milligram dry weight of tissue per hour. (The anaerobic glycolysis ($Q_{CO_2}^{N_2}$) and the aerobic glycolysis ($Q_{CO_2}^{O_2}$) are also determined manometrically and are expressed as microliters of gas per milligram dry weight of tissue per hour. In general, chemical measurements of lactate formation agree reasonably well with the glycolysis determined manometrically.)

production measured chemically. In the German literature a subscript M is used as the English L, signifying the German word for lactic acid, *milchsäure.* Subscript A refers to total acid production and in common practice is used interchangeably with CO_2 subscript. The R.Q., or respiratory quotient, is $\frac{Q_{CO_2}}{Q_{O_2}}$ and gives an indication of the type of foodstuff being respired. The R.Q. approaches unity for carbohydrate oxidation, 0.82 for pure protein oxidation, and 0.71 for fat. Of the many other quotients that have been used only two are of current interest. The absolute Pasteur effect is $Q_{CO_2}^{N_2} - Q_{CO_2}^{O_2}$; this is the simplest and most meaningful expression of the Pasteur effect (the inhibition of glycolysis caused by oxygen). The Meyerhof oxidation quotient (M.O.Q.) is

$$\frac{Q_{CO_2}^{N_2} - Q_{CO_2}^{O_2}}{1/3\ Q_{O_2}},$$

it is a measure of the inhibition of glycolysis (Pasteur effect) per mole of oxygen uptake. The respiration is divided by 3 in the M.O.Q. on the theoretical basis that if the lactate were being oxidized 3 moles of oxygen would be required (actually, there is no evidence that the inhibition of lactate accumulation is through direct oxidation of the compound).

Other quotients will not be used in the present work, but are defined for completeness. When the denominator of the Meyerhof oxidation quotient is not divided by 3, it becomes simply the Meyerhof quotient, that is,

$$\frac{Q_{CO_2}^{N_2} - Q_{CO_2}^{O_2}}{Q_{O_2}}.$$

The per cent Pasteur effect is defined as

$$\frac{100\ (Q_{CO_2}^{N_2} - Q_{CO_2}^{O_2})}{Q_{CO_2}^{N_2}}.$$

The fermentation excess was defined by Warburg (470) as $Q_{CO_2}^{N_2} - 2Q_{O_2}$. Originally it was thought that this quantity was positive in tumors and negative in normal tissues, but later studies indicated that such was not the

From the figures on anaerobic glycolysis of animal and human tumors presented in Tables 1, 2, and 3, it is quite apparent that tumor tissues, almost without exception, have a high rate of lactate production in nitrogen. Animal tumors display rates of anaerobic glycolysis consistently in excess of 20. While a considerable number of human neoplasms have rates between 10 and 20, it seems clear that, when due account is taken of the variable percentage of tumor tissue (30–100%) of this less favorable experimental material, the glycolytic rate of the human tumor cell is in the same range as that of animal tumors. Finally, the purest form of tumor tissue known, the ascites cell, displays rates of anaerobic glycolysis of between 30 and 80. The significance of such glycolysis may be obtained from Warburg's calculation that a tumor such as the Flexner-Jobling, with a rate of anaerobic glycolysis, $Q_{CO_2}^{N_2}$ of 31, is consuming glucose equal to 12.4% of its dry weight per hour (481). Aerobically, this tumor consumes glucose equal to 7% of its dry weight per hour, while the figures for the Jensen rat sarcoma are 16% anaerobically and 10% aerobically.

Slices of tumor tissue display a considerable rate of glycolysis in oxygen as well as in nitrogen, as indicated by the values of $Q_{CO_2}^{O_2}$. This aerobic glycolysis of the tumor slice is more impressive than the anaerobic glycolysis, especially when it is compared with normal tissues, in which aerobic glycolysis is a rarity. However, aerobic glycolysis is a less constant experimental quantity (127), much more dependent on the medium and on other experimental variables than is anaerobic glycolysis.

Despite this variability of aerobic glycolysis as an experimental quantity, animal and human neoplastic tissues almost without exception display a significant aerobic glycolysis. With most tumor tissues this aerobic glycolysis exceeds a Q value of 10; tumors in

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case. Finally, both anaerobic and aerobic glycolysis can be referred to the equivalent respiration and defined respectively as

$$\frac{Q_{CO_2}^{N_2}}{1/3\ Q_{O_2}} \quad \text{and} \quad \frac{Q_{CO_2}^{O_2}}{1/3\ Q_{O_2}}.$$

Further information on these quantities may be found in Burk's excellent review (59). The many other derived quotients do not appear to serve any useful purpose and have gradually been dropped from the modern literature.

TABLE 1
RESPIRATION AND GLYCOLYSIS OF ANIMAL TUMORS

	$-Q_{O_2}$	$Q^{O_2}_{CO_2}$	$Q^{N_2}_{CO_2}$	Absolute Pasteur effect	Meyerhof oxidation quotient	Ref.
Fowl tumors						
Rous sarcoma	5	20	30	10	6.0	467
Tumor I (rapid progression)	7	21	28	7	3.0	331
Tumor IX (slow progression)	9	14	23	9	3.0	331
Solid mouse tumors						
Crocker sarcoma	16	17	28	11	2.1	108
Tar carcinoma	20	15	25	9	1.4	108
Sarcoma 37	15	12	28	16	3.2	108
Bonné tar sarcoma	13	10	22	12	2.8	108
Tar sarcoma 173	15	15	30	15	3.0	108
Sarcoma 2529	14	16	32	16	3.4	108
Carcinoma 113	12	5	14	9	2.2	108
Melanotic sarcoma	9	6	16	10	3.5	108
Harding-Passey melanotic melanoma	5	2	11	9	5.8	62
Cloudman S-91 melanotic melanoma	5	3	12	9	5.3	62
Algire-91A melanotic melanoma	7	5	15	10	4.0	62
Earle L sarcoma	6	8	12	4	2.5	62

TABLE 1 (*Continued*)

	$-Q_{O_2}$	$Q_{CO_2}^{O_2}$	$Q_{CO_2}^{N_2}$	Absolute Pasteur effect	Meyerhof oxidation quotient	Ref.
Solid mouse tumors (*Continued*)						
Barrett C3HBA adenocarcinoma	9	10	18	7	2.3	62
Mouse ascites tumors						
Ehrlich	7	30	70	40	17.1	474
Thymoma (4 days)	13	8	33	25	5.8	287
Thymoma (14 days)	13	28	83	55	12.7	287
Krebs-2		22	53	31	—	534
Rat tumors						
Flexner-Jobling carcinoma	7	25	31	6	2.6	481
Jensen sarcoma	9	16	34	8	2.7	469
Philadelphia 1 sarcoma	12	14	25	11	2.7	146
3-4-Benzpyrene sarcoma	9	9	25	16	5.3	252
Maruya sarcoma	20	22	43	21	3.1	252
Ebina tar carcinoma	14	14	29	15	3.2	252
Walker 256 carcinosarcoma	9	24	42	18	6.0	117

TABLE 2
SPONTANEOUS MOUSE TUMORS (331)

No.	Histology[a]	$-Q_{O_2}$	$Q_{CO_2}^{O_2}$	$Q_{CO_2}^{N_2}$	$\frac{Q_{CO_2}^{N_2} - Q_{CO_2}^{O_2}}{Q_{O_2}}$	Rate of mitosis[b]	Growth rate[c]	Metastasis
1	Cystic hem. alveolar ca.	17.3	0	11.6	0.7	+	?	−
2	Adenocarcinoma	7.6	0	15.8	2.0	+	+	−
3	Cystic adenoca.	6.1	0	21.5	3.5	++	++	−
4	Cystic pap. alveolar ca.	12.4	0	15.2	1.2	+	?	−
5	Cystic adenoca.	14.9	0	12.1	0.8	±	+	−
6	Adenocarcinoma	14.6	0	15.6	1.1	±	+	−
7A	Cystic hem. alveolar ca.	17.8	3.9	18.4	0.3	++	+++	−
8A	Adenocarcinoma	17.6	3.7	19.2	0.9	±	+++	−
9	Adenocarcinoma	13.9	2.8	23.0	1.5	++	?	−
10	Alveolar ca.	26.0	9.6	27.3	0.7	++	+++	−
11	Cystic adenoca.	17.4	4.9	15.7	0.6	±	+++	+
12	Cystic adenoca.	10.0	2.5	23.4	2.1	±	±	−
7	Alveolar ca.	8.4	3.5	12.3	1.1	+++	?	−
13	Adenocarcinoma	18.0	7.6	27.6	1.1		+++	
14	Adenocarcinoma	6.1	6.1	20.7	0.6	±	±	+
15	Adenocarcinoma	7.6	7.6	27.6	1.1	+	+	−

[a] "A" following a tumor number indicates observations on autografts of the original tumor, and "B" observations were made on local recurrence following surgical removal.

Abbreviations: ca. = carcinoma, hem. = hemorrhagic, pap. = papillary.

[b] For mitosis in tumors, ± indicates only occasional mitotic figures, +++ indicates large numbers, and + and ++ are grades in between the two extremes.

[c] Growth rate is based on a 4-week period of observation; ± means slight growth, +++ tumors doubled in size in 4 weeks, and others are intermediate grades.

TABLE 2 (*Continued*)

No.	Histology[a]	$-Q_{O_2}$	$Q_{CO_2}^{O_2}$	$Q_{CO_2}^{N_2}$	$\frac{Q_{CO_2}^{N_2} - Q_{CO_2}^{O_2}}{Q_{O_2}}$	Rate of mitosis[b]	Growth rate[c]	Metastasis
16	Adenocarcinoma	8.6	8.6	35.1	1.3	+	?	−
17	Adenocarcinoma	6.9	6.9	33.0	1.5	+	?	−
18	Cystic alveolar ca.	16.3	8.3	30.4	1.3	++	+++	+
19	Cystic pap. adenoca.	9.7	6.2	24.6	1.9	++	++	+
20B	Adenocarcinoma	15.5	10.2	34.5	1.9	+	+++	+
21	Cystic hem. adenoca.	17.7	12.7	23.4	0.6	+	±	−
22	Alveolar ca.	15.6	11.2	26.0	1.0	+	++	−
23	Cystic adenoca.	8.3	6.1	32.5	3.2	±	+++	−
20	Adenocarcinoma	20.0	16.0	35.2	1.0	+	?	−
24	Hem. cystic adenoca.	12.4	10.2	29.0	1.3	±	+++	+
25	Pap. hem. adenoca.	14.8		36.2	1.5	+	++	+
10B	Alveolar ca.	10.7	11.2	38.0	2.5	++	+++	−
26	Alveolar ca.	17.5	17.1	30.6	0.8	+	?	−
27	Sarcoma (?)	4.4	4.8	23.5	4.2		++	−
10A	Alveolar ca.	11.2	12.4	31.1	1.7	++	+++	−
22A	Alveolar ca.	7.6	8.2	18.7	1.4	+	++	−
28	Adenocarcinoma	8.0	10.6	34.9	3.0	++	+++	−
29	Alveolar ca.	11.6	15.6	22.6	0.6	±	±	−
27A	Giant cell sarcoma	14.1	20.3	33.1	0.9		+++	−
30	Spindle cell sarcoma	3.2	7.2	32.1	7.7	+	+++	−
	Average	13.7	7.5	24.7	1.6			

TABLE 3
RESPIRATION AND GLYCOLYSIS OF HUMAN TUMORS

	Per cent tumor	$-Q_{O_2}$	$Q_{CO_2}^{O_2}$	$Q_{CO_2}^{N_2}$	Absolute Pasteur effect	Meyerhof oxidation quotient	Ref.
Carcinomas							
Lip	50	4	12	23	11	8.2	398
Tongue	50	1	9	12	3	9.0	398
Mucous membrane of mouth	50	5	10	18	8	4.8	481
Mucous membrane of cheek	75	5	14	18	4	2.4	398
Stomach	—	—	—	30	—	—	398
Stomach	80	5	8	18	10	6.0	398
Colon	—	8	17	27	10	3.8	481
Rectum	—	5	16	27	11	6.6	481
Rectum	30	2	6	11	5	7.5	398
Rectum	—	6	7	17	10	6.0	398
Rectum	—	—	7	22	15	—	398
Rectum	80	0	9	22	13	—	398
Rectum	—	17	17	27	10	1.8	398
Rectum	—	7	5	16	9	3.9	398
Rectum	—	9	16	29	13	4.3	398
Rectum	40	1	5	11	6	—	398
Skin	67	4	16	21	5	3.8	481
Skin	50	4	15	19	4	3.0	481
Skin	50	7	12	17	5	2.2	481
Skin	30	4	13	22	9	6.7	481
Skin	—	5	5	13	8	4.8	481

TABLE 3 (*Continued*)

	Per cent tumor	$-Q_{O_2}$	$Q_{CO_2}^{O_2}$	$Q_{CO_2}^{N_2}$	Absolute Pasteur effect	Meyerhof oxidation quotient	Ref.
Carcinomas (*Continued*)							
Skin (penis)	50	8	19	29	10	3.7	481
Skin (penis)	35	6	12	15	3	1.5	481
Skin (penis)	60	2	12	18	6	9.0	481
Larynx	—	3	11	21	10	10.0	481
Larynx	80	8	15	19	4	1.5	481
Larynx	80	4	12	18	6	4.5	398
Vagina	60	6	18	22	4	2.0	398
Breast	30	2	4	10	6	9.0	398
Breast	95	7	9	15	6	2.6	398
Breast	—	11	13	19	6	1.6	126
Breast	80	2	7	7	0	0	126
Breast	—	2	5	11	6	9.0	126
Breast	—	6	5	10	5	—	126
Breast	—	2	7	10	3	4.5	126
Breast (metastatic node)	—	5	14	15	1	0.6	126
Cystadenocarcinoma of ovary[a]							
Grade I	—	3	11	—	—	—	116
Grade II	—	3	13	—	—	—	116
Grade III	—	4	11	—	—	—	116
Sarcomas							
Round cell	—	5	—	28	—	—	481
Fibrosarcoma	—	1	4	4	0	0	481

[a] Aerobic glycolysis expressed in lactic acid determined chemically rather than as total acid production.

TABLE 3 (*Continued*)

	Per cent tumor	$-Q_{O_2}$	$Q_{CO_2}^{O_2}$	$Q_{CO_2}^{N_2}$	Absolute Pasteur effect	Meyerhof oxidation quotient	Ref.
Sarcomas (*Continued*)							
Large spindle cell	—	1	7	5	—2	—	398
Small round cell	80	10	13	17	4	1.2	398
Mixed cell	100	6	6	12	6	3.0	398
Large round cell	100	7	6	12	6	2.6	398
Benign tumors							
Nasal polyp	—	5	5	14	10	6.0	481
Nasal polyp	—	4	5	13	8	6.0	398
Bladder papilloma	—	9	7	17	10	3.3	398
Bladder papilloma	—	13	16	26	10	2.3	481
Ovarian cystadenoma[a]	—	4	2	—	—	—	116

[a] Aerobic glycolysis expressed in lactic acid determined chemically rather than as total acid production.

ascites form may possess values as high as 30. The one important exception is a group of mouse tumors (Table 2) which display no aerobic glycolysis. The absence of significant aerobic glycolysis in certain mouse tumors is probably a manifestation of the high oxidative rate of these tissues which, combined with a normal inhibitory effect of respiration on glycolysis, results in the complete suppression of the large anaerobic glycolysis under aerobic conditions. Murphy and Hawkins (Table 2) attempted to correlate the respiration and glycolysis of a group of spontaneous mouse tumors with growth rate, mitotic rate, and tendency to metastasize (331). Unfortunately, no correlation between the metabolic and the biological properties of the neoplasms was found.

B. Normal Tissues—Adult and Embryonic

For any significance to be attached to this high anaerobic and aerobic glycolysis of tumors, similar rates of glycolysis must be shown not to be common properties of normal tissues. In Table 4 the studies with normal tissues are tabulated and indicate that normal tissues do indeed display a different pattern from that of tumors, although exceptional cases can be found in which normal tissues have several metabolic properties of the tumor slices. A large group of normal tissues, including many of those with which the biochemist is most familiar (liver, kidney, pancreas, submaxillary gland, and thyroid) show an anaerobic glycolysis rate of under 5, and no appreciable aerobic lactate production. Another important group of normal tissues including the spleen, ovary, and endocrine organs forms a second group with higher anaerobic glycolysis (Q values up to 15), but again with little aerobic glycolysis. The tissues of nervous origin, e.g. brain and retina, comprise a separate category showing a quite high rate of anaerobic glycolysis and, in the case of retina, a high rate of aerobic glycolysis also. Interestingly enough, the high rate of aerobic glycolysis of retina seems to be a manifestation of the higher mammalian body temperature, since Warburg's studies (262) have shown that frog retina exhibits no aerobic glycolysis at 25°, while it does at 40°.

