Pyruvate Dehydrogenase, the Citrate Condensing Enzyme and the Utilization of ^{14}C-Labeled Lactate, Pyruvate and Alanine by Slices of Lactating Mammary Gland and Adenocarcinoma of Mouse Mammary Gland[1] (37153)

LEVY KOPELOVICH
(Introduced by M. Lipkin)
Division of Biological Chemistry, Sloan-Kettering Institute for Cancer Research, New York, New York 10021

Aerobic glycolysis has been demonstrated to be characteristic of neoplastic tissues (1). Exceedingly few normal tissues display this capacity (1). The high rates of aerobic glycolysis and lactate production observed in tumor cells have, in general, been attributed to defective mechanisms for the oxidation by the mitochondria of reducing equivalents synthesized in the cytosol (1–5). However, some of these proposed mechanisms may be questionable, nor could they apply to all tumors (1, 6, 7).

As part of a study on the metabolic characteristics of the mammary adenocarcinoma and its preneoplastic nodule outgrowths (8–13), we have assessed the oxidative decarboxylation pattern of lactate, pyruvate and alanine in tissue slices of lactating mammary gland and the mammary adenocarcinoma of C3H/Crgl mice. The utilization of these substrates by both tissues was correlated with the activity of enzymes directly responsible for their entry into the Krebs cycle: namely, pyruvate dehydrogenase, [pyruvate:lipoate oxidoreductase (acceptor-acetylating) (EC 1.2.4.1)] and the citrate condensing enzyme [citrate oxaloacetate-lyase (CoA-acetylating) (EC 4.1.3.7)]. We propose that a decreased oxidation of pyruvate, lactate, and alanine by the mammary adenocarcinoma could be due to the decrease in the activities of pyruvate dehydrogenase and the citrate condensing enzyme, and possibly other mechanisms as well (1–7).

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Materials and Methods. Animals and their treatment. All mice (C3H/Crgl) were obtained from the Cancer Research and Genetics Laboratory, University of California, Berkeley, CA. Normal lactating mice (18 days, suckling 6–8 young) and virgin mice bearing spontaneously occurring tumors were used throughout (8). The mice received a diet of Purina Laboratory Chow and water *ad libitum*.

Preparation of tissue slices and analytical methods. The mice were killed by cervical fracture. Mammary and tumor tissues were removed and placed in ice-cold 0.25 *M* sucrose, and slices were prepared as previously described (8). Tissue slices weighing 250 mg were placed in incubation flasks provided with a center well for the collection of respiratory CO_2. The incubation medium consisted of a Krebs–Henseleit bicarbonate buffer, pH 7.4 (14), that contained 25 μmoles of the labeled substrates in a total volume of 2.0 ml. Where specified, 25 μmoles of glucose was added. The labels of the substrates and their specific radioactivities are given in the tables. The flasks were gassed for 30 sec with a mixture of O_2 and CO_2 (95:5, by vol), then capped and incubated for 3 hr with mechanical shaking at 37°. At the end of the incubation period, 0.3 ml of a mixture of redistilled ethanolamine and 2-ethoxyethanol (1:1, by vol) was injected through the serum cap into the center well. This was immediately followed by the injection of 0.1 ml of 1 *N* HCl and heat inactivation for 30 sec at 90°. The following analytical procedures were performed as

described elsewhere (15): determination of respiratory $^{14}CO_2$ activity, isolation and determination of total ^{14}C activity in fatty acids and chromatographic analyses of amino acids and lactate in the incubation medium.

