in malic-DH activity could explain the lower rates of oxygen consumption reported by Dole *in vivo*(4) and Schirmer *in vitro*(9). Further, if energy from oxidation of substrates is limited by this decrease in malic-DH activity, one could also explain the depressed T_m for PAH and for glucose. Whether a loss in activity of malic-DH by ischemic kidneys does indeed explain the depressed rate of O_2 consumption of cortical slices(9) awaits further investigation.

Summary. 1) Rates of anaerobic CO_2 production by tissue obtained from dog kidneys with evidence of cortical ischemia have been compared with tissue from non-ischemic kidneys. Anaerobic CO_2 production rate was consistent with the following coupled oxidation-reduction reaction: α-ketoglutarate + oxaloacetate + GDP + P_i → malate + succinate + CO_2 + GTP. 2) Cortical washed homogenates from non-ischemic kidneys produced 14.6 ± 1.8 μmoles CO_2/100 mg dry wt hr (M ± SE); cortical homogenates of ischemic kidneys yielded 6.0 ± 3.0 μmoles of CO_2 ($P<0.05$). Similar studies done on slices revealed no such difference between ischemic and non-ischemic kidneys. 3) The activity of α-KG-dehydrogenase was the same in washed cortical homogenates of both ischemic and non-ischemic kidneys. In contrast, malic-dehydrogenase activity was significantly lower in cortical homogenates of ischemic kidneys. 4) Thus, cortical blanching is associated with a specific change in cortical mitochondrial enzymic activity so that both cortex and medullary homogenates have decreased capacities for complete oxidation of substrates. This specific difference in the enzymic activity of mitochondria from the ischemic cortex may explain, in part, the low activity of the above reaction in ischemic cortical homogenates and may be one of the initial metabolic changes which occurs in the ischemic kidney.

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Lipid Composition of the Carcass of Mice Bearing the Krebs-2 Carcinoma.* (32248)

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The profound alterations in the lipid composition of rats bearing transplantable tumors such as the Walker carcinoma 256 have been well known for some time(1-3). These lipid changes are characterized chiefly by a loss of host lipid as the tumor grows and this loss of lipid is mainly in the neutral lipid fraction. Also a high degree of lipemia results as a consequence of the host lipid decrease. These lipid alterations commence when the tumor reaches a size roughly about

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10% of the body weight of the host. Costa and Holland(4) reported that the Krebs-2 carcinoma reduced the host lipid of mice by 50% as early as 7 days after transplantation when the tumors were quite small. This decrease in total lipid was followed by another premortal loss at the fifth week of tumor growth. The present study was undertaken to determine the nature of the lipid alterations of mouse carcass induced by the growth of the Krebs-2 carcinoma.

Materials and methods. Swiss HA/ICR adult mice of nearly the same average weight (38 g) were used for the normal mice and for the growth of the Krebs-2 carcinoma exactly as described by Costa *et al*(4). These animals were supplied by Dr. Costa of this Institute. The lipid extracts were prepared in the following manner. After removal of the tumors at the first, third and fifth week, these mice and the controls were homogenized in 300-400 ml water in a large Waring Blendor. The homogenate was filtered and the residue and filtrate were extracted with residue-free and glass distilled lipid solvents. The lipids were extracted from the carcass residue by the proceduce of Rouser *et al*(5) using chloroform (C), methanol (M) 2/1, v/v as solvents, maintaining low temperatures and preventing oxidation of the lipids. The water filtrate was extracted with chloroform in a separatory funnel. This extract was added to the CM solution of the residue, made up to a known volume with CM and an aliquot equivalent to 10% (amount kept small to facilitate handling and drying to constant weight) of the total lipid extract was dried at or below room temperature in a rotary evaporator(5). The chloroform-methanol extracts were dried overnight under N_2 over solid KOH, reextracted with the same solvent mixture and then dried again. Finally the dried residue was extracted with chloroform. In this way proteins extracted with the lipid solvents are denatured and rendered insoluble.