The hematopoietic tissues also show significant glycolysis (287). Bone marrow glycolyzes aerobically and anaerobically, and studies on various types of bone marrow indicates that this glycolysis is caused by the myeloid elements (484). Likewise, tonsil, particularly hyperplastic tonsil (481), displays anaerobic and aerobic gly-

TABLE 4
RESPIRATION AND GLYCOLYSIS OF NORMAL TISSUES

	$-Q_{O_2}$	$Q_{CO_2}^{O_2}$	$Q_{CO_2}^{N_2}$	Absolute Pasteur effect	Meyerhof oxidation quotient	Ref.
Liver (rat)	12	1	3	3	0.7	481
Pancreas (dog)	3	0	4	4	4.0	481
Pancreas (rabbit)	5	0	3	3	2.2	481
Thyroid (rat)	13	0	2	2	0.5	481
Submaxillary (rat)	4	0	2	2	1.5	481
Adrenal (rabbit)	10	0	13	13	3.9	175
Pituitary (rabbit)	12	0	13	13	3.2	175
Parathyroid (rabbit)	8	1	10	9	3.4	175
Testicle (rat)	11	2	7	5	1.4	175
Ovary (mouse)	1	0	6	6	18.0	175
Kidney (rat)	21	0	3	3	0.5	481
Kidney medulla (guinea pig)	9	12	22	10	2.8	130
Kidney medulla (cat)	2	7	12	5	10.0	130
Spleen (rat)	13	0	9	9	2.1	175
Thymus (rat)	5	1	8	8	4.1	481
Tonsil (human)	5	3	13	9	3.5	481
Hyperplastic tonsil (human)	9	9	18	9	2.8	481
Lymph gland (human)	4	2	5	3	2.0	481
Bone marrow (rabbit)	4	4	7	3	2.5	352
Bone marrow, erythroid (rabbit)	9	0	7	7	2.3	484
Bone marrow, myeloid (rabbit)	6	9	22	13	6.5	484
Bone marrow, white (rat)	11	3	21	18	4.9	175
Leukocytes (rat)	9	2	20	18	6.0	175

TABLE 4 (*Continued*)

	$-Q_{O_2}$	$Q_{CO_2}^{O_2}$	$Q_{CO_2}^{N_2}$	Absolute Pasteur effect	Meyerhof oxidation quotient	Ref.
Sperm (rat)	6	1	5	4	2.0	175
Brain gray matter (rat)	11	3	19	17	4.5	481
Celiac ganglion (rabbit)	4	0	12	12	9.0	175
Retina (rat)	31	45	88	43	4.2	481
Retina at 25° C. (frog)	6	0	18	18	8.8	262
Retina at 40° C. (frog)	4	42	42	0	0	262
Embryo (chick)	10	1	21	20	5.8	481
Embryo, 3.0 mg. (rat)	12	0	13	13	3.2	59
Embryo, 0.9 mg. (rat)	13	6	23	17	3.9	59
Placenta (mouse)	7	3	15	12	4.8	175
Chorion	18	0	33	33	5.5	480
Embryonic liver, 5th day (rat)	16	1	14	12	2.4	447
Embryonic liver, 7th day (rat)	12	0	8	8	2.0	447
Embryonic lung (rat)	10	0	14	14	4.2	175
Muscle fascia (rat)	Trace	Trace	Trace	—	—	481
Synovial membrane (cow)	0.5	1.7	2.3	0.6	3.6	74
Cartilage (cow)	0.01	0.20	0.34	0.14	—	75
Smooth muscle stomach (human)	1.3	0.9	2.3	1.4	3.2	398
Smooth muscle intestine (cat)	2.6	0.4	6.1	5.7	6.6	398
Mucous membrane stomach (mouse)	10	3	14	11	3.4	398
Mucous membrane intestine (rat)	12	2	4	2	0.5	481
Mucous membrane jejunum	15	18	14	— 4	— 0.8	131
Mucous membrane colon (cat)	11	3	14	12	3.1	398

colysis; it is also well established that the adult leukocytes show active aerobic glycolysis (29–31). It appears that all these actively glycolyzing hematopoietic tissues contain in common cells of the leukocyte series.

Of embryonic tissues, the whole chick and rat embryo as well as fetal lung and liver possess rates of anaerobic glycolysis between 10 and 20. But of these tissues only the small rat embryo (0.9 mg.) shows any anaerobic glycolysis, and this, according to Warburg (471) is an artifact caused by the medium since there is no aerobic glycolysis by the embryo with intact fetal membranes. Chorion (480) and placenta (175) both show high rates of anaerobic glycolysis, while placenta shows an appreciable although small aerobic rate. Villee (460) has made a detailed study of the glycolysis and respiration of a number of human embryonic tissues (liver, heart, kidney, diaphragm, lung, and cerebral cortex) at various periods of the gestation. Table 5 indicates that there is a significant aerobic glycolysis of all the human embryonic tissues at all the periods of gestation studied. (The respiration of human embryonic tissues displayed no characteristic pattern during gestation. The respiratory rate of all human tissue is much lower than that of rodent. Thus, while the Q_{O_2}, on a per milligram dry weight basis, fell from 5.3 at 7–10 weeks to 3.2 at term, the respiration of fetal heart was 3.4 during early gestation and 5.3 at term; lung, 2.9 at 7–10 weeks and 2.0 at term; diaphragm, 1.9 at 7–10 weeks and 2.0 at term; and cerebral cortex, 4.9 at 7–10 weeks and 4.6 at term.) The suggestion has been made that active glycolysis is a manifestation of rapid tissue growth, either benign or malignant (205).

A final group of normal tissues with glycolytic rates typical of the tumor group should be considered. Dickens found that the mucous membrane of the jejunum showed a consistent and well maintained rate of both anaerobic and aerobic glycolysis (131). The mucous membrane of more distal sections of the gastrointestinal tract gave much more variable results. An active aerobic and anaerobic glycolysis in both Ringer's solution and homologous serum was also found by Dickens for the kidney medulla (130) of several animals. Finally, both synovial membrane (74) and cartilage (74, 75, 133) show appreciable rates of both aerobic and anaerobic glycolysis but, in view of the low over-all oxidative rate, the comparison of these tissues with more actively metabolizing tissues is difficult.

TABLE 5
AEROBIC LACTATE PRODUCTION[a] IN HUMAN FETAL TISSUES (460)

Tissue	Substrate	Fetal age in weeks					
		7.5–10	11–12	13–15	16.5–19	20–40	40
Liver	Glucose and pyruvate	24.1	19.7	18.9	22.0	17.8	24.0
	Alanine and pyruvate	7.9	18.3	9.8	—	—	—
Heart	Glucose and pyruvate	58.5	59.1	49.0	44.2	49.9	99.3
	Alanine and pyruvate	41.3	—	37.5	—	—	—
Lung	Glucose and pyruvate	16.3	12.0	14.9	18.4	—	19.6
	Alanine and pyruvate	7.4	11.7	6.0	—	—	—
Kidney	Glucose and pyruvate	19.7	25.0	21.0	17.6	16.8	21.5
	Alanine and pyruvate	7.6	8.2	—	—	—	—
Diaphragm	Glucose and pyruvate	22.0	21.4	15.6	16.3	12.9	26.5
	Alanine and pyruvate	—	—	3.7	3.3	—	—
Cerebral	Glucose and pyruvate	15.3	20.9	17.3	16.2	26.3	29.3
Cortex	Alanine and pyruvate	6.1	—	4.0	—	—	—

[a] In micromoles lactate per gram wet weight per hour.

Attempts have been made to determine to what extent the anaerobic and aerobic glycolysis of normal tissues may represent injury to the tissue caused by the medium (128, 471). It has been possible to show that several tissues, e.g. testis and chorion (128, 337) glycolyze aerobically in Ringer's solution but not in serum, while early embryos glycolyze even in serum but not in amniotic fluid with intact fetal membranes (337). On the other hand, with most tissues, including retina (337), intestinal mucosa (131), and renal medulla (130), the measurements of rates of glycolysis in bicarbonate-Ringer and phosphate-Ringer solutions and in serum have yielded results which are qualitatively similar.

C. Homologous Series of Normal and Neoplastic Tissues

Studies of the glycolytic behavior of tumors and of homologous normal tissue afford the best basis for comparison of the glycolysis of tumor and normal tissue. The first of these studies contained in Table 6 illustrates the changes that take place in the glycolytic and oxidative behavior of liver during butter yellow carcinogenesis (61, 63, 132). Both the hepatoma and the mixed butter yellow tumors show a marked aerobic and anaerobic production of lactate, a property not possessed by the normal liver. Whether the preneoplastic liver of animals fed butter yellow has an increased anaerobic glycolysis is not clear. The four pertinent studies are evenly split, two indicating the presence of increased anaerobic glycolysis (61, 252), and two reporting its absence (132, 353). The question of an increased anaerobic glycolysis of preneoplastic liver over that of normal liver is a difficult one because of the lack of agreement on the level of anaerobic glycolysis of the normal tissue (61, 252, 353, 398, 486). Detailed investigations indicate that the rate of anaerobic glycolysis of normal liver depends on the medium (486), and on the glycogen content of the liver (353). Under all the conditions studied, liver shows a glycogenolysis, not a *glucolysis* (i.e. the lactate formation is independent of the presence of glucose). A medium high in potassium, phosphate, and bicarbonate favors glycogenolysis and anaerobic lactate formation. The anaerobic lactate formation also varies directly with the glycogen content of the liver; this glycogen effect is most marked in the medium containing the ions listed above, is present but less marked in Ringer-bicarbonate and in serum, and is absent in certain other media. Thus it is difficult to evaluate the small changes in aerobic gly-

colysis which have been reported in the preneoplastic liver during azo dye carcinogenesis since, in most instances, the glycogen content has not been adequately controlled (61, 252). However, all investigators agree that the butter yellow preneoplastic liver lacks significant aerobic glycolysis, while the hepatoma glycolyzes both aerobically and anaerobically (61, 132, 252, 353).

A rather surprising observation is the finding that regenerating liver displays an oxidative and glycolytic behavior similar to that of normal liver. The rates of aerobic and anaerobic glycolysis are low and similar to the rates of normal liver (61), and most observers agree that at the time of maximal mitotic activity (2–3 days) there is no increase (61) or but slight increase (385) in the rate of respiration. One observer has reported that the oxygen uptake of regenerating liver is higher (358) than in the sham-operated control from the 14th to the 21st day after hepatectomy, but it is difficult to assign significance to this observation since regeneration is maximal at 2–3 days and generally considered to be complete at the end of 2 weeks. This similarity between the energy metabolism of normal and regenerating liver suggests that a cautious attitude must be assumed in expecting simple correlations between energy metabolism and growth.

Also included in Table 6 are studies on a series of spontaneous mouse hepatomas (132). An elevated rate of anaerobic and aerobic glycolysis was found in only one of six such tumors studied. The authors make the point that the amino azo dye rat tumors were histologically malignant, showed loss of many of the specialized biochemical functions of normal liver (urea, acetoacetate, and carbohydrate synthesis and uric acid oxidation), and had the typical aerobic and anaerobic glycolysis of tumors; the mouse tumors, on the other hand, were well differentiated, retained the many biochemical special functions, and also maintained the glycolytic character of normal liver.

Studies with human skin and human endometrium (132, 138) are consistent with the azo dye rat liver studies; i.e., in both cases the malignant tissue has acquired a high rate of aerobic glycolysis not present in the homologous nontumor tissue. In the case of skin the neoplasm also displays an anaerobic glycolysis not present in normal skin, while the leukoplakic skin (considered to be a preneoplastic condition) shows a modest anaerobic glycolysis.

Several authors have studied the behavior of rabbit skin during

TABLE 6

RESPIRATION AND GLYCOLYSIS OF HOMOLOGOUS SERIES OF NORMAL AND NEOPLASTIC TISSUES

	$-Q_{O_2}$	$Q^{O_2}_{CO_2}$	$Q^{N_2}_{CO_2}$	R.Q.
Normal and neoplastic rat liver (61, 63)				
Normal liver	6.0	1.5	1.0	0.70
Azo dye-fed normal (yeast protected)	4.9	1.6	2.1	0.98
Azo dye-fed normal lobe (adjacent tumor)	7.7	1.3	2.3	0.82
Azo dye-fed cirrhotic	6.5	1.5	3.1	0.82
Azo dye-fed necrotic tumor	4.2	3.9	6.0	0.77
Azo dye-fed mouse transplant	4.7	2.1	7.7	0.66
Azo dye-fed metastasis	5.0	2.5	8.7	0.83
Azo dye-fed adenocarcinoma-hepatoma	7.3	3.3	10.0	0.84
Azo dye-fed hepatoma	6.4	6.0	12.1	0.87
Regenerating liver (2 days)	4.5	0.8	0.6	0.38
Regenerating liver (3 days)	6.4	1.5	1.8	0.64
Fetal	6.0	0.6	8.1	1.0
Postembryonic (24 hr.)	6.9	1.2	2.3	0.66
Normal and neoplastic rat liver (132)				
Nontumor part of liver	10.2	0.5	5.2	0.89
Intermediate state (cirrhosis)	10.5	1.0	6.2	0.83
Liver tumors	12.0	6.1	12.5	0.79

TABLE 6 (*Continued*)

	$-Q_{O_2}$	$Q_{CO_2}^{O_2}$	$Q_{CO_2}^{N_2}$	R.Q.
Spontaneous mouse hepatomas (132)				
Hepatoma #1	8.0	— 0.1	2.1	—
Control liver #1	7.5	0.1	2.1	—
Hepatoma #2	16.5	0.5	5.6	0.82
Control liver #2	14.9	0.5	6.7	0.89
Hepatoma #4	8.6	5.3	6.1	—
Control liver #4	12.7	— 1.9	3.2	—
Hepatoma #5	13.4	— 3.3	1.7	—
Control liver #5	12.0	— 1.2	2.2	—
Hepatoma #6	6.1	— 2.4	0.9	0.72
Control liver #6	13.0	— 6.3	1.6	0.67
Lymph nodes from lymphatic leukemia of mice				
Normal node (458)	5.5	2.1	5.8	—
Nodes from transmitted leukemia				
Line A	6.4	1.9	8.3	—
Line I	5.5	5.6	12.8	—
Line M—liver	5.8	8.4	19.9	—
Line M—spleen-D	6.7	6.2	15.3	—
Spontaneous leukemia C-58 (457)				
Normal (6–8 wk.)	5.5	2.1	5.7	—
Normal (6–9 mo.)	4.8	3.1	7.8	—
Spontaneous leukemia (8–15 mo.)	5.6	4.3	10.2	—

TABLE 6 (*Continued*)

	$-Q_{O_2}$	$Q_{CO_2}^{O_2}$	$Q_{CO_2}^{N_2}$	R.Q.
Lymph nodes etc. (*Continued*)				
Normal (199)	6.3	2.0	4.7	—
Transmitted leukemia (199)				
AK mice	5.8	3.2	7.5	—
RAK mice	6.3	2.5	10.4	—
Spontaneous leukemia (199)				
AK mice	6.0	3.6	10.5	—
RAK mice	6.4	1.3	8.5	—
Human leukocytes[a] (29)				
Normal	0.40	3.01	—	—
Chronic myelocytic leukemia	0.12	1.27	—	—
Chronic lymphocytic leukemia	0.11	0.48	—	—
Tissue culture studies				
Chick fibroblasts (480)	15	5	23	—
Chick fibroblasts (270)	16	21	47	—
Embryonic heart (480)	30	13	52	—
Earle's "cancer" cells (474, 521) (high malignancy)	7	30	93–100	—
Earle's "cancer" cells (474, 521) (low malignancy)	13	10	36– 42	—
Normal and neoplastic rabbit skin (251)				
Normal skin	1.0	1.4	1.5	0.89
Hyperplastic skin	3.3	1.8	2.3	0.86
Virus-induced fibroma	0.6	1.3	2.9	0.92
Benign Shope papilloma	3.0	2.8	6.9	0.84

[a] Homogenized cells, expressed millimoles per 10^{10} cells per hour.

TABLE 6 (*Continued*)

	$-Q_{O_2}$	$Q^{O_2}_{CO_2}$	$Q^{N_2}_{CO_2}$	R.Q.
Normal and neoplastic rabbit skin (251) (*Continued*)				
V-2 carcinoma from Shope papilloma	3.2	4.6	10.2	0.67
(Brown-Pierce carcinoma)	—	—	11.8	—
(Andrews sarcoma)	—	—	12.0	—
Normal and neoplastic rabbit skin (34)				
Normal skin	0.9	0.45	1.3	0.7
Shope papilloma	0.6	0.3	1.25	0.6
Normal and neoplastic human skin (132)				
Normal skin of vulva	0.86	0.27	1.15	—
Leukoplakia of vulva	1.29	1.65	5.0	—
Squamous carcinoma of vulva	2.69	12.4	20.7	0.92
Viral skin lesions (107)				
Normal chicken lesion	1	0.4	1.5	—
Vaccinia lesion	4.9	4.1	10.9	—
Fowl pox	1–6	2.9–8.5	7.3–18.0	—
Human wart	1.2–1.7	1.6–2.2	3.8–4.1	
Normal and neoplastic human endometrium (138)				
Proliferative	16.9	1.4	9.7	—
Secretory	16.3	1.0	8.5	—
Decidua	16.4	0.8	14.2	—
Hyperplasia of endometrium	16.5	1.1	11.2	—
Adenomyosis	16.5	1.2	10.6	—
Carcinoma of endometrium	13.3	10.0	14.2	—

the induction of viral tumors (Table 6). There is disagreement as to whether the benign Shope papilloma differs from the normal skin with regard to its aerobic and anaerobic glycolysis. In one extensive study (251), the papilloma was found to display an increase in both aerobic and anaerobic glycolysis over normal skin, while another report (34) found identical glycolysis in papilloma and normal rabbit skin. However, it seems well established that the malignant V-2 carcinoma that develops from the Shope papilloma possesses the typical tumor metabolism of high aerobic and anaerobic glycolysis. The anaerobic glycolysis of the V-2 carcinoma is in the same range as that of other rabbit malignancies. Crabtree (107) has studied the metabolism of viral skin lesions, and his findings cast some doubt on the specificity of a tumor metabolism characterized by high aerobic and anaerobic glycolysis. Thus, the nonneoplastic viral skin lesions he studied all displayed a significant aerobic and anaerobic glycolysis.