Preparation of tissue homogenates and assay procedures of pyruvate dehydrogenase and the citrate condensing enzyme. Washed lactating mammary gland and mammary adenocarcinoma slices (8) were homogenized in 3 vol of 0.25 *M* sucrose. The homogenates were fractionated centrifugally (16) as follows: 350*g* for 10 min (Fraction I)[2]; 10,000*g* for 15 min (Fraction II); and 105,000*g* for 60 min (Fraction III). Mitochondria were isolated as described elsewhere (16). For assay of both pyruvate dehydrogenase (17) and the citrate condensing enzyme (18), Fraction I and mitochondria were treated by osmotic shock followed by sonication to disrupt particulate membranes. In some of the determinations of the citrate condensing enzyme, tissue slices were homogenized in 5 vol of 95% ethanol and 0.5 *M* KCl (1:5, by vol) as described elsewhere (18). All preparatory procedures were carried out at 4°. Pyruvate dehydrogenase activity was measured essentially as described by Korkes (17). Reactions were carried out in flasks provided with a center well and incubated at 37° in an atmosphere of O_2 and CO_2 (95:5, by vol). The incubation mixture consisted of 50 μmoles of phosphate buffer (pH 7.4) that contained 1.5 μmoles of $MnCl_2$, 10 μmoles of β-mercaptoethanol, NAD as indicated, 0.05 μmole of CoA, 0.1 μmole of thiamine pyrophosphate, 115 IU of lactate dehydrogenase, 18 IU of phosphotransacetylase, 25 μmoles of pyruvate-1-^{14}C and the enzyme preparation, in a total volume of 1.0 ml. All assays were measured against blank mixtures to which either no phosphotransacetylase or NAD were added. $^{14}CO_2$ was collected and assayed for ^{14}C activity.

Condensing enzyme activity was measured as described by Srere, Brazil and Gonen (18). One unit is equal to the utilization of 1 nmole of substrate/min at 30°. The assay system consisted of 200 μmoles of Tris-HCl buffer (pH 8.0), 2 μmoles of DTNB (5-5 dithio-bis-2-nitrobenzoic acid), 0.18 μmole of acetyl-CoA, 20 μmoles of oxaloacetate and enzyme preparation, in a total volume of 2 ml. All determinations were corrected for acetyl-CoA deacylase (EC 3.1.2.1) activity by incubating the enzyme preparation without oxaloacetate. The extinction coefficient of DTNB at 412 n*M* used in the calculations was 13.6×10^6 per mole.

Unless otherwise specified, measurements of initial enzyme activities were performed under conditions of zero order kinetics, and were proportional to enzyme (protein) concentration. Protein was determined by the biuret method (19) with defatted human serum albumin as standard (20).

Results. The utilization of ^{14}C-labeled pyruvate, lactate and alanine by slices of lactating mammary gland and mammary adenocarcinoma. Figure 1 shows the effects of varying lactate concentrations on $^{14}CO_2$ evolution from lactate-1-^{14}C by slices of lactating mammary gland and mammary adenocarcinoma. At all lactate concentrations (0.5 to 25 m*M*), more $^{14}CO_2$ was formed by lactating mammary gland slices than by mammary adenocarcinoma slices. The formation of $^{14}CO_2$ from lactate-1-^{14}C by both tissues was proportionately similar throughout the entire range of lactate concentration. Addition of glucose stimulated $^{14}CO_2$ production by lactating mammary gland slices, while it decreased that by mammary adenocarcinoma slices (Fig. 1). In both tissues, the addition of lactate above a concentration of 10 m*M*, in the absence or in the presence of glucose, did not effect a further increment in $^{14}CO_2$ yields (Fig. 1). Similar results were obtained with pyruvate-1-^{14}C in slices of both the lactating mammary gland and the mammary adenocarcinoma. In all subsequent experiments, therefore, all substrates were incubated at a concentration of 12.5 m*M*.

Table I shows the results of experiments in which lactating mammary gland slices were incubated with ^{14}C-labeled lactate, pyruvate and alanine in the absence and in the presence of glucose. Although glucose increased $^{14}CO_2$ yields from lactate-1-^{14}C and pyruvate-1-^{14}C, it caused a pronounced decrease of $^{14}CO_2$ production from the second and third

[2] In this paper, also referred to as whole homogenate fraction.