Costa *et al* used chloroform-ethanol for extraction of the carcass residue(4). For this purpose the residue was obtained by filtering one-fourth of the total volume of the carcass homogenate in water. This residue was then refluxed in a soxhlet extractor for 16 hours with chloroform-ethanol 3/1, v/v. The liquid phase obtained from the above filtration was extracted with chloroform in a separatory funnel. The chloroform and chloroform-ethanol extracts were combined and the solvents were removed at 69°C under reduced pressure to constant weight. The material thus obtained was assumed to be fat. However, such an extract would contain not only lipids, but also denatured proteins, sugars, amino acids and inorganic salts(5,6).

The carcass total lipid was then separated into the neutral lipid and phosphatide fractions by silicic acid chromatography. Prior to the separation of the various neutral lipid classes by silicic acid chromatography by the procedure of Hirsch *et al*(7), the free fatty acids (FA) were removed from the neutral lipids by the method of McCarthy and Duthie(8). The various neutral lipid fractions were collected in bulk and the solvents removed at low temperature(5) after which the lipid fractions were checked by thin layer chromatography as developed by Rouser(5). The methyl esters of the FA of the triglycerides, sterol esters and free FA were made by transesterifying these lipids with 10 ml anhydrous methanol containing 5% H_2SO_4 and 2 ml benzene(9). This mixture was refluxed at 95-100°C for 2.5 hours in a Dow Corning 200 fluid silicone bath. The methyl esters thus obtained were purified by column chromatography on silicic acid by the procedure of Luddy *et al*(10).

The FA composition of the methyl esters was determined by gas chromatography at 190°C with a Perkin-Elmer Model 810 Chromatograph equipped with hydrogen flame ionization detectors. Polar columns, 6 ft $\times$ 1/8 in. stainless steel, containing butanediol succinate polyester (Perkin-Elmer Corp.) on 80/100 mesh chromosorb W were employed. The methyl esters of the FA were dissolved in CS_2 to make roughly a 2% solution and 2 to 10 μl were injected per analysis which was usually done in duplicate. The identification of the FA of the methyl esters was based upon a comparison of the retention values (16:0 = 1) with known FA methyl esters(11,12). The

TABLE I. Effect of Krebs-2 Carcinoma on Mouse Carcass Neutral Lipids and Phosphatides.*

Sample No.†	Total lipids, mg/aliquot‡	Neutral lipids %	Phosphatides %
1. Controls (5)	283 ± 60	77.4 ± 2.3	22.6 ± 2.2
2. Bearing tumor 1 wk (5)	249 ± 36	78.0 ± 1.5	22.0 ± 1.4
3. " " 3 wk (6)	289 ± 14	82.2 ± 1.2	17.8 ± 1.2
4. " " 5 wk (4)	264 ± 13	69.2 ± 3.6	30.9 ± 4.1

* Results are given as the mean ± standard deviation for mice of comparable weight.
† Number of different animals used for analysis is given in parentheses.
‡ This represents the total lipid determined per 1/10 of the mice carcasses *after removal of the tumors.* Mice originally weighed 38 g which was maintained except at the 5th week when the large tumors increased the total body weight (carcass + tumor).

TABLE II. Effect of Krebs-2 Carcinoma on Mouse Carcass Neutral Lipid Fractions.*

Sample No.†	Free fatty acids‡ %	Cholesterol esters§ %	Triglycerides§ %	Cholesterol§ %	Mono- and diglycerides§ %
1. Controls (5)	4.9 ± 2.2	18.8 ± 1.6	71.3 ± 1.9	6.0 ± 1.4	1.8 ± .4
2. Bearing tumor 1 wk (5)	3.1 ± .7	19.7 ± 3.2	73.3 ± 2.1	7.9 ± 1.3	3.0 ±1.2
3. " " 3 wk (6)	3.9 ± .9	13.6 ± 4.8	78.9 ± 2.9	6.9 ± 1.1	3.1 ± .5
4. " " 5 wk (4)	5.4 ± 1.1	23.4 ± 4.7	71.2 ± 2.6	5.8 ± 2.6	1.8 ± .3

* Results are given as the mean ± standard deviation.
† Number of different animals used for analysis is given in parentheses.
‡ The free fatty acids were removed prior to neutral lipid fractions; given as % of neutral lipid.
§ Fractionation of the neutral lipids by silicic acid chromatography; given as % of the neutral lipid minus free fatty acids.

areas under the various peaks were measured with a Disc Integrator. To confirm the presence of suspected unsaturated FA, the methyl esters were reduced with Adam's catalyst and hydrogen by the micro procedure of Farquhar *et al*(13). The resulting saturated methyl esters were chromatographed and then the chromatograms compared with the original samples(11,12).