The metabolism of lymph nodes from normal mice and mice with spontaneous and transmitted lymphatic leukemia has been studied extensively by Victor and Potter (452–458). These investigators observed that a quantitatively small but consistent increase in the anaerobic and aerobic glycolysis of the lymph nodes (Table 6) was associated with the development of spontaneous or transmitted lymphatic leukemia. The increases observed were greater with the transmitted leukemias than with the spontaneous ones; aerobic glycolysis was less consistently increased than was anaerobic lactate production. Hall and Furth (199), also studying lymph nodes from spontaneous and transmitted lymphoid leukemia, confirmed these observations on glycolysis. They observed, in addition, that there was no difference in the oxygen uptake of normal or leukemic nodes, and that the Pasteur effect was greater in leukemic than in normal lymph nodes. Burk et al. (66), on the other hand, found that there was no increase in the anaerobic glycolysis of the spleen and lymph nodes of mice with radiation- and methylcholanthrene-induced leukemia, but that the anaerobic glycolysis of the leukemic liver was increased 2 to 8-fold over the non-leukemic value. These latter workers also found that the aerobic glycolysis of preleukemic, leukemic, and leukemoid lymph nodes, liver, and spleen was increased 50–100% over the normal value. In contrast to these studies with leukemic lymph nodes, which have generally shown an increase of the glycolysis of the neoplastic node over the

normal node, are the studies with isolated leukocytes. The normal leukocyte has been found to have an active aerobic and anaerobic glycolysis when studied either as the intact cell (302), or in homogenate form (29–31). The aerobic and anaerobic glycolysis of the leukocyte in chronic lymphocytic and chronic myelocytic leukemia has been found to be considerably decreased from this high value of the normal white cell (29, 302).

Studies on the glycolysis of cells in tissue culture are of great interest, although the relationship of the metabolism of cells in tissue culture to the metabolism of the same cells *in vivo* is unclear at the present time. Early experiments indicated that in tissue culture both chick fibroblasts (270, 480) and embryonic heart (480) display a high aerobic and anaerobic glycolysis. Other investigators have reported significant aerobic glycolysis in explants of chick embryonic tissues (357, 511, 512).

Recently a very extensive study on the metabolism of human embryonic and malignant cells in tissue culture has appeared (286). These workers found that irrespective of the standard of reference (total nucleic acid, cell number, protein nitrogen, or dry weight) the average rates of glucose consumption and acid production were always higher for embryonic cells than for tumor cells (Table 7). (With regard to the glucose oxidized [glucose consumption-acid production], on a "per cell" basis, the malignant cells oxidized more glucose than did the embryonic cells, while on a total nucleic acid basis the reverse was true. On a dry weight basis embryonic and malignant cells oxidized about the same amount of glucose.) It thus seems that under tissue culture conditions embryonic cells exceed malignant cells in their rate of aerobic glycolysis. How this is to be reconciled with Warburg's conclusions (471) that the intact embryo, handled carefully and *in situ* in the embryonic membranes, displays no aerobic glycolysis, is not clear. A possibility, although not a very convincing one, is that the aerobic glycolysis of the explants is a manifestation of damage to the respiratory apparatus of the cell in tissue culture. In the present author's opinion one of the following would be a more convincing explanation: (1) the embryo contains tissues which both produce and utilize lactate while the explant represents a selection of a single tissue with high aerobic glycolysis; (2) in the process of adapting to tissue culture the metabolism of the explant is modified from the parent embryonic tissue; (3) the details of tissue culture techniques (few cells bathed

TABLE 7

METABOLIC QUOTIENTS FOR HUMAN EMBRYONIC AND MALIGNANT CELLS IN TISSUE CULTURE (286)

	Quotients in moles per hour					
	Q glucose utilized		Q total acid produced		Q glucose oxidized	
	Per mg. nucleic acid phosphorus	Per mg. dry weight	Per mg. nucleic acid phosphorus	Per mg. dry weight	Per mg. nucleic phosphorus	Per mg. dry weight
Embryonic skin fibroblasts	70.0	—	113.5	—	13.1	—
Embryonic kidney cells	68.2	—	109.8	—	13.4	—
Embryonic lung fibroblasts	74.5	—	118.6	—	15.2	—
Mean (embryonic cells)	70.4	6.4	114.0	9.9	13.6	1.30
HeLa[a] carcinoma cells	19.1	—	16.0	—	11.0	—
HEP1[a] carcinoma cells	19.2	—	16.8	—	10.8	—
HEP2[a] carcinoma cells	19.7	—	20.8	—	9.8	—
Mean (carcinoma cells)	19.3	2.9	17.3	2.6	10.6	1.57

[a] HeLa and HEP1 carcinoma cells were derived from patients with epidermoid carcinoma of the cervix, while HEP2 cells were derived from a patient with epidermoid carcinoma of the larynx.

by a large amount of medium) allow diffusion of lactate into the medium, removing the controlling mechanism of the intracellular lactate concentration (286); (4) there are experimental artifacts in one or the other measurements (286). These explant studies also suggested a qualitative difference between embryonic and neoplastic cells in their response to insulin (227). With embryonic cells, insulin caused decreases in acid production relative to glucose utilization and in the ratio of lactic acid to keto acid in the medium (compared to controls to which hormone had not been added). With carcinoma cells, on the other hand, increases in these ratios accompanied by more intensive glycolysis were observed after insulin addition.

The relationship of the glycolytic behavior typical of the tumor slice to the development in tissue culture of malignant behavior on subsequent transplantation is intriguing. It will be noted (Table 6) that the aerobic and anaerobic glycolysis of a clone of fibroblasts of high malignancy (97% takes, on subsequent transplantation) was three times that of a clone of low malignancy (1% takes) (473, 474, 521). The two clones were descended from the same cell. The clone of high malignancy contained three times as much aldolase (11,300 versus 3700 Warburg units per milliliter of cells) and twice as much α-glycerophosphate dehydrogenase (2600 versus 1400 Schade units per milliliter of cells) as the low malignancy strain. In addition, the respiratory rate of the high malignancy line (Q_{O_2} = 5 to 10) was lower than that of the low malignancy line (Q_{O_2} = 10 to 15).

D. Fructolysis and Concentration Dependence of Glycolysis

The preceding sections have been concerned only with the glycolysis of glucose (glucolysis). Surviving normal and neoplastic slices and ascites cells are able to glycolyze mannose and fructose as well, at varying rates. Two separate studies have indicated that slices of Flexner-Jobling carcinoma (471) and Krebs-2 ascites cells (534) are able to form lactate at the same rate from mannose as from glucose. Warburg himself has reported different results on different occasions on the rates of fructolysis by slices of Flexner-Jobling carcinoma. One report stated that this tumor attacks fructose at the same rate as glucose (325), while at another time it was reported that the rate of fructolysis was only 14% of the rate of glucolysis (481). Dickens (124) studied a group of tumor slices,

and found a rate of fructolysis which varied from 0 to 40% of the rate of glucolysis by the same tumor. The Mill Hill fowl fibrosarcoma and the Jensen rat sarcoma (Table 18) were among the tumors that displayed more rapid fructolysis. Several strains of ascites cells (Ehrlich ascites and sarcoma 180 ascites) have been reported to display a rate of fructolysis at sugar concentrations below 0.005 *M*, which is slightly less than 50% of the rate of glucolysis (263). Other recent studies have reported that slices of mouse sarcoma 303 (321) and of Krebs-2 ascites cells (534) possess the same rates of glucolysis and fructolysis at high sugar concentrations, while at lower concentrations the rate of fructose breakdown lags considerably behind the rate of glucose breakdown. Failure to adequately control the concentration of glycolytic substrate may explain the divergent results with regard to the relative rates of glucolysis and fructolysis.

Table 8 contains results of studies on the relationship of glycolysis to sugar concentration. In separate experiments with tumor slices it has been found that the Jensen rat sarcoma (124) and the Flexner-Jobling carcinoma display dependence of the rate of glycolysis on sugar concentrations below 0.011 *M*. Studies with ascites cells have generally shown this experimental material to be remarkably free of concentration dependence of glucose glycolysis. Thus the glycolysis of the TA-3 ascites cell (43) and of the Krebs-2 ascites cell (534) were independent of sugar concentration down to a glucose concentration of 0.005 *M* and the Ehrlich ascites cell was independent down to 0.0002 *M* (246, 263). The only exception is the Lettré-Ehrlich strain (43) which does appear to display a moderate concentration dependence of glycolysis. Fructose glycolysis is moderately concentration dependent (263, 534) even in the ascites cell. It is reasonable to assume that the lack of concentration dependence of ascites cell glucolysis compared to the concentration dependence of glucolysis of the slice is because of the much greater diffusion difficulties of the latter tissue.

E. Summary

In summarizing the preceding sections it is apparent that the high aerobic and anaerobic glycolysis of the tumor slice, although not unique, is nonetheless a very impressive biochemical finding. When allowance is made for the admixture with normal tissue, the anaerobic glycolysis of tumor tissues is almost always in excess of

TABLE 8
CONCENTRATION DEPENDENCE OF GLYCOLYSIS IN NEOPLASTIC SLICES AND ASCITES CELLS

Tumor	Conditions	Sugar	Dependence	Ref.
Flexner-Jobling	Slice (N_2)	Glucose	Dependent (below 0.011 *M*)	471
Jensen Rat Sarcoma	Slice (N_2)	Glucose	Dependent (below 0.011 *M*)	124
Ehrlich	Ascites (N_2)	Glucose	Independent (0.005–0.055 *M*)	
		Fructose	(rate at 0.005 = 40% of rate at 0.055 *M*)	263
TA-3	Ascites (O_2)	Glucose	Independent (0.0005–0.0025 *M*)	43
Ehrlich (Lettré)	Ascites (O_2)	Glucose	Dependent (rate at 0.01 *M* = 12 times rate at 0.0001 *M* and 1.5 times rate at 0.001 *M*)	43
Ehrlich	Ascites (O_2)	Glucose	Independent (down to 0.0002 *M*)	246
Krebs-2	Ascites (N_2)	Glucose	Independent (0.0005–0.1 *M*)	534
		Fructose	Independent (0.015–0.1 *M*)	
			Dependent (below 0.015 *M*)	

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		Fructose	Independent (0.015–0.1 M)	
			Dependent (below 0.015 M)	

20 (expressed as $Q_{CO_2}^{N_2}$) and, except for mouse tumors, the aerobic glycolysis usually exceeds 10. Exceedingly few normal tissues display this combined behavior of aerobic and anaerobic glycolysis. In the group of normal tissues displaying a high aerobic and anaerobic glycolysis are jejunal mucosa, renal medulla, retina, myeloid bone marrow, and tonsil. The last two normal tissues may derive their glycolysis from the cells of the leukocyte series present, since the leukocytes of the blood are known to glycolyze actively also. Embryonic cells in tissue culture and viral skin lesions represent two other exceptions of non-neoplastic conditions in which a high rate of aerobic and anaerobic glycolysis is found. It is not unexpected that free-living cells such as the adult leukocyte and cells in tissue culture would have an exceptional energy metabolism.

2. Tumor Glycolysis *in Vivo*

Two types of studies on the *in vivo* glycolysis of tumors are available. The Coris and, later, Warburg compared the level of the blood lactate and blood glucose of the tumor-draining vein with that of the corresponding normal vein. The Coris (105, 106) found that the blood from the vein draining a Rous chicken sarcoma had, on the average, a 23-mg.% lower blood glucose and a 16.2-mg.% higher blood lactate than the blood from the vein of the non-tumor-bearing wing. Similarly, a large human fibrosarcoma of the forearm showed a blood sugar 12 mg.% lower and a blood lactate 8.6 mg.% higher than that from the same vein of the normal arm. Warburg (483) compared the arterio-venous (a-v) differences of the blood glucose and blood lactate for the Jensen rat sarcoma with the jugular, renal, ileal, and portal areas of the same animal. The non-tumor areas showed a-v differences in blood sugar that varied from 2 mg.% for the jugular system to 18 mg.% for the ileal area. In other studies, the blood draining from the Jensen sarcoma showed an average blood glucose level of 54 mg.% in contrast to the arterial value of 124 mg.%, an a-v difference of 70 mg.% which indicated that 57% of the blood glucose is removed in its passage through the tumor. Calculation indicates (483) that the tumor *in vivo* glycolyzes 7% of its dry weight of glucose per hour at a blood glucose level of 0.2% (200 mg.%), a figure that is in good agreement with the rate of *in vitro* glycolysis of slices of this tumor ($Q_{CO_2}^{O_2}$ of 16 = 10% of dry weight per hour). With regard to lactate

production, the non-tumor areas all lowered the blood lactate in its passage through the tissue, while the Jensen sarcoma raised the blood lactic acid level from 30 to 76 mg.% in its transit through the tumor. In recent studies (342), a rise in the blood lactate was also found in tumor-bearing mice following the administration of glucose. Active *in vivo* glycolysis was also indicated in mouse Ehrlich ascites cells by the extensive randomization of isotope in the isolated liver glycogen after *in vivo* administration of specifically labelled glucose (209). Finally, it has been possible to demonstrate that the presence of intraperitoneal implants of the Novikoff hepatoma or the Walker 256 carcinosarcoma reduced the blood sugar level of rats with alloxan diabetes (186), and that the content of liver glycogen (185) and its rate of deposition after glucose injection (184, 531) was much lower in tumor-bearing than in normally fed rats.

An ingenious method has been devised for the study of *in vivo* tumor glycolysis: that of measuring with a small glass electrode the changes in tissue pH produced by glycolysis in the living tumor. Using such a capillary glass electrode, Voegtlin *et al.* (461), found that the tissue pH of several animal sarcomas and carcinomas (including the Jensen rat sarcoma and the Flexner-Jobling carcinoma) was about 7.0, and became more acid shortly after the injection of glucose, mannose, xylose, fructose, or maltose but not after galactose or lactose. Figure 1 shows later and more refined studies on the effect of glucose administration on the pH of normal liver and hepatic tumor of the rat (235). Whereas normal rat liver has a resting pH of 7.4 which does not change upon glucose administration, the rat hepatoma has a resting pH of 7.0 which falls to 6.4 upon glucose administration. Mouse hepatoma is somewhat more variable but the average pH is 6.7 at rest and 6.2 after glucose. Levels of tumor pH below 6.2–6.4 could not be obtained, and this probably represents the maximal acidity at which glycolysis can continue. (Warburg *et al.* (481) showed that the *in vitro* glycolysis of slices of Flexner-Jobling carcinoma decreases with falling pH and is almost completely inhibited at a pH of 6.0.) By using improved techniques of simultaneous measurement of the pH in a number of areas of the tumor with multiple electrodes, these studies have been extended (143) to a larger group of eight additional transplantable rat tumors (a lymphosarcoma, four sarcomas, two hepatomas, and one carcinoma). The results were similar to

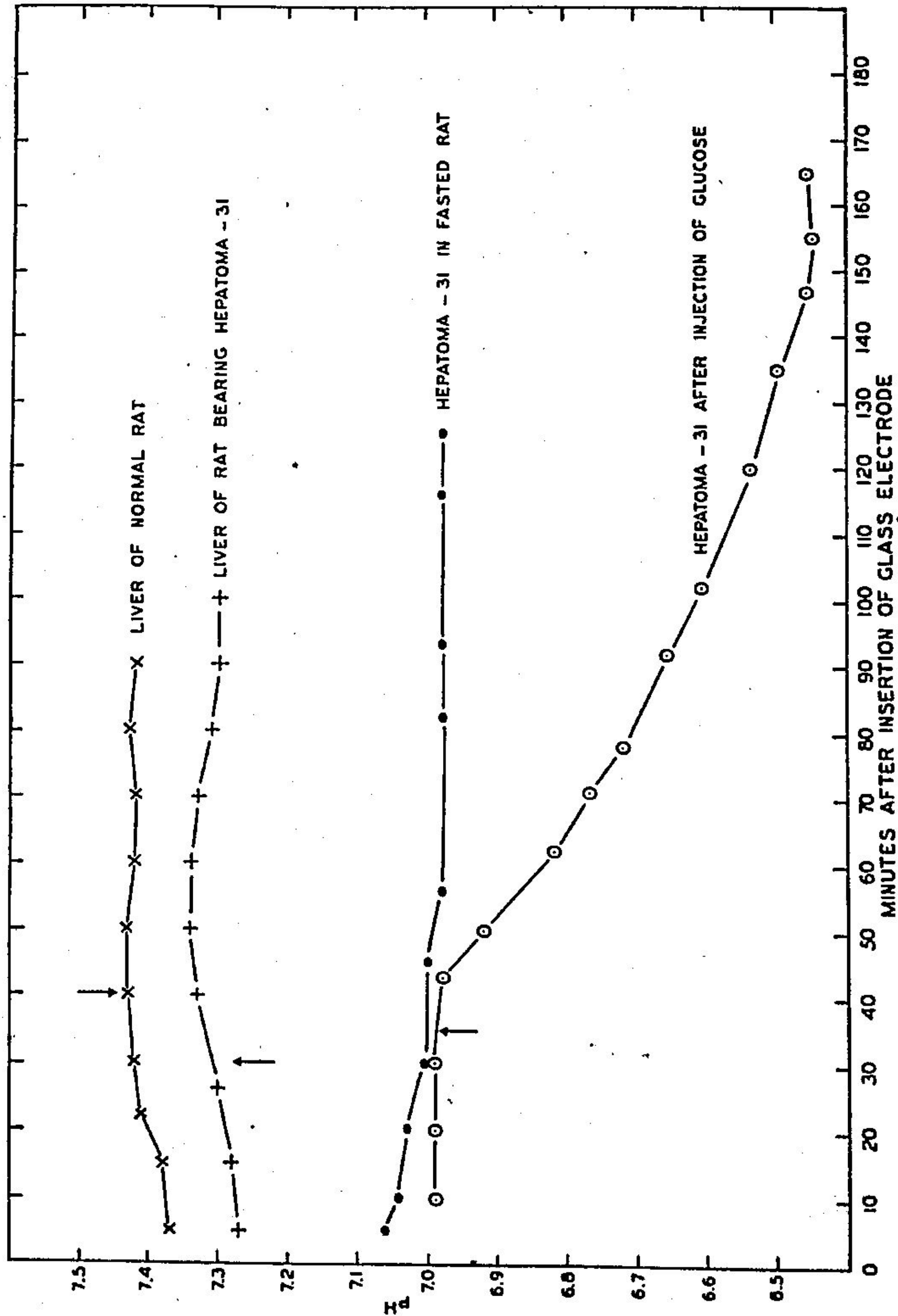


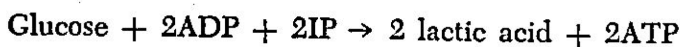
FIG. 1. Effect of glucose on tissue pH of normal rat liver and hepatoma. From Kahler and Robertson (235).

the earlier work. The average intratumor pH was 6.99 before and 6.55 at a time 3 to 4 hours after, the administration of glucose to the tumor-bearing animal. In contrast, before glucose the pH of the leg muscles and of liver was 7.40, while after glucose administration the pH of the leg muscles fell only to 7.26. A group of Japanese workers (445, 446) reported similar findings. Human tumors have also been found to have a lower pH than normal human tissues (323). It would appear desirable to study the behavior of other actively glycolyzing normal tissues, such as brain, for comparison with the behavior of tumor in this regard.