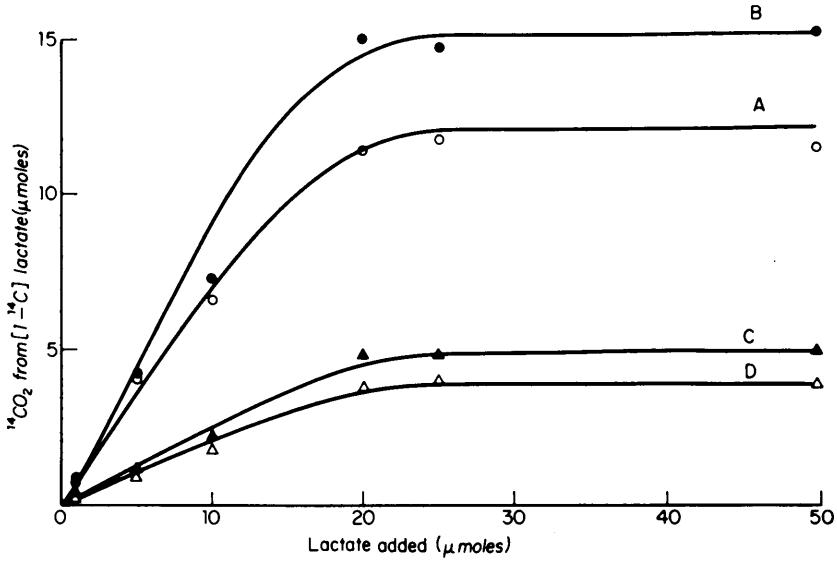

FIG. 1. The effect of lactate concentration on $^{14}CO_2$ production from lactate-1-^{14}C in the absence and in the presence of glucose by lactating mammary gland and mammary adenocarcinoma slices: 250 mg of slices were incubated for 3 hr in 2.0 ml of Krebs–Henseleit bicarbonate buffer (pH 7.4) in a gas phase of O_2 and CO_2 (95:5, by vol). Lactate-1-^{14}C was added as indicated. Where specified, glucose was added at a concentration of 12.5 μmoles/ml. (A, B) Lactating mammary gland plus and minus glucose, respectively; and (C, D) mammary adenocarcinoma minus and plus glucose, respectively.

carbons of lactate, pyruvate and alanine. The addition of glucose enhanced significantly the incorporation into fatty acids of the second and third carbons from lactate, pyruvate and alanine (Table I). Nonetheless, even in the absence of glucose, appreciable amounts of carbon-2-^{14}C and -3-^{14}C from these substrates were incorporated into fatty acids (Table I).

Unlike the lactating mammary gland, CO_2 formation from all three carbons of lactate, pyruvate and alanine by the mammary adenocarcinoma was lower in the presence than in the absence of glucose (Table I). Although the second and third carbons of these substrates were incorporated into fatty acids by the neoplasm, this incorporation was only slightly enhanced in the presence of glucose (Table I). The rates of fatty acid formation from the second and third carbons of lactate, pyruvate, and alanine by the mammary adenocarcinoma were considerably lower than those by the lactating mammary gland (Table I). Similar results were obtained with either acetate or glutamate as substrates (10).

Chromatographic analyses of some amino acids formed from ^{14}C-lactate by slices of lactating mammary gland and mammary adenocarcinoma. As shown in Table II, about 35% of the lactate added (average for all three carbons), was recovered from the incubation medium of lactating mammary gland slices at the end of 3 hr. The corresponding value for the mammary adenocarcinoma was about 65%. This indicates that lactating mammary gland slices utilized lactate at a rate twice that of mammary adenocarcinoma slices. However, the incorporation of ^{14}C-lactate into alanine was considerably greater in the neoplasm than in the lactating mammary gland. Thus, the values of the ratio of unutilized ^{14}C-lactate to ^{14}C-alanine formed were about 45 and 6 for the normal and neoplastic tissue, respectively (Table II). The incorporation of ^{14}C-lactate into glutamate and aspartate, presumably via the Krebs cycle, was also considerably higher in the mammary adenocarcinoma (Table II). In this respect, lactate uptake in the neoplasm was only 35%, and one-third of that amount could be accounted for as alanine. In the lac-

