations. The depression of \( Q_o \) with increasing concentrations of glucose in hepatoma is of some interest. It mirrors an effect noted by Elliot and Baker (11) in Philadelphia sarcoma and may be due to the diversion of ATP from oxidative processes to phosphorylation of glucose.

Since the oxidation of glucose in liver was singularly low, it appeared important to compare the hepatoma with other extra-hepatic tissues in regard to rates of glucose oxidation and aerobic glycolysis (31). These data are presented in Table 4. Brain slices which have an aerobic glycolysis comparable to hepatoma oxidized glucose to \( \text{CO}_2 \) 5 times as fast as hepatoma, while heart slices which show a rate of glucose oxidation comparable to hepatoma accumulated lactate only \( \frac{1}{2} \) as fast. Brain and heart thus showed a proportionality between the rates of glucose oxidation and aerobic glycolysis which hepatoma did not. It must be remembered, however, that the rate of lactate accumulation in tissue slices respining under aerobic conditions merely reflects the degree of disparity between the activity of the glycolytic system and the activity of the system oxidizing pyruvate at the particular concentration of pyruvate present in the tissue. The data indicate that the activity of the terminal oxidation system (enzymes of the Krebs tricarboxylic acid cycle) is fully comparable to that of normal liver and cardiac muscle, though less active than that of brain. It is clear, however, that this system does not keep pace with the rate at which \( \text{C}_3 \)-fragments are presented to it by the highly active glycolytic system of the hepatoma.

The finding of a very low rate of glucose oxidation in liver slices in the face of an active terminal oxidation system was very intriguing. The inertness of liver slices towards glucose as regards aerobic glycolysis, \( {\text{C}^4}{\text{O}}_2 \) production, and stimulation of respiration recalled the work of a number of investigators which had failed to show any effect of added glucose upon aerobic or anaerobic glycolysis in either slices (6, 35, 44, 54) or homogenates (91, 49) of liver. The finding of Stoesz and LePage (49) that hexose diphosphate was rapidly glycylized in homogenates of liver tissue, on the other hand, suggested that the enzymatic equipment for glycolysis in liver was intact from the level of aldolase on down. Likewise, the rapidity with which hexose forms glycogen in the intact rat (9), the ease with which radioactive glucose equilibrates with liver glycogen in liver slices (50), and the positive uptake of glucose in the present experiments with fasted liver slices, indicated that the enzymes concerned with the conversion of glucose to glycogen in liver tissue are active. It appeared, therefore, that the site of the physiological blockage of glycolysis in liver tissue was between glucose-6-phosphate and hexose diphosphate in the Embden scheme. Studies of the rates of anaerobic glycolysis in homogenates of many tissues, including hepatoma, with glucose-6-phosphate, fructose-6-phosphate, and hexose diphosphate as substrates indicated that, although fermentation of hexose-diphosphate was most active in all tissues, the most marked lag between the values obtained for hexose diphosphate and fructose-6-phosphate or glucose-6-phosphatase was noted in rat liver (31). Assays for phosphohexokinase were then carried out on tissue extracts by the method of Racker (43) with the results shown in Table 5. Of the tissues tested, rat liver had the lowest content, with hepatoma about 8 times as active as liver. The fact that lipogenesis from glucose is more active in hepatoma than in rat liver, as shown in Table 5, is also consistent with the data on distribution of phosphohexokinase. It is of interest that duck liver, which is phylogenetically slightly more primitive, has a higher content of phosphohexokinase than rat liver, and that this is associated with increased ability of slices to oxidize and glycolyze added glucose (see Table 4).

Although Orr and Strickland (35) observed high rates of anaerobic glycolysis in livers rich in glyco-
gen from fed rats, a finding which has been questioned by Burk (6), we observed very little effect of high glycogen content upon aerobic glycolysis in our experiments, as shown in Table 6. The slightly greater endogenous lactate formation in liver slices from fed rats may represent breakdown of triose and other intermediates below the level of hexose diphosphate which may be present in greater quantity in livers from fed rats. Addition of C\textsubscript{14}-glucose to slices of liver from fed rats did not materially affect the aerobic glycolysis, and the rate of its oxidation to C\textsubscript{14}O\textsubscript{2} was somewhat less, as would be predicted from the dilution of added glucose-C\textsubscript{14}, with glucose appearing in the medium through glycogenolysis of liver glycogen.

The synthesis of glycogen in liver slices from pyruvate, lactate, and CO\textsubscript{2} is well annotated (4). In our own studies the anabolism of pyruvate and lactate toward carbohydrate was more active in liver than in tumor. One may well inquire, assuming the activity of phosphohexokinase is limiting in glycolysis in liver, how the reverse reactions are successfully carried out. The answer is found in the fact that two enzymes are required for the interconversion of fructose-6-phosphate and fructose diphosphate, viz.:

\[\text{Phosphohexokinase} + \text{ATP} \rightarrow \text{Fructose-6-PO}_4 \rightarrow \text{Fructose-1,6-(PO}_4)_2.\]

\[\text{Phosphatase}\]

Inactivity of the phosphohexokinase system in no way vitiates the reverse reaction, because it is catalyzed by a different enzyme. This system is analogous, of course, to the hexokinase system, viz.:

\[\text{Hexokinase} + \text{ATP} \rightarrow \text{Glucose} \rightarrow \text{Glucose-6-phosphate.}\]

\[\text{Phosphatase}\]

The operation of these systems in normal liver is such that a conservation of glucose for secretion into the blood and nourishment of the extra-hepatic tissues is achieved. The relative inactivity of the phosphohexokinase system in liver serves as a ball valve to prevent h e x o s e c a r b o n , once synthesized from smaller molecules, or captured from the blood stream, from being broken down via the reactions of glycolysis. The oxidative requirements of the liver are apparently met by combustion of fat and 2- and 3-carbon acids.