The remarkable extent of the glycolysis of the tumor cell *in vivo* is demonstrated in studies on mice bearing Line I lymphatic leukemia (454). These animals, in the course of ether anesthesia, displayed a fall of blood sugar to a value one-third that of normal animals. In experiments on Ehrlich ascites cells (246) Kemp and Mendel have found that extremely rapid rates of diffusion of glucose into the ascitic fluid and of lactate from the ascitic fluid into the blood are necessary to meet the sugar requirements of the neoplastic cell. Their observations indicated that 14 mg. of glucose could diffuse into, and 13 mg. of lactate out of, 5 ml. of ascites fluid in an hour. In the living animal the sugar level of the ascitic fluid was found to be 5–7 mg.%, a level that is just sufficient to allow maximal glycolysis in this tumor cell. [The Ehrlich ascites cell glycolyzes maximally above 4 mg.% (479)].

3. Tumor Glycolysis in Extracts and Homogenates

Figure 2 represents the over-all Embden-Meyerhof scheme for glycolysis in a mammalian tissue such as muscle, as it is understood today. Chart 1 (see Appendix) is a historical review of the major discoveries which formed the experimental basis for the Embden-Meyerhof formulation of the mechanism of muscle and yeast glycolysis. Since 1 mole of glucose gives rise to 2 moles of lactate the over-all reaction can be written:



$$\text{Glucose} + 2\text{ADP} + 2\text{IP} \rightarrow 2 \text{ lactic acid} + 2\text{ATP}$$

Certain reactions in the scheme deserve particular attention. The hexokinase and phosphohexokinase reactions resemble each other in that both require ATP and both are essentially irreversible. ATP is regenerated from ADP in the phosphopyruvic and phosphoglyceric transferase reactions and, since each mole of glucose yields 2

moles of triose, a net gain of 2 moles of ATP is realized for each glucose molecule consumed. The two molecules of inorganic phosphate, required for the phosphorylation of the 2 moles of ADP to ATP, enter the glycolytic scheme at the triose phosphate dehydro-

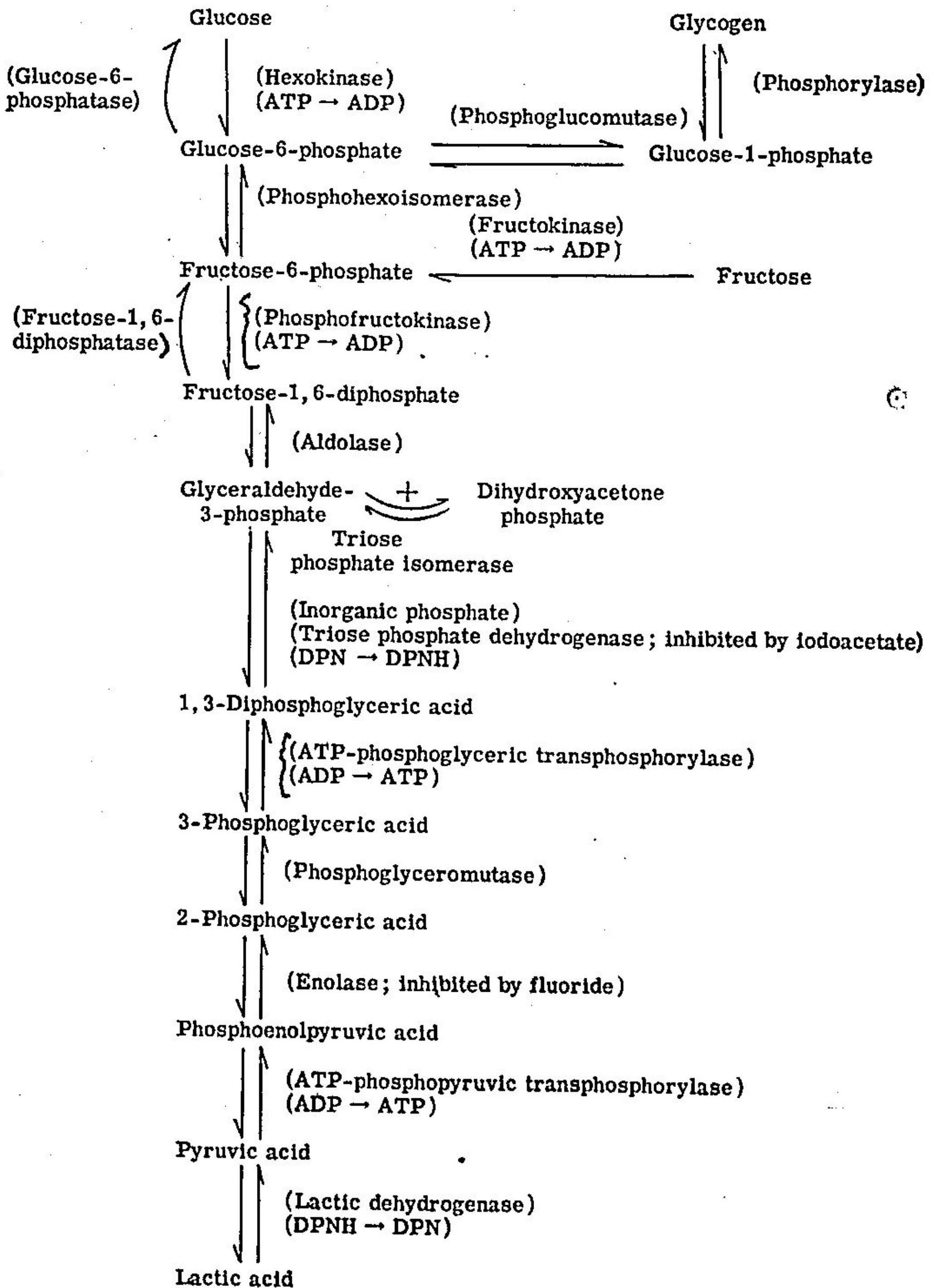


FIG. 2. Anaerobic glycolysis in muscle (Embden-Meyerhof scheme).

genase reaction (the reaction that is most sensitive to iodoacetate). The triose phosphate dehydrogenase reaction is also noteworthy as the only oxidative step in the glycolytic pathway. The DPNH formed in this step is used to convert pyruvate to lactate, regenerating the DPN, thus enabling DPN to function catalytically in this anaerobic pathway. The aldolase reaction is important as the reaction in which the hexose molecule is cleaved. Fluoride inhibits the enolase step but, because of the nature of the equilibrium between the two phosphoglyceric acids, it is 3-phosphoglyceric acid that accumulates.

As in many fields of biochemistry, the early work on tumor glycolysis in relatively intact systems such as the tissue slice has been followed by studies on more purified systems, tissue extracts, and homogenates. Although early experiments (335) suggested that tumor glycolysis was nonphosphorylating, the more recent work of Boyland, of Meyerhof, and of LePage has established clearly that tumor glycolysis is qualitatively similar to glycolysis in normal tissues, as the latter is understood today.

The first studies on glycolysis in tumor extracts which have current interest are those of Boyland and Boyland (52–54). These authors discovered early in their work that extracts of the Crocker 180 mouse sarcoma contained active enzymes which destroyed adenylpyrophosphate (ATPase) and cozymase (DPNase), and that for active glycolysis to be maintained in the extract ATP and DPN had to be added. However, if these substances were added, active glycolysis of glucose, hexose monophosphate, and hexose diphosphate by the extract was obtained, and a rate of lactate production (8–10 mg. lactic acid per gram wet tissue per hour) comparable to the rate of the slice (11 mg. per hour calculated from a $Q_{CO_2}^{N_2}$ of 20–22) could be obtained.

Meyerhof's important studies on tumor glycolysis are a natural evolution of his earlier experiments on glycolysis in yeast and brain, and it is difficult to consider the tumor work without a discussion of these earlier studies. Meyerhof was originally interested in the Harden-Young effect (200), a phenomenon seen in glycolyzing yeast extracts and dried yeast but not seen in whole living cells. The Harden-Young effect describes the two phases of yeast fermentation observed in such extracts: an initial "phosphate" period of rapid glucose fermentation in which there is stoichiometric ac-

cumulation of hexose phosphate (hexose monophosphate and hexose diphosphate), and a later "hexose diphosphate" period in which either the inorganic phosphate or the free sugar has been exhausted and which is characterized by a much slower rate of fermentation. Meyerhof (314, 315) demonstrated that the Harden-Young effect in yeast extracts was caused by the destruction of the labile adenosine triphosphatase (ATPase) in the course of preparation of the extract, showing that in the preparation of the extract the ATPase remains with the solid particles which are discarded and that the rate of fermentation of HDP by an extract correlates well with the ATPase level of the preparation. Furthermore, the addition of potato ATPase to a yeast extract abolished the Harden-Young effect and resulted in a rapid and continued fermentation of HDP equal to the maximal rate of sugar fermentation of the initial "phosphate" period. Meyerhof was able to obtain a quick-dried preparation of Brewer's yeast in which the ATPase activity was preserved and which showed fermentation both without a break (no Harden-Young effect) and without phosphate ester accumulation. Moreover, if ATPase inhibitors such as octyl alcohol or phenylurethane were added to this preparation of quick-dried yeast, a Harden-Young effect appeared. Arsenate, which removes the phosphate requirement for glycolysis and eliminates the Harden-Young effect of ordinary yeast extracts by yielding a spontaneously hydrolyzable arsenate ester, removes the requirement of an active ATPase. Meyerhof concluded that for active, continued glycolysis, without the accumulation of hexose diphosphate, a balanced activity of ATPase and hexokinase is necessary. In ordinary yeast extracts the ATPase is fragile and is destroyed, while an active hexokinase remains. Under these conditions glycolysis slows with a pile-up of hexose diphosphate since the inactive ATPase fails to provide sufficient inorganic phosphate and ADP. In whole living yeast or in the quick-dried preparation the ATPase provides sufficient inorganic phosphate and phosphate acceptor, and glycolysis proceeds unimpeded without hexose diphosphate accumulation.

With brain (317–319, 322) a very different balance was found to obtain with regard to the ATPase activity. Fortified whole homogenates and slices of brain show a similar glycolytic behavior: a moderate rate of lactate formation from free sugar ($Q_L^{N_2} = 8$), with more rapid fermentation of glucose than of fructose (at sugar con-

centrations below 2.5%). If, however, the homogenate is freed of its particles by centrifugation, or the activity of the particles is destroyed by freezing or hypotonicity, then the resulting extract shows a much more rapid glycolysis ($Q_L^{N_2} = 50$), and now glucose and fructose are fermented at the same rate. It was concluded that the factor responsible for the slow glycolysis of the whole homogenate was the ATPase, which is known to be concentrated in the particles of the brain homogenate. Repeated ATP additions to the whole homogenate, for the purpose of maintaining the ATP concentration, resulted in an increase in the rate of glycolysis ($Q_L^{N_2} = 20$), with equal rates of glucolysis and fructolysis at all sugar concentrations. This increase in the rate of glycolysis of free sugar could also be achieved in the whole homogenate by the addition of such inhibitors of ATPase as decyl alcohol. Thus in brain slices and whole homogenates, glycolysis is limited by a lack of ATP which is caused by excess of ATPase activity. The lower rate for the glycolysis of fructose under these conditions was ascribed by Meyerhof to a lower affinity of fructose for ATP in the presence of hexokinase.

With a group of transplanted rat and mouse tumors the following situation prevailed (320, 321): whole homogenates and centrifuged extracts of tumor both showed a rapid glycolysis of HDP ($Q = 30$–50) and hexose monophosphate ($Q = 80$), but no glycolysis or only very transient glycolysis of free sugar. This lack of glycolysis of free sugar in tumor homogenates and extracts is caused by an active ATPase which destroys the ATP but which, unlike the ATPase of brain, is in solution or fine suspension and therefore does not centrifuge with the particles. Rapid glycolysis of free glucose can be obtained in tumor extracts or homogenates if hexokinase is added or if the ATPase is inhibited with octyl alcohol, toluene, or azide ($Q = 40$–60). The soluble ATPase of tumor is more easily inhibited by higher alcohols than is the particulate ATPase of brain, and is also inhibited by lipid solvents and digitonin.

Thus Meyerhof concludes (313) that active glycolysis of free glucose is the result of a fine balance between hexokinase, which uses ATP to initiate glycolysis, and ATPase, which splits ATP. Insufficient ATPase results in a glycolysis inhibited by lack of inorganic phosphate and phosphate acceptor, while excess ATPase results in a glycolysis limited by lack of ATP to phosphorylate glucose.

Both brain and tumor homogenates have an excess of ATPase but, whereas the brain ATPase is particulate, the tumor ATPase can not be centrifuged and is more susceptible to inhibition by octyl alcohol.

Extensive studies by LePage have established without question the primary phosphorylative nature of tumor glycolysis. The data from Table 9 on the analyses of organic phosphate compounds indicate that the levels of the sugar phosphate and adenine nucleotides in a group of transplanted and spontaneous tumors are quite comparable to the levels found in normal tissues (280). In another study (281) AMP, ADP, ATP, glucose-6-phosphate (G-6-P) and 1,2-propanediol phosphate were actually isolated from Flexner-Jobling rat carcinoma with results that confirmed the analytical studies. It was also shown (282) that anoxia *in vivo* resulted in a rise of the lactic acid content and a fall in the levels of phosphorylated hexose and pyrophosphate phosphate of the Jensen sarcoma and the Flexner-Jobling carcinoma, suggesting that phosphorylating glycolysis was functioning *in vivo*.

A system was developed by LePage *et al.* (284, 343, 440) for demonstrating phosphorylation coupled to anaerobic glycolysis in homogenates of tumors. The medium contained fluoride, pyruvate, DPN, ATP, nicotinamide, glucose, and low levels of hexose diphosphate. In this system up to 0.8 μmoles of phosphate were esterified per micromole of lactate formed, while if the fluoride was omitted glycolytic rates ($Q_{CO_2}^{N_2}$) of 43 to 85 were obtained for Flexner-Jobling, Walker 256, and Jensen rat tumors. In cell fractionation studies (285), the soluble fraction of the Flexner-Jobling carcinoma (S_2) was the only fraction found to have glycolytic activity by itself, although the addition of microsomes, mitochondria, or nuclei each increased the activity of the supernatant fraction (Table 10). This localization of the glycolytic system in the soluble particle-free supernatant fraction was also found in rabbit liver (285) and is consistent with Meyerhof's findings given above. Kennedy and Lehninger observed similar localization of the glycolytic system in rat liver (247). The supernatant fraction of the cytoplasm (soluble fraction plus microsomes) contained 82% of the glycolytic activity (hexose diphosphate as substrate) of the unfractionated homogenate, while this fraction also contained 96% of the aldolase. The mitochondria and nuclei were both free of significant glycolysis and aldolase activity.

TABLE 9

GLYCOLYTIC INTERMEDIATES IN NORMAL AND NEOPLASTIC TISSUES[a] (280)

Components	Brain	Muscle	Liver	Kidney	Heart	Primary mouse carcinoma	Primary liver carcinoma (rat)	Human breast carcinoma	Flexner-Jobling carcinoma	Walker 256 carcinosarcoma	Jensen sarcoma	Mouse ear carcinoma
Lactic acid	141	188	230	155	578	833	590	1458	862	824	637	704
Glycogen[b]	531	3480	28450	81	2460	232	468	1620	67	65	43	56
Acid-soluble phosphorus	2390	5070	3040	2530	3200	1830	2810	2030	2650	2430	2130	2950
Inorganic phosphorus	495	748	417	497	730	723	828	382	1035	622	580	726
Organic phosphorus	1895	4322	2623	2033	2470	1108	1983	1649	1615	1808	1550	2224
Phosphocreatine	311	1630	274	116	219	88	0	46	92	116	78	94
Adenylic acid	151	155	144	213	329	97	278	137	131	171	183	251
Adenosine diphosphate	27	59	330	48	65	12	133	51	49	25	46	135
Adenosine triphosphate	179	542	8	138	105	93	54	67	106	152	142	161
Glucose-1-phosphate	61	80	42	42	175	47	100	101	104	130	106	156
Glucose-6-phosphate	185	250	423	264	249	335	555	470	278	454	393	500
Fructose-6-phosphate	30	33	24	17	53	11	34	16	7	14	17	37
Hexose diphosphate	6	7	17	4	7	5	21	15	5	6	5	11
Phosphoglyceric acid	98	140	183	102	209	167	116	161	119	148	98	165
"Coenzymes"	17	17	35	16	25	—	—	—	—	—	—	—
Free pentose phosphate	42	22	48	72	50	—	—	—	—	—	—	—
Per cent of organic phosphate accounted for	80	81	70	68	65	95	78	77	72	86	91	89

[a] As micromoles per gram.
[b] As hexose.