TABLE I. Metabolism of ^{14}C-Labeled Lactate, Pyruvate and Alanine by Slices of Lactating Mouse Mammary Gland and Mammary Adenocarcinoma.[a]

Labeled substrates	Glucose added (μmoles)	Lactating mammary gland; Percentage of added ^{14}C recovered in:		Mammary adenocarcinoma; Percentage of added ^{14}C recovered in:	
		CO_2	Fatty acids	CO_2	Fatty acids
Lactate-1-^{14}C	0	44.0	0	17.4	0
	25	53.0	0	13.4	0
Lactate-2-^{14}C	0	22.3	15.4	11.0	0.7
	25	5.0	56.0	8.0	0.9
Lactate-3-^{14}C	0	17.5	15.7	8.0	0.8
	25	3.5	58.0	4.6	1.3
Pyruvate-1-^{14}C	0	74.0	0	36.5	0
	25	79.0	0	30.0	0
Pyruvate-2-^{14}C	0	33.2	14.7	16.6	0.8
	25	11.2	50.0	15.9	1.2
Pyruvate-3-^{14}C	0	25.5	15.2	11.2	0.6
	25	10.4	54.0	10.7	0.8
Alanine-2-^{14}C	0	8.9	11.5	4.4	0.8
	25	4.1	39.1	3.7	1.1
Alanine-3-^{14}C	0	7.7	12.1	3.6	0.6
	25	2.7	41.2	2.4	0.9

[a] Slices (250 mg) were incubated for 3 hr at 37° in a medium consisting of 2.0 ml Krebs–Henseleit bicarbonate buffer (pH 7.4) and 25 μmoles of the labeled substrates recorded above. The specific radioactivity for all substrates was 1×10^4 cpm/μmole. Glucose was added as indicated. Gas phase, O_2 and CO_2 (95:5, by vol). Each value is the average of 3 closely agreeing results from experiments with different mice.

tating mammary gland, on the other hand, 65% of the lactate was taken up, while only a negligible portion of that amount was incorporated into alanine (Table II). It would appear, therefore, that not only were the absolute rates of lactate conversion into amino acids greater in the neoplasm, but that, on a proportionate basis, they were greater still, with respect to aspartate and glutamate in particular. It should be noted that in the neoplasm, incorporation of ^{14}C-lactate into glutamine (presumably via glutamate) was greatly reduced as compared with the normal tissue (Table II). Thus, the values of the ratio of glutamine to glutamate were 6.5 and 0.27 for the lactating mammary glands and the mammary adenocarcinoma, respectively.

Activity levels of pyruvate dehydrogenase and the citrate condensing enzyme in homogenate fractions of lactating mammary gland and mammary adenocarcinoma. Pyruvate dehydrogenase. As has been indicated elsewhere (17), relatively large amounts of lactate dehydrogenase need to be employed in the dismutation assay. At an NAD concentration of 2.5 m*M*, a twofold increase of lactate dehydrogenase in the assay (60 to 115 IU) did not result in an increase in the reaction rate. These same results were obtained whether isolated mitochondria or whole homogenate fractions were employed. For both the normal and the neoplastic tissue, an increase in the concentration of NAD, from 0.025 to 2.5 m*M* (Table III), was accompanied by a parallel increase in $^{14}CO_2$ generation from pyruvate-1-^{14}C. If this effect were due to lack of NAD generation in the assay system, then by doubling the amount of lactic dehydrogenase (at 2.5 m*M* of NAD), it should have been possible to increase the rate of reaction. However, this was not the case in our experiments. There was no further increase in the activity of pyruvate dehydrogenase at NAD concentrations above 2.5 m*M*. For each tissue, pyruvate dehydrogenase activity in both the whole homogenate and the mitochondrial fractions was linear for up to 4 hr.