**GENERAL CONCLUSIONS ABOUT THE OXIDATIVE PATHWAY IN HEPATOMA SLICES**

Is there a “defect” in the respiration of tumor tissue as Warburg thought? In the hepatoma the magnitude of the pathway for oxidation of 2-, 3-, and 4-carbon intermediates of carbohydrate metabolism is within the range found for normal tissues, the values for hepatoma being very close to those found in liver. The relative rates of C\textsubscript{14}O\textsubscript{2} production from acetate and pyruvate labeled in each of their carbon atoms in hepatoma slices, the isolation of radioactive citrate from tumors oxidizing radioactive fatty acids by Weinhouse, Millington, and Wenner (55), and the identification of most of the enzymes which catalyze reactions of the Krebs tricarboxylic acid cycle in tumor tissue (40, 47, 55) suggest, furthermore, that the mechanism of the terminal oxidation of substrate in tumor tissue is the same as in normal tissues. A defect in the efficiency of coupling of oxidation to high energy phosphate bond generation in tumor tissue cannot, however, be ruled out without further studies.

**BIOCHEMICAL DIFFERENCES BETWEEN LIVER AND HEPATOMA**

A summary of some of the metabolic differences between liver and hepatoma is presented in Chart 7. Although from our studies there appears to be no absolute change in the qualitative aspects of metabolism in the hepatoma, in some cases, the degree of change, quantitatively, in the routing of metabolic traffic is almost tantamount to a qualitative change. In normal liver, glycogen is synthesized from blood glucose and blood lactate, stored, and resecreted as glucose. Very little is glycolyzed. As the liver undergoes neoplastic change, glycolysis increases, anaerobic before aerobic (25), until in the full-blown state the liver tumor is diverting all its glucose uptake into the glycolytic pathway, with a negligible storage of glycogen (17). Carbohydrate synthesis from pyruvate is also reduced. To account for this, our data suggest that both the hexokinase and phosphohexokinase systems become more active in the liver tumor and succeed in diverting glucose into glycolytic channels where glucose carbon becomes available for lipogenesis, amino acid synthesis, purine and pyrimidine synthesis, or further oxidation to CO\textsubscript{2}. The rate of glycolysis exceeds the rate of utilization of C\textsubscript{3}-fragments sufficiently to maintain a high concentration of C\textsubscript{3}-reactants in the tumor tissue (38). The lower contents of oxidative...
enzymes and coenzymes in the hepatoma apparently does not compromise the ability of this tissue to carry out the terminal oxidation of carbohydrate at rates comparable to that of the liver cell. Fewer side reactions appear to occur in the combustion of C1-fragments and C2-fragments via the tricarboxylic acid cycle in the hepatoma than in the liver. Despite oxidative activity, glycolysis continues, presumably because the breakdown of ATP for growth and other anabolic reactions cannot be matched by oxidative phosphorylation (38). Urea synthesis is abolished (17), and the tumor becomes vascularized with an arterial blood supply (2). In all respects the hepatoma has become an extra-hepatic tissue.

The work reported today constitutes only a small beginning in the application of tracer techniques to the problem of the intermediary metabolism of tumor tissue. It seems to me that the working hypothesis for future work in this field should be that the biochemical differentiation of the tumor is peculiar to its needs and that the motivation for the clarification of that biochemical pattern be to understand its operation rather than merely to draw comparisons with a variety of normal tissues.

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![Chart 7](chart7.png)

**Chart 7.**—Summary of the differences in carbohydrate metabolism between liver and hepatoma. The boxes represent adjacent tumor and liver cells and the space between them a blood vessel. The thickness of the lines represents the approximate reaction rates and the arrows their direction. The acids of the Krebs tricarboxylic acid cycle are abbreviated as follows: OAA = oxalacetate, CIT = citrate, α-KETO = α-ketoglutarate, SUCC = succinate, FUM = fumarate, and MAL = malate. Other abbreviations are: FA = fatty acids, CHOL = cholesterol, SR = side reactions.


44. Rosenthal, O., Untersuchungen über Milchsauregärung


Fig. 1.—Rat liver tumor induced by feeding m'-methyl-p-dimethylaminoazobenzene. Zenkers fixative and Masson's trichrome stain with a green filter. Mag. X770. This is almost exclusively hepatoma with very little necrosis. There is exuberant young fibrous tissue proliferation. A portal vein filled with tumor cells is seen to the right of center.

Fig. 2.—Rat liver tumor induced by feeding m'-methyl-p-dimethylaminoazobenzene. Zenkers fixative and Masson's trichrome stain with a green filter. Mag. X770. This shows mainly cholangioma, the bile duct epithelial cells being involved in the neoplastic change. There is a marked stromal reaction and an area of necrosis at the upper right.
Oxidation of C\textsuperscript{14}-labeled Carbohydrate Intermediates in Tumor and Normal Tissue

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