Results and discussion. The effect of the growth of the Krebs-2 carcinoma for 1, 3, and 5 weeks on the carcass neutral lipids and phosphatides is shown in Table I. Only the mice bearing the carcinoma for 5 weeks showed a somewhat salient but not too pronounced decrease in the neutral lipids and an increase in the phosphatides. Actually an increase in the neutral lipids occurred prior to the fifth week. Costa *et al*(4) reported nearly a 50% loss in total lipid after one week's growth of the carcinomas which were just measurable with a further substantial decrease after 5 weeks of tumor growth (premortal). The distribution of the various neutral lipids of the control and tumor bearing mice did not differ appreciably (Table II). Triglycerides accounted for the largest share of the neutral lipids with the sterol esters being second. Free FA and mono- and diglycerides were present at relatively low levels.

The FA composition of the carcass triglycerides as a function of the growth of the carcinoma showed no significant differences (Table III). Oleic acid and 18:2 accounted for nearly 78% of the triglyceride FA composition. Stearic acid was present in fair amounts (15%) whereas the other FA represented the remainder.

The FA composition of some of the samples of the free FA of the carcass lipids showed considerable variation (Table IV) but no significant changes resulted as a consequence of tumor growth. Oleic acid and 18:2 accounted for about 65% of the FA composition and 16:0 and 18:0 about 25%. The sterol ester fraction of the carcass lipids had a rather complex FA composition in that iso 14:0, iso 16:0, 15:0, 17:0 and anteiso 21:0 were present. However no significant differences in the FA of the sterol esters of the carcass resulted from the growth of

TABLE III. Fatty Acid Composition* (Per Cent of Total Fatty Acids) of Mouse Carcass Triglycerides of Mice Bearing Krebs-2 Carcinoma.†

Sample No.‡	14:0	16:0	16:1	18:0	18:1	18:2	20:1
1. Controls (4)	.4 ± .2	14.1 ± .6	4.0 ± .4	1.4 ± .1	32.9 ± 1.0	45.4 ± .9	.8 ± .1
2. Bearing tumor 1 wk (5)	.6 ± .1	13.3 ± 1.3	3.7 ± .3	1.4 ± .1	33.4 ± 1.4	45.8 ± .8	.8 ± .1
3. Bearing tumor 3 wk (6)	.8 ± .2	15.7 ± 1.2	4.9 ± .5	.9 ± .1	31.1 ± 1.4	44.9 ±1.3	.8 ± .1
4. Bearing tumor 5 wk (4)	.8 ± .1	14.2 ± .4	3.6 ± .4	1.4 ± .3	32.7 ± .4	45.7 ± .6	.8 ± .1

* Traces of 14:1 were calculated with 14:0; traces of iso 14:0, 15:0, iso 16:0, 17:0, 18:3, 20:0, 20:2, 20:3 and 20:4 were also present.
† Results are given as the mean ± standard deviation.
‡ Number of different animals used for analysis is given in parentheses.

TABLE IV. Fatty Acid Composition (Per Cent Total Fatty Acids) of Mouse Carcass Free Fatty Acids of Mice Bearing Krebs-2 Carcinoma.*

Sample No.†	16:0	16:1	18:0	18:1	18:2	20:0	20:1
1. Controls‡ (3)	18.3 ±5.6	1.7 ± .4	10.8 ±4.9	26.7 ±3.6	32.8 ±6.3	4.7 ±3.9	3.0 ±2.3
2. Bearing tumor 1 wk‡ (3)	16.0 ±3.6	3.1 ±1.0	6.5 ±4.2	30.0 ±1.9	36.8 ±5.2	3.0 ±2.9	2.5 ±1.5
3. Bearing tumor 3 wk§ (2)	12.0 ± .0	2.1 ± .6	6.5 ± .7	35.0 ±1.0	42.0 ±1.0	.6 ± .2	1.4 ± .5
4. Bearing tumor 5 wk‖ (4)	19.0 ± .9	1.5 ± .6	12.4 ±2.6	26.6 ±2.1	34.7 ±4.5	1.2 ± .4	2.8 ±1.1

* Results are given as the mean ± standard deviation.
† Number of different animals used for analysis is given in parentheses.
‡ About 2% of 14:0, 18:3 and undetermined fatty acids were included in these analyses.
§ Traces of 18:3 and undetermined fatty acids were included in these analyses.
‖ About 2% of 17:0, 18:3 and undertermined fatty acids were included in these analyses.

the carcinoma (Table V). The unsaturated FA (16:1, 18:1, 18:2, 18:3, 20:1 and 22:1) accounted for 64% of the total FA whereas the saturated FA made up the rest.