It should be noted that tissue homogenates

TABLE II. Chromatographic Analysis of Some Water-Soluble Compounds Derived from Incubation of ^{14}C-Labeled Lactate with Lactating Mouse Mammary Gland and Mammary Adenocarcinoma Slices.[a]

Lactate carbon labeled	Compounds on chromatogram	Percentage of added ^{14}C recovered in experiments with: Lactating mammary gland (A)	Mammary adenocarcinoma (B)	Ratio B/A
1	Lactate	38	71	1.9
2	Lactate	29	51	1.8
3	Lactate	37	68	1.8
1	Alanine	0.7	7.6	10.9
2	Alanine	0.8	7.9	9.9
3	Alanine	0.8	10.2	12.8
1	Glutamate	0	0	—
2	Glutamate	2.5	7.7	3.1
3	Glutamate	4.0	13.5	3.4
1	Glutamine	0	0	—
2	Glutamine	9.6	1.2	0.1
3	Glutamine	10.7	2.2	0.2
1	Aspartate	0	0	—
2	Aspartate	0.3	0.5	1.7
3	Aspartate	1.3	2.3	1.8

[a] The variously labeled ^{14}C-lactates were incubated as described in Table I and the text.

of tumors in general, but not of normal tissues, have been shown to require high amounts of NAD, *i.e.*, about 2 m*M*, to facilitate the oxidation of various substrates (21). However, our experiments showed that the increase in pyruvate dehydrogenase activity in response to increasing amounts of NAD was proportionately similar for both tissues, and that at all NAD concentrations, pyruvate dehydrogenase activity in the lactating mammary gland whole homogenate or mitochondrial fractions, was about 3-fold that in the neoplasm (Table III).

Citrate condensing enzyme. Table IV shows that the specific activity of the citrate condensing enzyme in the lactating mammary gland was 7-fold higher than that of the neoplasm in both the whole homogenate and the mitochondrial fractions. When oxaloacetate was omitted from our assay system, the values obtained, presumably a measure of acetyl-CoA deacylase activity, were about 2.5-fold higher in the neoplastic tissue than in the lactating mammary gland (unpublished data). As shown in Table V, the reciprocal addition of varying amounts of tumor homogenate fractions to the corresponding fractions of the normal tissue did not affect the level of the citrate condensing enzyme activity. These results indicate that the lower activity observed in the neoplasm could not have been due to inhibitory substances in the homogenate fractions of the mammary adenocarcinoma.

Discussion. Previous reports concerning the oxidative decarboxylation pattern of several glycolytic intermediates in normal and neoplastic tissues have been equivocal [for review, see (1)]. Those studies pertaining to hepatomas and normal liver in particular have failed to consider the formation of glucose from pyruvate, lactate and alanine. Most hepatomas, unlike normal livers, display no gluconeogenesis from these substrates (22).

To our knowledge, the lactating mammary gland and the mammary adenocarcinoma, although different morphologically and functionally (8, 9, 23, 24), in general, retain similar metabolic pathways, and neither tis-

TABLE III. Effect of NAD Concentration on the Conversion of Pyruvate-1-^{14}C to $^{14}CO_2$ by Lactating Mammary Gland and Mammary Adenocarcinoma Whole Homogenate Fractions.[a]

NAD (m*M*)	Pyruvate-1-^{14}C (nmoles) converted to $^{14}CO_2$/2hr/mg protein: Lactating mammary gland (A)	Mammary adenocarcinoma (B)	Ratio A/B
0.025	40.5	—	—
0.05	45.6	20.8	2.2
0.15	80.1	32.2	2.7
0.75	83.4	36.0	2.3
2.50	190.0(381)[b]	59.5(129)[b]	3.2(2.9)[b]