TABLE 10

GLYCOLYSIS[a] IN CELL FRACTIONS OF FLEXNER-JOBLING CARCINOMA AND RABBIT LIVER (285)

	Flexner-Jobling Carcinoma				Rabbit liver			
	Lactic acid produced per flask (μmoles)	Net P uptake per flask (μmoles)	Nitrogen (μg.; %)	RNA (μg.; %)	Lactic acid produced per flask (μmoles)	Net P uptake per flask (μmoles)	Nitrogen (μg.; %)	RNA (μg.; %)
Homogenate	7.4	3.6	1503(100)	55(100)	6.3	0.2	2580(100)	47(100)
Nuclei (N)	1.3	1.2	559(37)	21(38)	0.8	0.3	574(22)	13(28)
Mitochondria (M_w)	0	—0.1	124(8)	6(11)	0	0.1	272(11)	5(10)
Microsomes (P_w)	0.1	—0.8	130(9)	9(17)	0.2	—0.3	373(15)	15(32)
Supernatant fluid (S_2)	2.7	2.1	759(51)	18(32)	3.3	0.4	1260(49)	15(32)
$N + M_w$	2.0	1.2	—	—	0.8	0.5	—	—
$N + P_w$	2.3	0.6	—	—	1.7	—0.1	—	—
$N + S_2$	4.2	2.4	—	—	4.8	0.4	—	—
$M_w + P_w$	0.4	—0.5	—	—	0.6	—0.2	—	—
$M_w + S_2$	4.3	2.8	—	—	4.3	0.1	—	—
$P_w + S_2$	4.9	2.2	—	—	6.0	0.3	—	—
$N + M_w + P_w + S_2$	7.3	3.3	1572(105)	54(98)	6.6	0.2	2479(96)	48(102)

[a] Glycolysis obtained in 40 minutes with 30 mg. of tissue or fractions therefrom in a medium containing 12 μmoles of hexose diphosphate and 30 μmoles of glucose.

stress, but in the light of other data this was interpreted as evidence of a stability of the hormonally controlled insulin:anti-insulin system, which was postulated as controlling glucose utilization. In a later study this stress-modifiable insulin:anti-insulin mechanism, believed to regulate glucose utilization in the well-differentiated S-91 melanoma, was found to be absent in the more anaplastic melanomas and in the Krebs-2 ascites tumor (517). It was concluded that in normal tissues glycolysis was carefully regulated by a hormonal insulin:anti-insulin mechanism. In well-differentiated tumors (melanoma S-91) the same metabolic control appeared to operate, but these tumors were unable to maintain glycolytic homeostasis during stress. Progressive anaplasia of neoplastic tissues (the melanoma series was studied) was associated with a graded loss of sensitivity of the glucose metabolism to such hormonal regulation. Thus the most anaplastic tumors displayed a complete loss of control of hexokinase activity with a resultant high, sustained rate of glycolysis. Because of the work of Hochstein on mitochondrial glycolysis (215), Burk *et al.* (64) feel that the site of action of this hormonal regulatory system is the mitochondrial hexokinase. These authors believe that the *sine qua non* of cancer metabolism is a high rate of initial glucose phosphorylation due to a decreased restraint or inhibition of the mitochondrial hexokinase reaction by anti-insulin hormones. In the present author's opinion, the significance of this particular mechanism of hexokinase regulation in cancer metabolism remains to be determined.

In studying the anaerobic glycolysis of hexose diphosphate or of glucose in the fluoride-blocked homogenate system (with pyruvate added, to accept the hydrogen of DPNH), Groth and LePage (196, 197) found that a considerable amount of pyruvate disappeared which could not be accounted for as lactate. In the Flexner-Jobling carcinoma this excess pyruvate disappearance was found to be largely caused by propanediol phosphate formation, while in the non-tumor tissues studied a considerable part of this excess pyruvate was decarboxylated.

Clowes and Keltch (100, 101) have studied the phosphorylating mechanism of the Walker 256 carcinosarcoma and have compared it with rat liver and rat brain in respect to the importance of oxidative and glycolytic phosphorylation, the site of localization within the cytoplasm, and the resistance to dinitrocresol (an uncoupler of oxidative phosphorylation). They found that in this tumor, under

TABLE 11

ANAEROBIC GLYCOLYSIS[a] WITH GLUCOSE AND PHOSPHORYLATED SUGARS BY HOMOGENATES OF RAT TISSUES (283)

	Experiment 1	Experiment 2		Experiment 3				
	30 μ*M* glucose, 12 μ*M* HDP	6 μ*M* HDP	30 μ*M* glucose, 6 μ*M* HDP	No substrate	6 μ*M* HDP	30 μ*M* glucose, 6 μ*M* HDP	4.5 μ*M* G-6-P, 6 μ*M* HDP	4.5 μ*M* F-6-P, 6 μ*M* HDP
Heart	119	9.1	9.9					
Skeletal muscle	112	9.0	8.7					
Diaphragm	163	5.5	5.6	0.1	6.2	6.6	8.9	9.5
Kidney	98	6.5	6.8	0.1	6.0	6.0	8.3	8.2
Liver	78	6.4	6.8	0.1	7.7	7.6	9.0	9.6
Brain	76	6.0	9.8	0.1	6.8	9.1	8.6	9.6
Flexner-Jobling carcinoma	74	6.2	9.5	—	—	—	—	—
Walker 256 carcinosarcoma	68	—	—	—	—	—	—	—
Jensen sarcoma	80	—	—	—	—	—	—	—

[a] In experiment 1 lactate accumulation is measured manometrically and expressed in microliters CO_2 released per milligram dry weight of tissue per hour. In experiments 2 and 3 it is measured chemically and expressed as micromoles released per 30 mg. wet weight of tissue, except for diaphragm where 20 mg. is used.

stress, but in the light of other data this was interpreted as evidence of a stability of the hormonally controlled insulin:anti-insulin system, which was postulated as controlling glucose utilization. In a later study this stress-modifiable insulin:anti-insulin mechanism, believed to regulate glucose utilization in the well-differentiated S-91 melanoma, was found to be absent in the more anaplastic melanomas and in the Krebs-2 ascites tumor (517). It was concluded that in normal tissues glycolysis was carefully regulated by a hormonal insulin:anti-insulin mechanism. In well-differentiated tumors (melanoma S-91) the same metabolic control appeared to operate, but these tumors were unable to maintain glycolytic homeostasis during stress. Progressive anaplasia of neoplastic tissues (the melanoma series was studied) was associated with a graded loss of sensitivity of the glucose metabolism to such hormonal regulation. Thus the most anaplastic tumors displayed a complete loss of control of hexokinase activity with a resultant high, sustained rate of glycolysis. Because of the work of Hochstein on mitochondrial glycolysis (215), Burk *et al.* (64) feel that the site of action of this hormonal regulatory system is the mitochondrial hexokinase. These authors believe that the *sine qua non* of cancer metabolism is a high rate of initial glucose phosphorylation due to a decreased restraint or inhibition of the mitochondrial hexokinase reaction by anti-insulin hormones. In the present author's opinion, the significance of this particular mechanism of hexokinase regulation in cancer metabolism remains to be determined.

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the conditions they employed, the major part of the phosphate uptake, both aerobic and anaerobic, was caused by the glycolysis of the soluble cytoplasmic fraction which was dinitrocresol-resistant. On the other hand, in rat brain and liver, although this glycolytic component was present, there was also an important aerobic dinitrocresol-sensitive substrate-dependent mechanism located in the particles (oxidative phosphorylation). Since DPN was not added, it is possible that these studies did not give a true picture of the tumor capacities for oxidative phosphorylation.

More recently, studies have been done on certain of the glycolytic enzymes of the ascites cell and the leukemic leukocyte. Wu and Racker (529) have found that the ascites cell resembles brain (111) in that the hexokinase is largely localized in the particles. In the ascites cell, the major part of the other glycolytic enzymes are located in the soluble cytoplasmic fraction (529). Detailed studies by Yushok (534) have revealed that the hexokinase of the ascites cell resembles that of brain in substrate specificity also. Thus the particulate hexokinase of the Krebs-2 ascites cell shows a ratio of Michaelis constant for glucose to Michaelis constant for fructose of 90, while the figure for brain hexokinase is similar, 200. Both hexokinases attack glucose and mannose with about equal facility. A study (110) has revealed that various sugars penetrate the ascites cell by a mechanism described by a first-order equation with an equilibrium concentration inside and outside of unity.

Beck has studied the glycolytic enzymes of the normal leukocyte and of leukocytes in chronic myelocytic and lymphocytic leukemia (26–28). He found no difference in the properties of any of the glycolytic enzymes. However, the level of hexokinase, which appeared from his kinetic studies to be the rate-limiting enzyme in both normal and abnormal cells, was found to be particularly low in the leukemic cells. This suggested to him that the low level of glycolysis which has been described in leukemic leukocytes is caused by a lack of hexokinase activity. This lack of hexokinase activity of the leukemic leukocyte would also inhibit glycolysis by depressing the level of available phosphate acceptor (ADP). Such a secondary depression of phosphate acceptor was thought to be a significant factor in the low rates of glycolysis of leukemic cells because these cells are deficient in other ADP-generating systems. It should be mentioned that the glycolysis of the leukocyte is quite exceptional among normal tissues, i.e., a very active glycolysis of the normal leukocyte exceeds that of the neoplastic cell.

4. Metabolic Inhibitors of Glycolysis

This section is devoted to studies with glycolytic inhibitors. The rationale of their use in tumor chemotherapy will be discussed at greater length in a later section (Part V), but for the moment it is sufficient to point out the obvious interest in attempting to inhibit the prominent glycolysis of tumors. The classic inhibitors of glycolysis, fluoride (enolase inhibition) and iodoacetate (glyceraldehyde phosphate dehydrogenase inhibition), will not be discussed since their great toxicity would presumably preclude their use as chemotherapeutic agents. Rather, a number of recently developed inhibitors of glycolysis will be considered of which 2-deoxyglucose is the best studied compound.

2-Deoxyglucose was first shown to inhibit the aerobic glycolysis of yeast (522) and of chick heart fibroblasts in tissue culture (152). Later studies with slices of mammalian normal tissues and tumors (523) are included in Table 12. Of the tissues studied, brain was

TABLE 12
EFFECT OF 2-DEOXYGLUCOSE ON GLYCOLYSIS OF SLICES OF RAT TUMOR AND NORMAL TISSUES (523)

Tissue	Ratio of 2-deoxyglucose to glucose	Inhibition (%)
Flexner-Jobling carcinoma	1 to 1	72
	0.5 to 1	46
	0.25 to 1	22
Walker 256 carcinosarcoma	1 to 1	89
	0.5 to 1	30
	0.25 to 1	0
Brain	0.25 to 1	95
Diaphragm	0.5 to 1	75
Liver (fructolysis)	1 to 1	39

the most sensitive, and fructolysis was found to be more sensitive than glucolysis. These early studies showed that 2-deoxyglucose affected the endogenous respiration of glucose by brain slices only at much higher concentrations than those which inhibited glycolysis, although fructose oxidation was moderately sensitive. The inhibition was competitive (could be removed by excess glucose) and involved both aerobic and anaerobic glycolysis.

Sols and Crane (435) have shown that 2-deoxyglucose is a sub-

strate for brain hexokinase. It has been found that the Michaelis constant of yeast hexokinase for glucose is lower than the constant for 2-deoxyglucose (524), which indicates that yeast hexokinase has a greater affinity for glucose than 2-deoxyglucose. This makes it unlikely that hexokinase is the site of action of 2-deoxyglucose inhibition of yeast glycolysis. Wick *et al.* have found that 2-deoxyglucose enters the extrahepatic tissue of the eviscerated animal (506) and of the isolated rat diaphragm (333) by a mechanism which is sensitive to insulin. In the rat diaphragm, 2-deoxyglucose leads to a 40% reduction in the uptake of glucose despite the injection of maximal insulin.

More recent studies by Wick *et al.* indicated that C^{14}-labelled 2-deoxyglucose was itself oxidized only in trace amounts, and that it did not influence the oxidation of labelled acetate (507) in kidney slices. These results suggested to the authors that 2-deoxyglucose was inhibiting an early glycolytic reaction, and studies with purified rat kidney phosphohexoisomerase did indicate that 2-deoxyglucose competitively inhibited ketose formation from glucose-6-phosphate. Other workers (340) confirmed phosphohexoisomerase inhibition by 2-deoxyglucose in intact ascites cells, but felt, on the basis of studies with homogenates of ascites cells, in which equal inhibition of the glycolysis of glucose, fructose-6-phosphate, and hexose diphosphate was displayed, that 2-deoxyglucose also inhibited a reaction beyond the cleavage of hexose diphosphate.

Despite the above evidence of phosphohexoisomerase inhibition, this site of action does not appear to explain adequately 2-deoxyglucose inhibition of glycolysis, in several regards. The most significant objection is that this locus fails to account for inhibition of fructolysis, while a second less serious point is that higher concentrations of 2-deoxyglucose are required for the isomerase inhibition than for the effect on glucolysis in the intact slice. It would still seem reasonable to look for the locus of action in the hexokinase reaction and/or the mechanism of glucose transport into cells. [Evidence suggestive of such a mechanism already has appeared (274, 340)].

In addition to the tissues mentioned above, 2-deoxyglucose has been shown to inhibit the glycolysis of Ehrlich ascites tumor cells and human leukemic cells *in vitro* (274). The compound has been tried against a number of experimental animal tumors (153) with some promising results. Growth of the Crocker rat sarcoma was

inhibited 60% (434) and that of the Walker 256 carcinosarcoma, 10–20% (21), while there was some prolongation of survival time of mice with lymphoid leukemia L-1210 (274) and with Krebs-2 ascites tumor (273). Metabolic and pharmacologic effects following the administration of 2-deoxyglucose in man have been described (266).

Only sporadic reports on other potential glycolytic inhibitors are at present available. 3-Deoxy-3*F*-glyceraldehyde (347) was found to have an action like that of fluoroacetate, inhibiting the Krebs cycle even though it is phosphorylated to the sugar phosphate. This compound is devoid of any inhibitory effect on glycolysis. The compounds 3-*O*-methyl glucose, 3-*O*-methyl fructose, 6-*O*-methyl glucose, and 6-deoxy-6*F*-glucose are all apparently not potent glycolytic inhibitors (274), though the last-mentioned compound has been found to inhibit competitively the oxidation of glucose-C^{14} in kidney slices (419). 2-Deoxy-galactose appears to be similar to 2-deoxyglucose in its effectiveness as an inhibitor of glycolysis in leukemic cells (274). Two analogs of *dl*-glyceraldehyde (glycidaldehyde and α-methoxyhydracrylaldehyde) were found to produce potent inhibition of both glycolysis and respiration of various whole ascites tumor cells by means of a mechanism that involved the formation in the aldolase reaction of a compound inhibitory to hexokinase (182). Fluoropyruvic acid has been reported to inhibit both the oxidative and reductive metabolism of pyruvate by acting as a non-competitive inhibitor of lactic dehydrogenase (71). The effects of a miscellaneous group of compounds on the glycolysis of the Krebs-2 ascites tumor cell have been described by Yushok (533).

5. Glycolytic Enzymes in the Blood

A. Aldolase

This section concerns studies on the levels of glycolytic enzymes in the blood of patients and animals with neoplasms. The enzyme which has received most study is aldolase. Elevation of this enzyme and of phosphohexose isomerase was first reported by Warburg and Christian (476) in animals with large Jensen sarcomas (greater than 2% of body weight). However, they reported that the enzyme was rarely elevated in neoplastic disease in man. On the basis of studies which showed that (1) the levels of aldolase in the tumor

artery and vein were equal and (2) the level of aldolase was only high enough to take care of the glycolysis of the tumor, these authors concluded that the source of the aldolase was probably skeletal muscle (the only tissue with an extremely high aldolase level).

More detailed studies (428) on the serum aldolase elevation have been performed by Sibley and Lehninger, taking advantage of a newly developed method (427) for the determination of the aldolase level. Studying sarcoma 39 and the Walker 256 carcinosarcoma, they found that an elevation of the serum aldolase was first detectable when the tumor reached a size of 5 gm. (2–3% of body weight, i.e., equivalent to a 1.5-kg. tumor in the human). If the tumor was excised or its growth inhibited by urethane, X-ray, or nitrogen mustard, the elevated aldolase declined to normal value. Furthermore, the aldolase was not elevated by a series of nonspecific states such as fasting, infection, anemia, pregnancy, adrenalectomy, splenectomy, or partial hepatectomy. Extending the study to man, they found elevation of the serum aldolase in about 20% of 104 cases of cancer, but it was also elevated in 2 cases of muscular dystrophy and 1 case each of gout and liver disease (Table 13). Baker and Govan (18) have found that the serum aldolase was elevated in 12 of 16 patients with proven metastatic carcinoma of the prostate, and all but 2 of these patients responded to hormonal therapy with a return of the enzyme level to normal. Unfortunately, there was no increase in the aldolase in early, nonmetastatic cancer of the prostate.