The difference between the results reported here and those obtained by Costa *et al*(4) result from the difference in the method of lipid extraction. The method employed by Costa *et al* would leave an extract containing lipids, proteins, salts, sugars and amino acids, whereas Rouser's procedures would eliminate much of the non-lipid materials. However, even using Rouser's procedure, Dextran gel chromatography is necessary to remove some remaining non-lipid water soluble substances(6) from carefully prepared lipid extracts of tissues. It would appear that not all tumors bring about a decrease in the lipids of the host or that the lipid loss may not occur in all species as a result of tumor growth or that metastases, such as are found as a result of the growth of the Walker carcinosarcoma(14), are necessary to deplete the lipids of the host. Costa *et al* were apparently measuring non-lipid carcass materials which decreased in content as a result of tumor growth.

Summary. The lipid composition of the carcass of mice bearing the Krebs-2 carcinoma for periods varying from 1 to 5 weeks was determined. The only change of questionable significance occurred after 5 weeks of tumor growth at which time the carcass neutral lipids were decreased and the phosphatides were increased. Prior to the 5th week the neutral lipids increased somewhat. No change was found in the amount of the carcass neutral lipid classes—the triglycerides, sterol esters, cholesterol, mono- and diglycerides and free fatty acids—as a result of the growth of the Krebs-2 carcinoma. Furthermore no significant change was found in the fatty acid composition of the free fatty acids, sterol esters and tri-

TABLE V. Fatty Acid Composition* (Per Cent Total Fatty Acids) of Mouse Carcass Cholesterol Esters of Mice Bearing Krebs-2 Carcinoma.†

Sample No.‡	14:0	iso 16:0	16:0	16:1	17:0	18:0	18:1	18:2	18:3	20:0	20:1	ante-iso 21:0	20:4	22:0	22:1
1. Controls (3)	1.3	1.2	7.0	18.4	1.4	3.0	17.8	8.3	1.7	4.1	7.3	1.6	8.2	1.7	9.6
	±.4	±.8	±1.0	±2.0	±.6	±.4	±1.1	±.9	±.1	±1.1	±2.2	±.6	±1.6	±.4	±1.0
2. Bearing tumor 1 wk (4)	1.4	2.9	9.0	16.5	.9	2.6	17.4	10.4	2.2	5.1	7.1	2.1	6.3	1.8	6.8
	±.4	±1.5	±1.2	±2.4	±.2	±1.0	±2.8	±2.7	±.5	±1.1	±1.7	±.7	±1.7	±.7	±1.1
3. " 3 wk (4)	1.9	2.2	7.4	18.5	.9	5.0	14.4	8.5	3.5	5.5	7.1	2.8	7.3	2.4	6.4
	±.9	±1.2	±1.1	±3.8	±.1	±1.5	±1.7	±1.4	±2.1	±.9	±.9	±.9	±1.3	±.7	±1.2
4. " 5 wk (3)	2.6	2.0	10.2	20.6	.9	2.1	13.8	7.1	3.2	4.8	7.4	3.2	7.2	2.3	5.5
	±.5	±.1	±.9	±.5	±.3	±.0	±.4	±.8	±.4	±1.1	±.8	±.4	±2.2	±.5	±.7

* These analyses include 3 undetermined fatty acids of a homologous series representing 4 to 6% of the total fatty acids of the control mice and mice bearing tumors. Other fatty acids found in trace amounts were iso 14:0 and 15:0.

† Results are given as the mean ± standard deviation.

‡ Number of different animals used for analysis is given in parentheses.

glycerides of the carcass lipids following growth of the carcinoma from 1 to 5 weeks.

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