[a] The incubation mixture contained 25 μmoles of potassium pyruvate-1-^{14}C (the specific radioactivity was 6×10^8 cpm/μmole), 50 μmoles of potassium phosphate buffer (pH 7.4), 1.5 μmoles of $MnCl_2$, 10 μmoles of β-mercaptoethanol, 0.05 μmoles of CoA, 0.1 μmole of thiamine pyrophosphate, 115 I U of lactate dehydrogenase, 18 I U of phosphotransacetylase, NAD as indicated above and either 19.2 mg protein of the lactating mammary gland whole homogenate fraction or 8.4 mg protein of the mammary adenocarcinoma whole homogenate fraction; all in total volume of 1.0 ml. The mixture was incubated with mechanical reciprocal agitation for 2 hr at 37°. Gas phase, O_2 and CO_2 (95:5, by vol). Each value is the average of 3 closely agreeing results from experiments with different mice. See text for preparation of homogenate fractions and other experimental details.

[b] Values in parentheses represent values of the isolated mitochondria.

sue displays any gluconeogenic capacity. Thus, the metabolic products from lactate, pyruvate and alanine in these tissues would consist largely of CO_2, fatty acids and proteins. For example, in experiments with ^{14}C-lactate, the amount of unutilized lactate and that converted to CO_2, fatty acids and amino acids closely approximated the initial amount of lactate present in the incubation medium (Tables I and II). This study has demonstrated the greatly reduced capacity of mammary adenocarcinoma slices to oxidize pyruvate, lactate and alanine. The possibility existed that the endogenous concentration of lactate, generally found to be considerably greater in neoplasms than in normal tissues (1) was responsible for the apparent decrease of lactate oxidation (*i.e.*, isotopic dilutions). However, as was pointed out in Fig. 1, the ratio of $^{14}CO_2$ formation in the lactating mammary gland to that in the neoplasm was similar at a large range of concentration of lactate-1-^{14}C. Indeed, reports from other laboratories on a large variety of tumors (25) indicate a maximum possible dilution of 1% under our experimental conditions. In addition, the differences in the amounts of ^{14}C-lactate recovered from the incubation medium of each tissue (Table III), corroborates the finding that the tumor tissue has a reduced capacity to utilize lactate.

In an attempt to localize the site of a possible metabolic lesion in the oxidation of these substrates by the neoplasm, we were guided by the following considerations: In the normal tissue, the values for the ratios of $^{14}CO_2$ from lactate-1-^{14}C/$^{14}CO_2$ from lactate-2-^{14}C, and of $^{14}CO_2$ from lactate-2-^{14}C/$^{14}CO_2$ from lactate-3-^{14}C were 2.5 and 1.3, respectively (Table I). The corresponding values of these ratios in the neoplasm were 2.2 and 1.4, respectively (Table I). Similar values for these ratios can be derived for ^{14}C-labeled pyruvate and alanine in both tissues (Table I). The similarity of the values of these ratios for all substrates indicates the following: Neither lactate dehydrogenase nor ala-

TABLE IV. Activity of the Citrate Condensing Enzyme in Lactating Mammary Gland and Mammary Adenocarcinoma Homogenate Fractions.[a]

Homogenate fraction	No. of mice	Lactating mammary gland	Mammary adenocarcinoma
Whole homogenate	5	44.6 ± 3.7	8.2 ± 0.9
Mitochondria	5	141 ± 10.9	20.9 ± 1.7
Ethanolic–KCl extract	2	157	24.7

[a] Citrate condensing enzyme activity was measured at 30°. One unit is equal to the utilization of 1 nmole of substrate/min/mg protein. The assay mixture consisted of 200 μmoles of Tris–HCl buffer (pH 8.0), 2 μmoles of DTNB, 0.18 μmoles of acetyl-CoA, 20 μmoles of oxaloacetate and enzyme preparation, in a total volume of 2 ml. All determinations were corrected for acetyl-CoA deacylase activity by incubating the enzyme preparation without oxaloacetate. See text for preparation of homogenate fractions and other experimental details.