Later work by Sibley *et al.* (425, 426) has established that the tumor may indeed be the source of the elevated serum aldolase, contrary to the opinion of Warburg. Studies with the new, more sensitive method for measuring aldolase indicated that the level of enzyme in the tumor venous blood was actually higher than in the arterial blood. Furthermore, assay of the enzyme level in the tumor (Walker 256 carcinosarcoma) showed the level to be higher than Warburg had found (Table 14), while extirpation of the tumor resulted in a prompt fall of the elevated serum aldolase to normal (comparable to the rate of fall following an injection of the crystalline enzyme). Sibley also found that there was an increase in the aldolase of animals following nephrectomy, indicating that the enzyme is probably in part excreted by the kidney.

Studies by Warburg and Hiepler (479) and by Schade (405)

have shown that there is a rise in the aldolase level of the ascitic fluid following the anaerobic incubation of Ehrlich ascites cells, but not following their aerobic incubation. These results were confirmed by Sibley *et al.* (425, 426), who demonstrated that slices of

TABLE 13
SERUM ALDOLASE IN HEALTH AND DISEASE (428)

Tissue and condition	Serum aldolase[a]
Non-neoplastic (123 cases)	
Normal	3.5–8.0
Pregnancy	3.4–8.4
Infections	3.2–8.2
Blood dyscrasia	3.3–9.4
Non-neoplastic disease	3.1–9.4
Progressive muscular dystrophy	20.8, 14.3
Gout	12.6
Jaundice	13.8
Neoplastic (21 of 104 cases)	
Carcinoma of breast	11.8, 12.0 36.0
Carcinoma of prostate	10.5, 11.2, 13.9, 12.0
Carcinoma of pancreas	11.1
Carcinoma of stomach	27.2, 12.2
Carcinomatosis	11.8
Carcinoma of lung	11.5
Carcinoma of esophagus	10.9
Chronic lymphatic leukemia	11.6
Hypernephroma	10.5, 17.7
Acute myelogenous leukemia	26.6
Chondrosarcoma	12.0
Carcinoma of rectum	14.7
Carcinoma of adrenal	14.0
Carcinoma of bladder	12.9

[a] Expressed in microliters of hexosediphosphate split per milliliter per hour.

Walker 256 carcinosarcoma released aldolase into the medium under anaerobic conditions, particularly when glucose was not present. Thus it would appear clear that the elevation of the serum aldolase results, at least in part, from the liberation of the enzyme from foci of necrosis and anaerobiosis of the tumor. That other factors are involved will be clear from the discussion of abnormalities of other serum enzymes which follows. Recently, Wu has

studied (527) the leakage of enzymes from ascites tumor cells under various conditions in an attempt to define whether this enzyme loss is due to cell lysis, simple diffusion, of differential leakage. He studied both glycolytic enzymes and enzymes of the hexose monophosphate shunt. (Actually, under the conditions of

TABLE 14

ALDOLASE LEVELS IN NORMAL AND NEOPLASTIC ANIMAL TISSUES (427)

Tissue	Aldolase[a]
Skeletal muscle	74,800
Brain	15,800
Heart	15,600
Liver	12,100
Red marrow	9,500
Adrenal	8,600
Kidney	7,800
Spleen	4,800
Thyroid	4,800
Thymus	4,700
Prostate	4,400
Parotid	3,800
Stomach	3,700
Bladder	3,100
Placenta	3,000
Testis	2,900
Lung	2,800
Uterus	2,100
Red cells	900
Pancreas	500
Fat	400
Serum	60
Sarcoma 39	14,000
Walker 256	15,600

[a] Expressed as microliters at hexosediphosphate split per gram of fresh tissue per hour.

his experiments the metabolism of ribose-5-phosphate took place outside the cells in the ascites supernatant fluid, the presence or absence of cells making little difference.) Wu found, as had previous investigators, that under anaerobic conditions these enzymes rapidly appeared in the ascites supernatant fluid when glucose was absent but not when glucose was present. Furthermore, under aerobic conditions with glucose absent, dinitrophenol enhanced the cellular loss of enzymes. Studying the rate of loss of a group of

enzymes from the cell under varying conditions of pH, temperature, and the presence and absence of air, glucose, and dinitrophenol, he felt the evidence favored a differential loss of ascites cell enzymes rather than loss due to simple diffusion or cell lysis. (Under the conditions of these experiments only a 2-fold variation was observed by Wu in the proportions of any two enzymes in the supernatant fluid.)

B. Phosphohexose Isomerase

Elevation of phosphohexose isomerase was also reported by Warburg (476), but as yet few studies on this enzyme in neoplasia have been made. Bodansky improved the method of measurement of the enzyme in serum (44) and measured it in a select group of patients with metastatic carcinoma of the breast (45) and prostate (46). In 10 patients with metastatic carcinoma of the breast the serum isomerase was felt to parallel the activity of the metastatic disease of bone (as judged by clinical impression and urinary calcium studies). In 6 patients with metastatic carcinoma of the prostate the enzyme level was also thought to correlate with the activity of the metastatic disease, as measured by acid and alkaline phosphatase levels. Serum phosphohexose isomerase has been found to be elevated in chronic myelocytic leukemia, though it is not elevated in chronic lymphatic leukemia (229). In animals bearing the Walker 256 carcinosarcoma the isomerase level began to rise on the seventh day after transplantation and reached a level nine times that of normal controls on the twentieth day (47).

C. Lactic Dehydrogenase

Although lactic dehydrogenase is a glycolytic enzyme, the serum level has little selectivity for the neoplastic state, probably because of the widespread distribution of the enzyme in non-neoplastic tissues (311). In 1954 Hill and Levi (212) reported that the serum lactic dehydrogenase was elevated in neoplastic disease and in pregnancy, but not in a group of other diseases. In the following year Hsieh *et al.* (226) observed that in mice with a transplanted tumor the lactic dehydrogenase increased promptly after transplantation and decreased with tumor regression. An early report by Wróblewski and LaDue (526) described elevated serum levels in myocardial infarction, diabetic acidosis, and hepatitis, in addition to acute stem cell leukemia and chronic myelogenous leukemia. In

a later study (525) the lactic dehydrogenase level was found to be elevated in serum, serous effusions, and spinal fluid of patients with neoplastic, inflammatory, and degenerative disease.

In mice with transplantable leukemia, elevation of lactic dehydrogenase occurred within 24 hours of inoculation and progressed until death (173). Another experiment (211) compared the level of lactic dehydrogenase following transplantation of L-4964 transplantable leukemia to two strains of AKR mice of varying resistance to the tumor. Both strains showed a rise of serum lactic dehydrogenase within 48 hours of inoculation, before there was any cytological evidence of leukemia. In the susceptible strain the lactic dehydrogenase continued to rise until death, while in the resistant strain the lactic dehydrogenase returned to normal limits with regression of the tumor. Other studies have indicated that induced and transplanted malignant tumors of mice and rats, other than leukemia, result in a rise in serum lactic dehydrogenase (225, 304). The tumors studied included cigar tar- and methylcholanthrene-induced tumors, spontaneous mammary gland tumors, and transplanted sarcomas, carcinomas, lymphomas, and gliomas. The lactic dehydrogenase was not elevated in benign tumors, pregnancy, or hepatic regeneration in animals.

In recent experiments, Hsieh *et al.* (224) obtained evidence that, in the case of a transplanted mouse tumor, the elevated lactic dehydrogenase was not simply the result of enzyme leakage from the tumor. These workers found that after excision of a transplanted tumor the elevated lactic dehydrogenase declined slowly, the level 6 weeks after excision being four times that of control animals. Even 6 months after the tumor had been removed the enzyme level was elevated. These observations with lactic dehydrogenase should be contrasted with the findings reported above with aldolase, where a prompt fall in the enzyme was observed following removal of the tumor. On the basis of their observations with lactic dehydrogenase (224), the authors conclude that the tumor probably induces formation of the enzyme by the host's normal tissues, so that after removal of the tumor there is not an immediate return to the normal situation.

There have been a number of recent clinical studies on the serum lactic dehydrogenase levels in human neoplastic disease. Thus, Bierman *et al.* (40) have found the level of this enzyme was elevated in only 23 of 128 normals (21 were children, who normally

have a higher level), and in 11 of 190 patients with non-neoplastic disease, while it was elevated in 110 of 156 patients with cancer, in 34 of 50 patients with lymphomas, and in 84 of 91 patients with leukemia. However, most observers feel that the specificity of elevation of the serum lactic dehydrogenase level for neoplastic disease is poor. Thus, J. H. Hill (213) reports that the highest percentage of elevations of the serum lactic dehydrogenase was found in the patients with cardiovascular disease, and that the elevation of this enzyme level in cancer patients was similar to that of a group of individuals with other diseases.

Wróblewski has elucidated several of the factors involved in the serum lactic dehydrogenase elevations observed in man (525). The simplest situation was that observed in experimental animals, in which a rise in lactic dehydrogenase was observed that was proportional either to the amount of cardiac necrosis following coronary ligation or to the amount of cardiac homogenate injected. A mechanism such as this probably accounts for part of the lactic dehydrogenase elevations seen in such clinical situations as myocardial infarction, skeletal muscle and surgical trauma, acute pancreatitis, and fulminant hemolytic states. In purely inflammatory disease without necrosis, Wróblewski has observed minimal lactic dehydrogenase elevations in man. [Other authors have observed elevations of lactic dehydrogenase in purely inflammatory disease (201)]. When necrosis and inflammation exist together in a tissue the lactic dehydrogenase may be elevated, but this elevation is not necessarily proportional to the tissue enzyme concentration. For example, even though the liver cell is richer in lactic dehydrogenase than in glutamic oxalacetic transaminase, it is the transaminase level that rises in infectious hepatitis while the dehydrogenase tends to remain normal. In such a situation a selective enzyme loss by the cell would seem to be involved. The rise of the lactic dehydrogenase in malignant disease is even more complex. Thus, though tumor necrosis may play a role in the enzyme elevation, in experimental malignant disease (induced leukemias and transplantable sarcomas and carcinomas), lactic dehydrogenase elevations may occur without necrosis and indeed may occur within 6 to 48 hours of the onset of the neoplastic state. Simultaneous measurements of the levels of serum, liver, and tumor lactic dehydrogenase levels in the course of sarcoma 180 transplantation led Wróblewski to conclude that the rise in enzyme level was due to

liver and/or tumor release of the enzyme. He feels that in human neoplasia the same mechanisms obtain.

D. Summary

Table 15, from the work of White (505), contains studies of the serum levels of aldolase, phosphohexose isomerase, lactic dehydrogenase, glutamic oxalacetic transaminase, and isocitric dehydrogenase in neoplastic, cardiac, and hepatic disease. (Elevations of the last two enzymes have also been reported in patients with cancer.) It will be noted that elevations of all five enzymes are observed in individuals with cardiovascular and acute hepatic disease as well as in patients with malignant disease. Not included in this table are occasional cases of hemolytic anemias, renal disease, muscular disease, and chronic hepatic disease. In another report White (504) points out that a pattern of serum enzyme abnormalities may be discernible in the various clinical situations. Thus, myocardial infarction tends to display an elevation of lactic dehydrogenase, glutamic oxalacetic transaminase, isomerase, and aldolase, while the level of isocitric dehydrogenase is usually normal. In acute liver disease there is usually marked elevation of isocitric dehydrogenase with minimal elevation of the lactic dehydrogenase. Muscular dystrophy is characterized by an elevation of lactic dehydrogenase, a marked elevation of aldolase and, usually, a normal isocitric dehydrogenase and isomerase. In disseminated cancer either of two different patterns is observed, depending on the presence or absence of liver metastasis. In the absence of liver involvement the pattern is similar to that observed in muscular dystrophy, i.e., elevated lactic dehydrogenase, normal or elevated aldolase, and

TABLE 15
ELEVATED SERUM ENZYME ACTIVITY IN HUMAN DISEASE (505)

	Cancer	Myocardial infarction	Angina pectoris	Infectious hepatitis
Aldolase	36 of 104[a]	36 of 69[a]	23 of 61[a]	5 of 14[a]
Phosphohexose isomerase	42 of 88	28 of 69	27 of 61	
Lactic dehydrogenase	57 of 75	68 of 69	59 of 61	13 of 14
Serum glutamic oxalacetic transaminase	16 of 51	51 of 69	40 of 61	7 of 14
Isocitric dehydrogenase		11 of 69		7 of 14

[a] Expressed as numbers of patients.

normal isocitric dehydrogenase. With malignant disease with hepatic involvement there is usually elevation of lactic dehydrogenase, aldolase, isocitric dehydrogenase, and hexose isomerase. The similarity of the pattern of enzyme elevations in cancer patients without liver involvement to that of patients with muscular dystrophy led White to consider that protein depletion and secondary muscular wasting may be a cause of the enzyme elevations in the former group of patients. Confirmation of such a mechanism was obtained by observing the effects on serum enzyme levels of intravenous administration of protein hydrolyzate. It was possible to bring elevated enzyme levels to normal (lactic dehydrogenase, aldolase, and glutamic oxalacetic transaminase) by intravenous feedings in patients with disseminated cancer without liver metastasis, but not in cancer patients with liver metastasis or in patients with muscular dystrophy. Thus White concludes that in clinical states in man several factors appear to be responsible for the elevated enzyme values. Among these are (1) necrosis of enzyme-rich tissue such as muscle, liver, or tumor, (2) inflammation with or without necrosis and, finally (3) distant effects of tumors in general resulting in the breakdown of host tissues, particularly muscle, to supply the requirements for growth of the neoplasm. The calculation of other investigators, based on the enzyme content of tissues, has confirmed that simple necrosis of tissue cannot alone account for the observed enzyme elevations (201).

It is clear that none of the above-mentioned enzyme elevations can in any sense be considered specific to or diagnostic of the neoplastic process. Perhaps it will be possible to achieve greater specificity with these enzyme measurements by some type of enzyme fractionation procedure. This is being attempted in the case of lactic dehydrogenase (210, 450), which is known to exist in serum in at least four electrophoretically separable components.

A discussion of tissue catalase levels in neoplastic disease has not been included in the present monograph. An excellent recent monograph is available (334).

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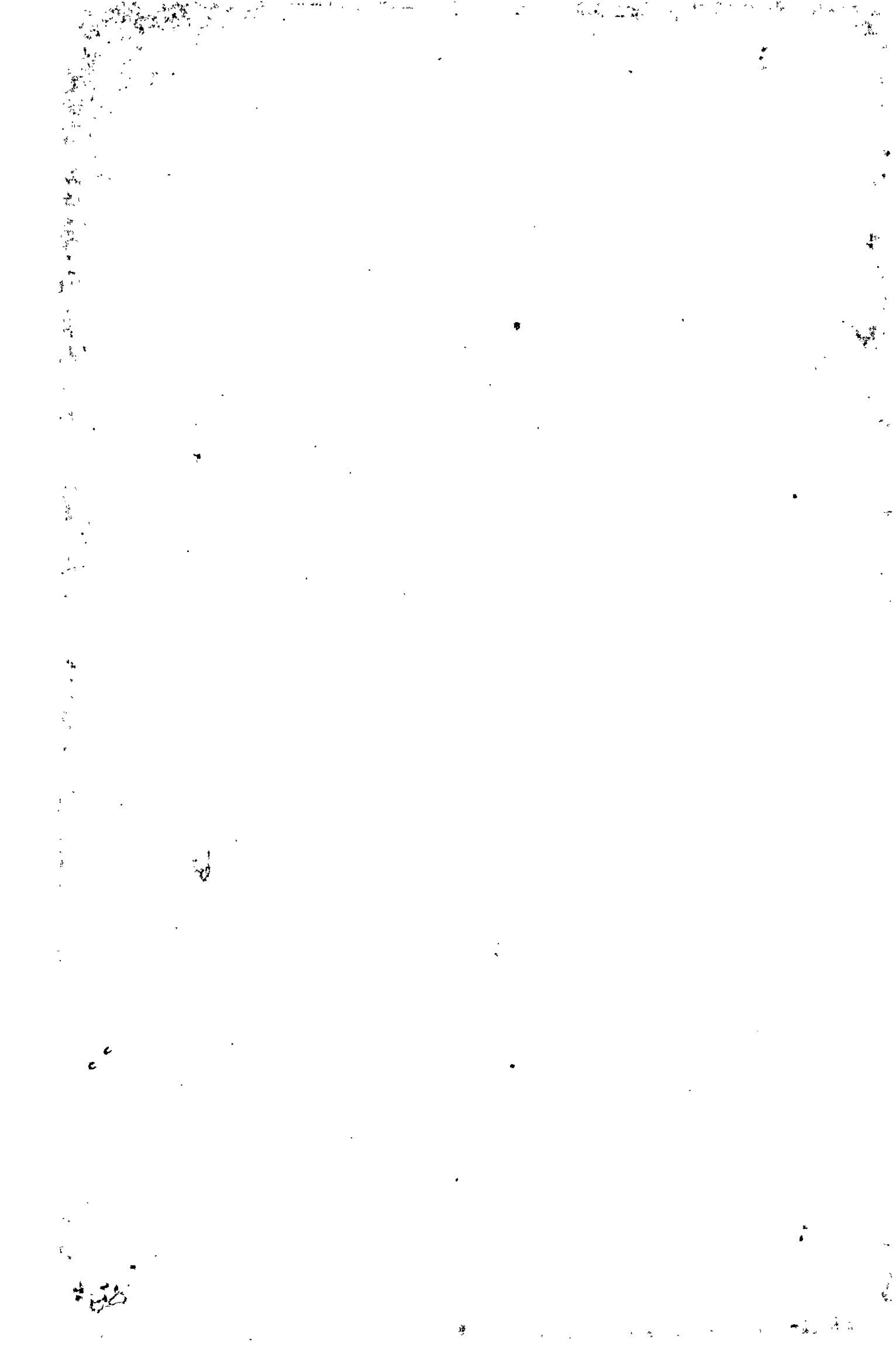

PART II: The Oxidative Metabolism of Tumors

I. The Over-All Respiratory Rate of the Tumor Slice*

It is important to establish at the outset that the over-all oxidative rate of the tumor slice is similar to that of most normal tissues (Tables 1–4 and 6). The impression that tumor respiration is quantitatively defective arose because the first tumors studied by Warburg happened to display a low rate of respiration (144). A study of Tables 1, 2, and 3 indicates that the Q_{O_2} of rat and chicken tumors varies between 5 and 10, while human tumors, perhaps because of the greater proportion of connective tissue and necrotic material, varies from 1 to 10. The Q_{O_2} of mouse tumors is considerably higher, in the range of 10–20, a finding consistent with the known inverse relationship between Q_{O_2} and body size (261). This high oxidative rate of mouse tumors taken together with an equal effectiveness of oxygen uptake in inhibiting glycolysis (M.O.Q. in the same range as other normal and tumor tissues), results in a low or absent aerobic glycolysis despite a very high anaerobic glycolysis. The normal tissues listed in Table 4 show oxidative rates in the same range as tumors, although examples of tissue with an oxidative rate above 10 are more frequent in normal tissues than in tumors. The most convincing evidence of the similar oxidative rates of normal and tumor tissues is seen in a comparison of the oxidative rates of tumors with tissue of origin (Table 6). Carcinoma of the endometrium and of the vulva and hepatoma all have an oxidative rate similar to the homologous normal tissue.

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* The respiratory rate of tissue slices is influenced by the suspending medium. Early investigators (78, 172, 237, 530) found that slices of the majority of tissues displayed a higher and more stable respiration in serum than in Ringer's solution. This effect of serum was later shown to be due to the bicarbonate content of serum (485). Stimulation of respiration in bicarbonate buffer greater than that in phosphate buffer has been demonstrated in the following tissues: rabbit brain (14), 50%; rat retina and Crocker mouse sarcoma (272), 100%; rabbit bone marrow and guinea pig liver (485), 40%; rat kidney and rat liver (485), 20%; cat brain and rabbit renal cortex (485), no stimulation; mouse fibroblasts in tissue culture (114), 32%; and Yoshida rat ascites tumor cells (114), 60%. The endogenous respiration of normal and leukemic human leukocytes was found to be maximally stimulated at a CO_2 concentration of 1–2% (39).

2. Nature of the Substrate of Tumor Respiration

A. Studies on the Respiratory Quotient of Normal and Tumor Tissues

Shortly after Warburg's original work on tumor glycolysis appeared, Dickens undertook an extensive series of investigations on the oxidative metabolism of slices of normal and neoplastic tissues (122, 125, 127–129, 131), as revealed through studies of the respiratory quotients (R.Q. = the CO_2 produced divided by the O_2 consumed). The results of these studies are summarized in Tables 16 and 17. On the basis of the respiratory quotient data and the rates of anaerobic glycolysis, Dickens divided normal tissues into three

TABLE 16
RESPIRATORY QUOTIENTS OF NORMAL AND TUMOR TISSUE[a] (126)

Tissue	R.Q.	$Q_{CO_2}^{N_2}$
Normal rat tissues		
Liver	0.79	3
Kidney	0.85	3
Intestinal mucous membrane	0.85	4
Submaxillary gland	0.87	7
Spleen	0.89	8
Testis	0.94	8
Embryo (rat; 10 mg.)	1.04	8
Embryo (chick; 10 mg.)	1.00	18
Brain cortex	0.99	19
Chorion (embryo weight, 30 mg.)	1.02	32
Retina	1.00	88
Tumors		
Jensen sarcoma (rat)	0.83	34
Slow-growing sarcoma (rat)	0.94	18
Rous sarcoma (chick)	0.93	30
Spindle cell tar tumor 173 (mouse)	0.91	21
Tar carcinoma 2141 (mouse)	0.87	22
Crocker sarcoma (mouse)	0.89	22
Sarcoma 37S (mouse)	0.86	27
Spontaneous tumor I (mouse)	0.91	20
Spontaneous tumor II (mouse)	0.87	16
Papillary carcinoma of bladder (man)	0.86	3.4
Carcinoma of the breast (man)	0.84	7.1

[a] Glucose present.

groups (127). Group I consists of tissues with a low respiratory quotient (approaching the R.Q. of fat, which is 0.71) and a low rate of anaerobic glycolysis. These tissues, which include liver, kidney, and mucous membrane, utilize little carbohydrate either oxidatively or glycolytically and, presumably, they mainly oxidize fat and protein. Group II is comprised of tissues with a respiratory quotient of unity (the R.Q. of carbohydrate is 1.0) and a high anaerobic glycolysis. These are normal tissues that utilize carbohydrate actively, both oxidatively, and glycolytically when oxygen is absent. This second group includes brain, retina, chorion, and embryo. The third group (III) is intermediate between I and II in both the rate of anaerobic glycolysis and the respiratory quotient, and presumably has an intermediate rate of utilization of carbohydrate. Submaxillary gland, spleen, and testis comprise this group. Dickens found that, in contrast to these normal tissues, tumors displayed a

TABLE 17

RESPIRATORY QUOTIENTS OF NORMAL AND TUMOR TISSUE (DICKENS AND POST-DICKENS; SEE ALSO TABLE 6)

	Glucose present		Glucose absent	
	$Q_{CO_2}^{N_2}$	R.Q.	R.Q.	Ref.
Normal tissues				
Synovial membrane	2.3	0.71	—	74
Jejunal mucous membrane (rat)	14	0.87	—	131
Tuberculous lymph node (rat)	14	0.91	—	127
Erythroid bone marrow (rabbit)	7	0.95	—	484
Myeloid bone marrow (rabbit)	22	0.96	—	484
Renal medulla (guinea pig)	22	0.99	—	130
Rat tumors				
Philadelphia 1 sarcoma	15	0.84	0.78	146
Walker 256 carcinosarcoma	—	—	0.80	146
Jensen rat sarcoma	34	0.84	0.80	127
Mouse tumors				
Tar carcinoma 173	—	0.78	—	108
Crocker carcinoma	—	0.75	—	108
Harding-Passey melanoma	11	0.89	—	62
Cloudman S-31 melanoma	12	0.76	—	62
Algire 91-A melanoma	15	0.83	—	62
Earle sarcoma	12	0.83	—	62
Barrett C3HBA adenocarcinoma	18	0.91	—	62

distinct pattern characterized by a high anaerobic glycolysis and a low rate of carbohydrate oxidation (an R.Q. midway between that of fat and that of carbohydrate). He further concluded, recognizing that adequate experimental verification of this point was not available, that it was only the carbohydrate portion of oxidation which was responsible for the inhibition of glycolysis. Dickens developed a scheme of tumor metabolism which may be expressed in modern terms in the following way: tumors possess a very active glycolytic pathway with only a limited ability to oxidize carbohydrate split product (pyruvate); the result is that more sugar is split by tumor than can be oxidized; this leads to an inadequate oxidation of carbohydrate, a decreased respiratory quotient, and a high anaerobic and aerobic glycolysis.

In considering Dickens' work several points should be raised. First, the respiratory quotient data itself has been questioned by Elliott and Baker (145), who found values for brain and retina below unity, while they claimed that occasionally the value for liver and tumor reached unity. There can be no doubt that the respiratory quotient is a rather unsatisfactory experimental quantity (145), showing chance variations of considerable magnitude (± 0.04) when compared to the small range of physiological significance (0.71–1.0). A specific feature which may explain the discrepancy in the R.Q. data from the two laboratories is the fact that Dickens and Simer (127) used a 5-hour incubation period, while Elliott and Baker (145) employed a 90-minute one. Most investigators have inclined to the acceptance of Dickens' data, but the point that it is only the carbohydrate part of the tissue oxidation which inhibits glycolysis has received little confirmation. Experimentally, if this last relation were true, it would be expected that the Meyerhof oxidation quotient (M.O.Q.) would vary from 0 for tissues with an R.Q. of 0.71 to some definite number for tissues with a pure carbohydrate R.Q. of 1.0. Though this data is difficult to obtain, as Dickens himself has pointed out, it *would* nevertheless appear that the M.O.Q. varies from 3 to 6 without regard to the respiratory quotient (R.Q.). Second, it will be noted in the data of Table 17 that jejunal mucous membrane and tuberculous lymph gland show the typical tumor metabolism: the respiratory quotient midway between the fat and carbohydrate values, combined with a high anaerobic (and aerobic) glycolysis.

Despite the uncertainties that surround certain aspects of the

problem, Dickens' R.Q. data would appear to have added to the understanding of tumor metabolism. Of the normal tissues with a high anaerobic glycolysis some (though not all), including brain, retina, embryo, bone marrow, and renal medulla, show an oxidative metabolism that is almost exclusively carbohydrate, as evidenced by an R.Q. of unity. Tumor also displays a high anaerobic glycolysis, *but* it does so in the presence of an R.Q. distinctly less than unity (0.80–0.90), indicating that a significant part of its energy supply comes from the oxidation of fat and protein. Thus tumor differs from this group of glycolyzing normal tissues in that it displays an ability to glycolyze rapidly without a predominantly carbohydrate respiration. This conclusion, that tumors actively oxidize fat and protein, is consistent with isotopic studies, which will be considered in the next section.

In a later paper (122) (Table 18), Dickens compared the effect of endogenous substrate, glucose, and fructose on the R.Q., Q_{O_2}, $Q_{CO_2}^{O_2}$, and $Q_{CO_2}^{N_2}$ of various tissues. The carbohydrate character of the respiration of brain and retina was confirmed by a fall of the oxidative rate and anaerobic glycolysis in the absence of added sugar. Kidney and spleen were less affected, while testis behaved like the nervous tissues in its dependence. Liver, as would be expected from its R.Q., was unaffected by the presence or absence of sugar. The respiratory rate of embryonic tissue showed little dependence on the presence of sugar, presumably because of stored substrate, though the anaerobic glycolysis declined sharply when sugar was omitted. The Jensen sarcoma, the only tumor studied, showed a respiratory rate independent of the presence of sugar, while its anaerobic glycolysis fell off when the sugar was not present. The independence of the respiratory rate of this tumor is consistent with a mixed substrate endogenous respiration, as formulated above.

The respiratory quotient of the tumor slice rises somewhat on the addition of glucose (Table 17), indicating that carbohydrate oxidation has in part replaced fat oxidation. In the ascites cell this shift of respiratory quotient is much more marked, the R.Q. shifting from a level of 0.80–0.90 to a pure carbohydrate quotient of unity or above (263).

TABLE 18

RESPIRATION AND GLYCOLYSIS IN GLUCOSE, FRUCTOSE, AND SUGAR-FREE MEDIA (122)

Tissue	Sugar	R.Q.	$-Q_{O_2}$	$Q_{CO_2}^{O_2}$	$Q_{CO_2}^{N_2}$
Normal adult tissues					
Brain cortex (rat)	Glucose	1.01	12.8	1.5	11.5
	Fructose	0.99	9.7	0.3	1
	None	1.00	3.9	— 0.4	0.5
Brain cortex (rabbit)	Glucose	—	7.5	1.5	12
	Fructose	1.01	8.5	0.5	3
	None	1.02	5	— 1	—
Retina (rat)	Glucose	1.00	22	22.5	64
	Fructose	1.01	16	0.3	3.5
	None	—	4	—	3.5
Testis (rat)	Glucose	0.88	11.2	7.2	12
	Fructose	0.82	9.3	2.7	4
	None	0.6–0.8	5.2	1.9	2
Kidney (rat)	Glucose	0.85	20.6	0	6
	Fructose	0.90	25.5	0.2	2
	None	0.80	17.6	— 1	1
Spleen (rat)	Glucose	0.91	11	5	8
	Fructose	0.83	11	4	3
	None	0.83	9	0.5	3
Liver (rat)	Glucose	0.68	7	3	1.5
	Fructose	0.78	7.5	3	4
	None	0.66	7.8	2	1.5

TABLE 18 (*Continued*)

Tissue	Sugar	R.Q.	$-Q_{O_2}$	$Q_{CO_2}^{O_2}$	$Q_{CO_2}^{N_2}$
Embryonic tissues					
Chick embryo (5 days)	Glucose	1.00	10	2	15
	Fructose	0.92	9.3	0	1.5
	None	0.89	8.3	0	1.5
Rat embryo (13–14 days)	Glucose	1.00	11	—	12
	Fructose	0.98	11.5	0	3
	None	0.7–1	9	0.5	2
Rabbit embryo	Glucose	1.00	8.5	—	13
	Fructose	1.00	7	—	2
	None	1.00	6.5	—	—
Rat yolk sac	Glucose	1.00	11	2	20
	Fructose	0.98	10	1	15
	None	0.93	11.5	0	2
Tumor tissue					
Jensen rat sarcoma	Glucose	0.84	9.6	11	31
	Fructose	0.84	11.1	7	13
	None	0.80	10.0	0	2

B. Isotope Studies

Since the respiratory quotient is a quantity subject to a number of uncertainties, it is fortunate that isotope studies (11, 43, 143, 161, 307, 308, 309) on the nature of the normal oxidative substrate of tumor are available. Bloch-Frankenthal and Weinhouse (43), and Medes *et al.* (309) have used radioactive materials to study the substrate preference of ascites cells and tumor slices.

In an early study (43) with radioactive glucose the conversion of this compound to $C^{14}O_2$ in ascites cells was studied (Table 19).

TABLE 19
EFFECT OF GLUCOSE CONCENTRATION[a] ON THE OXIDATIVE RATE AND RADIOACTIVE GLUCOSE OXIDATION OF EHRLICH ASCITES CELLS (43)

	Glucose concentration						
	0	0.001 *M*		0.0025 *M*		0.01 *M*	
	O_2 (μ*M*)	O_2 (μ*M*)	CO_2 (μatoms)	O_2 (μ*M*)	CO_2 (μatoms)	O_2 (μ*M*)	CO_2 (μatoms)
First hour	12.5	12.3	4.2	10.7	4.4	5.8	3.5
Second hour	10.6	10.8	3.1	10.7	4.0	6.2	3.6
Third hour	8.4	9.3	2.9	10.1	4.3	8.0	4.3
Fourth hour	6.3	6.9	0.7	8.3	3.4	7.8	5.9

[a] Duration of glycolysis: at 0.001 *M* glucose, 5–10 minutes; at 0.0025 *M*, 30 minutes; at 0.01 *M*, 90 minutes.

Over a 4-hour period it was found that oxygen uptake and glucose oxidation remained linear despite a rapid glycolysis resulting in a conversion of the glucose to lactate. Thus in the latter part of the experimental period the glucose had disappeared and the tumor was oxidizing lactate. Kinetic studies (Table 20) indicated that the

TABLE 20
INITIAL RATES OF GLYCOLYSIS, GLUCOSE OXIDATION, AND LACTATE OXIDATION IN EHRLICH ASCITES CELLS STUDIES WITH LABELLED SUBSTRATE (43)

Substrate concentration (molar)	Glucose to CO_2 (μatoms)	Lactate to CO_2 (μatoms)	Glucose to lactate (μatoms)
0.0001	0.25	—	0.39
0.0002	0.37	0.65	0.96
0.0005	0.46	0.89	2.10
0.001	0.42	1.16	3.15
0.0025	0.45	1.36	3.84
0.01	0.45	1.58	4.68
0.02	—	2.10	—

initial rates of glycolysis were not highly concentration-dependent, glycolysis being essentially maximal above a glucose concentration of 10^{-4} *M*. At all concentrations of glucose studied, glycolysis was much more active than glucose oxidation. It was found that the inhibition of respiration (Crabtree effect), which was consistently observed following glucose addition, was exerted on both glucose and endogenous substrate. Medes and Weinhouse (309) studied substrate competition in fatty acid oxidation in ascites cells. Using Ehrlich ascites cells, whose fatty acids had previously been labelled by feeding labelled palmitate, the authors observed that the addition of glucose, fructose, lactate, and acetate all inhibited the endogenous fatty acid oxidation, as determined from the $C^{14}O_2$ formation. In parallel experiments with unlabelled cells (fatty acids) and labelled glucose, and labelled cells and unlabelled glucose, it was possible to show that, at low and moderate concentrations of substrate, glucose oxidation roughly corresponded to the suppression of endogenous fatty acid oxidation, indicating a replacement of the latter by the former (Table 21). At relatively high concentrations of

TABLE 21
INHIBITION OF OXIDATION OF LABELLED ENDOGENOUS FATTY ACID BY GLUCOSE ADDITION, AND OXIDATION OF LABELLED GLUCOSE IN ASCITES CELLS (309)

Glucose concentration (molar)	Oxidation of labelled endogenous fatty acid		Oxygen uptake (μmoles)	Oxidation of labelled glucose $C^{14}O_2$ (μatoms)
	$C^{14}O_2$ (c.p.m.)	Inhibition (%)		
0	92,000		348	0
0.0005	86,000	6.5	348	30
0.002	68,600	25	341	85
0.005	52,000	43	290	124
0.01	28,000	70	158	114
0.025	27,800	70	141	116

glucose and fructose (but not lactate or acetate), the decrease in fatty acid oxidation was accompanied by an inhibition of oxygen uptake (Crabtree effect) but was without concomitant increase in sugar oxidation. Clearly, at low glucose concentrations glucose substitutes for fatty acid as the respiratory substrate, while at high glucose concentrations there is, in addition, an inhibition of total respiration. The authors suggest that fatty acid was the physio-

logical substrate for tumor respiration but that its utilization depends on the availability of glucose, which is a preferred substrate. In liver, kidney, brain, and heart, glucose did not suppress basal fatty acid catabolism, as it did in tumor (143). Emmelot and van Vals (161), studying ascites cells of rhabdomyosarcoma and mammary carcinoma, concluded, on the basis of R.Q. studies and $C^{14}O_2$ formation from acetate, that glucose depresses the endogenous fatty acid oxidation of the tumor cell. In another study, Medes *et al.* (307) found that slices of transplanted mouse tumors (a rhabdomyosarcoma, TA-3 carcinoma, hepatoma 98/15, and sarcoma 37), which had been labelled with C^{14}-palmitic acid *in vivo,* formed, on *in vitro* incubation, $C^{14}O_2$, which had the same specific activity as the fatty acids of the tumor. This indicates that under the conditions of these experiments fatty acids form an important part of the endogenous substrates of these tumors. Using labelled plasma lipids as substrate, Medes *et al.* (308) also studied the oxidation of endogenous fatty acid by Lettré-Ehrlich ascites cells, and confirmed their earlier work on the oxidation of endogenous palmitate. Exogenous fatty acid oxidation was found to account for 5–13% of the total respiration of the tumor cell. Summarizing their results with both exogenous and endogenous fat oxidation (308), these authors concluded that in the presence of adequate glucose about two-thirds of the respiratory CO_2 was derived from glucose and the remainder probably from fatty acids (endogenous and exogenous).