TABLE V. Effect of Mammary Adenocarcinoma Homogenate Fractions on the Citrate Condensing Enzyme Activity in Lactating Mouse Mammary Gland Homogenate Fractions.[a]

Homogenate fraction (ml) from				Enzyme activity	
Lactating mammary gland		Mammary adenocarcinoma			
Whole homogenate	Mitochondria	Whole homogenate	Mitochondria	Obsd	Calcd
0.01	0	0	0	8.9	8.9
0	0	0.01	0	1.9	1.9
0.01	0	0.01	0	10.5	10.7
0.02	0	0.01	0	20.7	19.5
0.01	0	0.10	0	25.6	27.4
0	0.01	0	0	15.1	15.1
0	0	0	0.01	1.4	1.4
0	0.01	0	0.01	17.7	16.5
0	0.01	0	0.03	20.7	19.3
0	0.01	0	0.05	21.4	22.0

[a] Activity is expressed as nanomoles of substrate utilized per minute. The calculated values were determined from the activities expected if the values were additive. See text and Table IV for preparation of homogenate fractions and other experimental details.

nine transaminase were rate limiting in the conversion of lactate and alanine to pyruvate by the neoplasm. This assumption is substantiated by the relatively high rates of alanine formation in the mammary adenocarcinoma from ^{14}C-labeled lactate (presumably via pyruvate) and by studies reported elsewhere on lactate dehydrogenase activities in both these tissues (8). In this connection, a low glycerol-3-phosphate shunt activity (4, 8) could have accounted for the decreased rates of oxidative decarboxylation of pyruvate, lactate and alanine by the mammary adenocarcinoma. However, this possibility is unlikely since all substrates, regardless of their redox state, were utilized in a similar fashion by the neoplasm (Table I). These ratios also indicate that reactions leading to CO_2 formation from the second and third carbons of lactate, pyruvate and alanine (via the Krebs cycle) are not primarily responsible for the reduced capacity of the neoplasm to utilize these substrates. Experiments on the activities of various Krebs cycle enzymes and on the oxidation of acetate-1-^{14}C and 2-^{14}C showed similar rates for both tissues (8–10). It would appear, therefore, that the derangement of the reactions involved in the utilization of these three substrates must be localized at a stage prior to their entry into the Krebs cycle, and past the lactate dehydrogenase and the alanine transaminase reactions. In this respect, Mehard, Packer and Abraham have shown (26) that the rate of oxygen consumption in the presence of pyruvate by mitochondria isolated from the mammary adenocarcinoma was about 2.6-fold lower than that from mitochondria isolated from lactating mammary gland, but that the rate of oxygen consumption in the presence of succinate was similar in isolated mitochondria of both these tissues.

Pyruvate dehydrogenase and the citrate condensing enzyme are principally involved in the conversion of pyruvate carbon to acetyl-CoA and, subsequently, into citrate and/or to alternative pathways. As was shown here, the specific activities of pyruvate dehydrogenase and the citrate condensing enzyme were about 3- and 7-fold lower, respectively, in the mammary adenocarcinoma than in the lactating mammary gland. The specific activity of acetyl-CoA deacylase was about 2- to 3-fold higher in the former tissue (unpublished data). As has been reported elsewhere (1), the mitochondrial content of various tumors is reduced; our results indicate that the mitochondrial content in this tumor is 50% lower than in the lactating mammary gland. Hence, if we were to have reported the activity levels of pyruvate dehydrogenase and the citrate condensing en-

zyme per total weight of the mitochondrial fraction, these enzyme activities in the neoplasm would have been shown to be lower still, by a factor of two. On the other hand, acetyl-CoA deacylase activity would have been similar in both tissues. We propose that an enzymatic alteration at the site of the pyruvate dehydrogenase and the citrate condensing enzyme, could create a bottleneck in the flow of carbons from the glycolytic pathway to the Krebs cycle and alternative metabolic routes. Accordingly, defective lipogenesis in the mammary adenocarcinoma (Table I) could be attributed to a stage(s) prior to that of acetyl-CoA synthesis from pyruvate and those following its formation (10, 11, 13). In this respect, Hepp *et al.* (27) have suggested that, in several tumors, reduced acetate thiokinase and high acetyl-CoA deacylase activities lead to acetate accumulation which will result in a deficiency of respiratory energy. The effect of various modifiers at these sites may also be considerable.

Thus, previous reports (1), as well as the results reported in this work, suggest that under certain conditions neoplastic tissues may exhibit a decreased citrate and possibly ATP formation. Citrate and ATP have been shown to inhibit phosphofructokinase, a key regulatory step in glycolysis (28). It would appear, therefore, that in tumors, a deficiency in respiratory energy, due to lack of substrates entering the Krebs cycle, could be compensated by augmented anaerobic and aerobic glycolysis. Further elucidation of this problem, however, would require extension of the studies reported here to a wide variety of tumors, the minimal deviation tumors in particular, as well as determination of the *in vivo* concentration of the intermediates involved, their turnover rates, pool sizes, and their affinities for the enzymes specified.

The preferential formation in the mammary adenocarcinoma of amino acids from ^{14}C-labeled lactate confirms the proven ability of neoplasms to synthesize protein in large amounts (1). Particularly interesting, is the observation that, compared with normal mammary gland, in the mammary adenocarcinoma there was pronounced alanine formation from ^{14}C-lactate. The increase in alanine formation, coupled with a decreased lactate, pyruvate and alanine oxidation, further substantiates the thesis of a metabolic lesion below pyruvate in this neoplasm. As shown here, glutamine formation from ^{14}C-labeled lactate is very low in the mammary adenocarcinoma compared with the lactating mammary gland. Low glutamine content in neoplasms in general, has been attributed to both its reduced synthesis and its rapid utilization (29).

Summary. The utilization of ^{14}C-labeled lactate, pyruvate and alanine by tissue slices of lactating mouse mammary gland and adenocarcinoma of the mouse mammary gland was investigated. In the absence of glucose, rates of $^{14}CO_2$ formation and fatty acid synthesis from these substrates in the mammary adenocarcinoma were considerably lower than those in the lactating mammary gland, regardless of the position of the labeled carbon. In the lactating mammary gland, addition of glucose caused a considerable decline in $^{14}CO_2$ formation from the second and third carbons of lactate, pyruvate and alanine, while it significantly stimulated their incorporation into fatty acids. Glucose also stimulated $^{14}CO_2$ formation from the first carbon of these substrates. In the mammary adenocarcinoma, however, $^{14}CO_2$ formation from the first carbon of lactate and pyruvate decreased upon glucose addition, while that from the second and third carbons was almost unaffected. The incorporation into fatty acids of the second and third carbons from these substrates in the neoplasm was only slightly stimulated by glucose. Lactate uptake by lactating mammary gland was twice that observed in the mammary adenocarcinoma, but amino acid formation from this substrate was significantly higher in the neoplasm. The synthesis of glutamine, however appears to be defective in the mammary adenocarcinoma. The data indicated considerably lower levels of pyruvate dehydrogenase (EC 1.2.4.1) and citrate condensing enzyme (EC 4.1.3.7), and possibly higher acetyl-CoA deacylase (EC 3.1.2.1) activities in the tumor than in the lactating mammary gland. The significance of this finding in relation to the entry of 3-carbon glycolytic intermediates into the Krebs cycle in both the lactating mammary gland and the mammary adenocarcinoma is discussed.

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