The role of amino acid oxidation in the respiratory metabolism of the tumor cell has received little study. Measurement of NH_3 accumulation, by Dickens and Greville (123), suggested that as much as one-half of the endogenous respiration of the tumor slice may be derived from amino acids. The only available recent report suggests that amino acids are handled by tumor cells in a manner similar to that of the handling of fatty acids. In this study Rapoport *et al.* (392) report that the endogenous respiration of tumor cells and reticulocytes is due party to amino acids. Furthermore, the endogenous respiration of glycine (as measured by $C^{14}O_2$ formation from radioactive glycine) was inhibited by the addition of glucose in the same way as was the endogenous respiration of fatty acid reported above. These authors feel that aerobic glycolysis supplies part of the energy need of the cell so that there is less need for amino acid oxidation.

3. Carbohydrate Oxidation in Tumor Tissue

A. Introduction: The Citric Acid Cycle

It has become clear over the past 20 years that the major oxidative pathway in animal and plant tissue is the pathway presented in Fig. 3, the citric acid or tricarboxylic acid cycle (258, 259). The

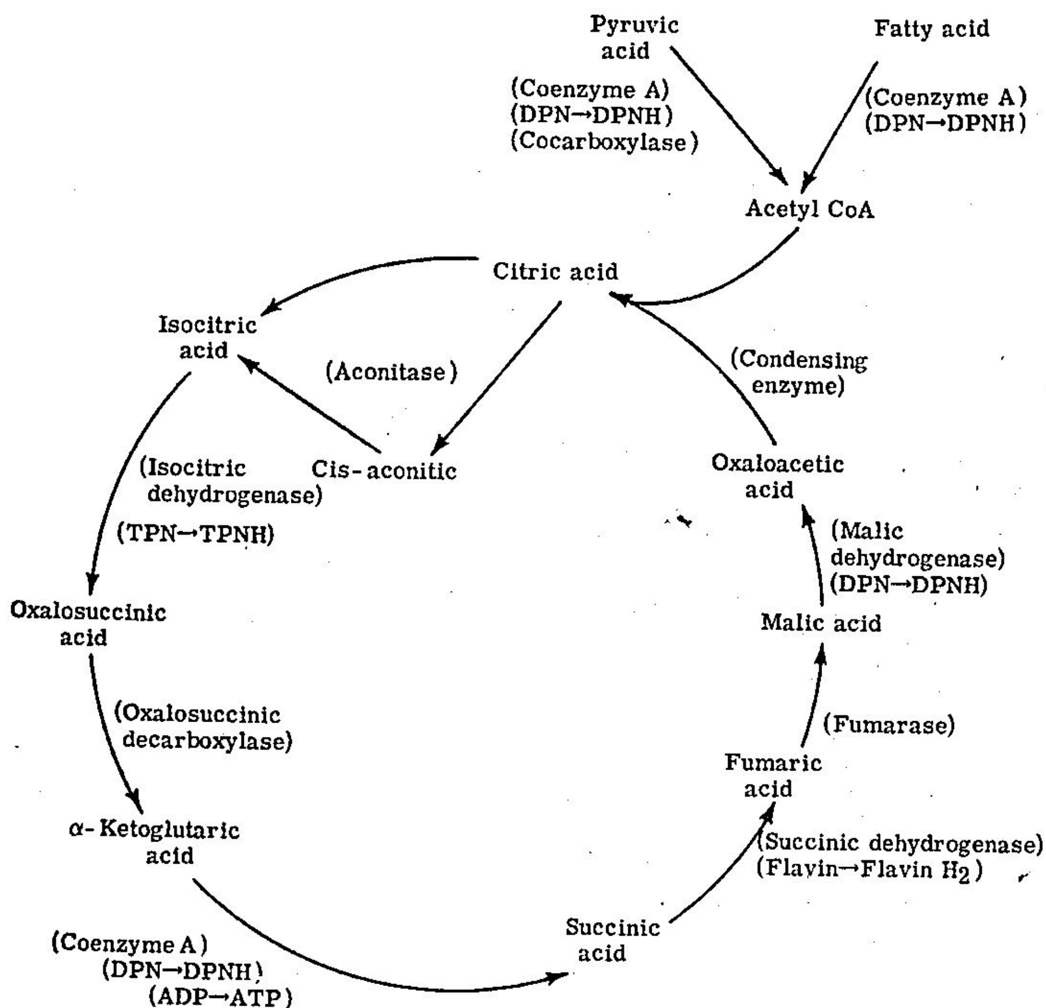

Fig. 3. Citric acid cycle.

decisive experiments of Krebs (261), which led to his formulation of the citric acid cycle in its modern form, are among the fascinating developments of modern biochemistry. A number of important observations had been made before Krebs's experiments. First, it was well known that whereas there was little oxidative response of pigeon muscle to many organic compounds, there was a marked oxidative response to a number of di- and tricarboxylic acids: citric, *cis*-aconitic, isocitric, α-ketoglutaric, succinic, fumaric,

oxaloacetic, pyruvic, glutamic, and aspartic. Furthermore, Szent-Györgyi had shown that the di- and tricarboxylic acids had a catalytic effect on the respiration (of pyruvate, for example) of pigeon breast muscle. Martius and Knoop had demonstrated that the same tissue rapidly oxidized citrate, isocitrate, *cis*-aconitate, and α-ketoglutarate, the first three of these acids giving rise to dicarboxylic acids. It was also known that succinic acid could be oxidized to fumaric acid (a reaction which was blocked by malonate), and that fumaric, malic, and oxaloacetic acids were linked in animal tissues by reversible reactions, as were the tricarboxylic acids citric, isocitric, and *cis*-aconitic. It remained for Krebs to establish the cyclic nature of the relationship of the di- and tricarboxylic acids through several observations. A critical point was clarified when Krebs demonstrated that oxaloacetic acid gave rise to citric acid as well as the other tri- and dicarboxylic acids. He then found that malonate inhibited pyruvate oxidation and that fumarate relieved this inhibition, but only in a stoichiometric manner (i.e., in the presence of malonate 1 mole of fumarate resulted in the oxidation of 1 mole of pyruvate). Finally, Krebs showed that even in the presence of a malonate block there was stoichiometric accumulation of succinate, when oxaloacetate, malate, or fumarate were added. Although the Krebs cycle was established without benefit of isotopic materials, later studies with such substances have offered ample confirmation of the correctness of the formulation.

The over-all reaction for the oxidation of 1 mole of pyruvate is as follows:

$$\text{Pyruvate} + 5O \longrightarrow 3CO_2 + 2H_2O$$

The cycle (Fig. 3) involves the condensation of a molecule of pyruvate (after oxidative decarboxylation to an activated two-carbon fragment) with a molecule of oxaloacetate to yield a molecule of citrate. The citrate then undergoes a series of oxidations and decarboxylations, eventually regenerating the oxaloacetate molecule. The energy of each of the oxidative steps is conserved in the form of high energy phosphate by the phosphorylation of ADP to ATP. The citric acid cycle is, according to present views, the important oxidative pathway of fat, protein, and carbohydrate. Fatty acid enters the citric acid as two-carbon acetyl coenzyme A (CoA) fragments, while the amino acids enter as various Krebs cycle intermediates after transamination and deamination.

The unifying concept of high energy phosphate, particularly

ATP, as the major energy intermediate in mammalian systems was developed by Lippmann and Kalckar. The central role of ATP in muscle contraction, nerve transmission, intestinal absorption, and numerous other chemical and physiological phenomena is at present unquestioned. In the oxidation of 1 molecule of glucose, 38 molecules of ADP are phosphorylated to ATP. In the complete passage of a pair of electrons up the electron transport chain (pyridine nucleotide, flavin, cytochrome *b, c, a,* and a_3), 3 moles of ATP are formed. This phosphorus:oxygen (P:O) ratio of 3 applies to each of the reactions generating DPNH, reduced diphosphopyridine nucleotide (pyruvate oxidative decarboxylation, malate oxidation, etc.). The oxidation of succinate bypasses the pyridine nucleotides, the electron pair going directly to flavin, and the P:O ratio for this reaction is only 2. This is balanced by the oxidative decarboxylation of α-ketoglutaric acid which, in addition to yielding three high energy phosphates through the DPNH formed, also generates an additional molecule of ATP at the substrate level, giving a P:O ratio of 4 for this reaction and a total yield of 15 for the complete oxidation of 1 mole of pyruvate (average P:O ratio, 3.0). In addition to the 15 moles of ATP realized for the oxidation of each pyruvate molecule, under aerobic conditions the DPNH formed in the glyceraldehyde dehydrogenase step of glycolysis is oxidized with a gain of 3 more ATP molecules. Thus, aerobically 1 mole of glucose gives a theoretical yield of 38 moles of ATP ($15 \times 2 = 30 + 3 \times 2 = 36 + 2$ moles of ATP from glycolysis = 38 moles). For further details on these aspects of oxidative metabolism the reader is referred to several excellent recent general references (20, 136, 174).

B. The Response of the Oxidative Rate of Normal and Tumor Tissue to Added Carbohydrate Substrate

The results of the early studies of Kisch on the effect of added lactate and pyruvate on the oxidative rate of tumor slices were equivocal (253). Later systematic investigations into the response of the oxidative rate of tumor to added substrate were performed by Elliott (146, 147, 149, 150). These experiments with slices of kidney cortex, liver, brain, testis, and two tumors (the Philadelphia 1 sarcoma and the Walker 256 carcinosarcoma) involved the measurement of respiratory quotient, oxidative rate, total acid, keto acid, and lactate production in the presence and absence of added sub-

TABLE 22
AEROBIC METABOLISM OF LACTIC, PYRUVIC, AND SUCCINIC ACIDS IN SLICES OF NORMAL AND NEOPLASTIC TISSUES (146, 149)

Tissue	Substrate	$-Q_{O_2}$	R.Q.	Q_{acid}	Q_{lactic}	Q_{keto}
Tumor tissues:						
Philadelphia 1 rat sarcoma	No addition	13.6	0.74	− 0.3	0.0	− 0.3
	Lactate	13.2	0.80	− 2.6	− 2.3	+ 0.7
	Pyruvate	12.8	1.08	− 1.3	+ 3.5	− 9.0
	Succinate	13.8	0.74	+ 0.4	+ 0.2	0.0
Walker 256 carcinosarcoma	No addition	11.5	0.82	− 0.7	0	− 0.2
	Lactate	11.5	0.82	− 1.5	− 0.9	+ 1.0
	Pyruvate	13.1	1.04	− 1.2	+ 3.6	− 7.5
	Succinate	11.0	0.80	− 0.9	+ 0.1	0
Normal rat tissues						
Kidney	No addition	21.5	0.82	+ 0.5	0	0
	Lactate	32.1	0.89	+ 9.0	− 9.3	+ 0.6
	Pyruvate	33.6	1.28	−20.3	+ 4.2	−26.0
	Succinate	32.8	0.68	−14.0	+ 3.7	+ 3.5
Liver (fed)	No addition	12.2	0.93	+ 2.8	+ 1.5	+ 0.5
	Lactate	14.7	0.94	− 3.2	− 6.1	+ 1.0
	Pyruvate	13.0	1.76	− 2.1	+ 7.5	−11.2
	Succinate	14.6	0.72	+ 2.2	+ 2.8	+ 0.7
Liver (fasted)	No addition	12.5	0.34	− 3.9	+ 2.3	+ 2.7
	Lactate	13.6	0.66	− 6.1	−10.7	+ 1.3
	Pyruvate	16.0	1.19	− 9.1	+ 7.2	−17.1
	Succinate	13.0	0.31	+ 3.1	+ 2.9	+ 1.6

[a] Glucose absent.

hepatomas, Jensen sarcoma, Flexner-Jobling carcinoma, Ehrlich ascites tumor, mouse amelanotic melanoma, sarcoma 37, a rhabdomyosarcoma, and a mouse mammary carcinoma. The success of these later workers was due in part to the addition of higher levels of DPN (0.002 *M*, as opposed to 0.0003 *M* used by Potter) and in part to the use of sucrose as the homogenizing medium and a more fortunate choice of substrate. The ability to separate the actively oxidizing particle, the mitochondrium, from the other dephosphorylating fractions of the cell was also an important factor in obtaining the most active preparations. Table 23 contained some typical studies on the oxidation of Krebs cycle intermediates by Wenner *et al.* (501). McKee *et al.* (301) have reported that addition of carbohydrate substrates (glutamate, lactate, pyruvate, oxaloacetate, aspartate, and alanine) stimulated the endogenous respiration of the Ehrlich ascites cell from 50 to 111%.

The important role played by malonate (261) and fluoroacetate (359) in elucidating the citric acid cycle led to the study of the effects of these metabolic inhibitors on the oxidative metabolism of tumors. Elliott (147) measured succinate formation in slices of normal and tumor tissue when succinoxidase was blocked by malonate. In a group of normal tissues and tumors (Philadelphia 1 sarcoma and Walker 256 carcinosarcoma), only kidney accumulated much succinate from pyruvate. These studies suffered from the fact that the Krebs cycle was incompletely understood at the time and, since Elliott failed to add oxaloacetate, succinate formation was limited by the carboxylation of pyruvate. Later studies by Busch and Potter (72) showed that malonate injection *in vivo* resulted in comparable levels of succinate accumulation (3–5 μM per gram of wet tissue per 2 hours) in liver, kidney, and Flexner-Jobling carcinoma. Potter also studied the accumulation of citrate (Table 24) following the injection of fluoroacetate (370). After the *in vivo* injection of a lethal dose of fluoroacetate, there was citrate accumulation in all normal tissues but liver, while there was no accumulation observed in the tumors studied (Walker 256 carcinosarcoma, Jensen rat sarcoma, and a primary rat hepatoma). This failure of the tumor to accumulate citrate was always accepted with some difficulty, in view of the high concentrations of citrate (118) that have been found in tumor tissue (Table 25). Later experiments by Busch *et al.* (70) indicated that in the slice, fluoroacetate led to the accumulation of citrate in rat tumors in quantities comparable to those for

TABLE 23

OXIDATION[a] OF KREBS CYCLE INTERMEDIATES BY MITOCHONDRIA OF NORMAL AND NEOPLASTIC TISSUE IN THE PRESENCE AND ABSENCE OF ADDED DPN (501)

Tissue	DPN	Substrate						
		None	Pyruvate	Citrate	α-Keto glutarate	Succinate	Fumarate	Malate
Mouse liver	−DPN	37	150	176	142	145	130	—
	+DPN	49	145	215	180	165	156	—
Rat liver	−DPN	43	139	146	118	147	106	160
	+DPN	45	154	153	154	128	154	164
Mouse kidney	−DPN	53	279	299	212	230	95	—
	+DPN	91	249	296	335	284	220	—
Rat brain	−DPN	40	38	22	43	114	35	69
	+DPN	50	146	116	116	104	101	127
Hepatoma 7A77	−DPN	15	30	14	100	125	15	33
	+DPN	44	250	153	214	116	143	176
Hepatoma 98/15	−DPN	19	17	—	—	82	48	—
	+DPN	36	196	153	119	79	98	82
Sarcoma 37	−DPN	28	51	—	—	170	—	—
	+DPN	45	150	68	99	204	—	136
Rhabdomyosarcoma	−DPN	16	21	4	85	170	—	56
	+DPN	36	105	137	172	118	—	99

[a] Values are expressed in microliters oxygen consumed per milligram mitochondrial nitrogen per 30 minutes.

TABLE 24

CITRIC ACID CONTENT[a] OF NORMAL AND NEOPLASTIC TISSUE BEFORE AND AFTER *in Vivo* FLUOROACETATE INJECTION (370)

Tissue	Before fluoroacetate	1 Hour after fluoroacetate
Normal		
Brain	57	160
Heart	49	448
Lung	75	206
Thymus	55	275
Liver	47	39
Kidney	56	714
Spleen	59	514
Testis	73	79
Blood	54	79
Muscle	31	42
Pancreas	53	
Neoplastic		
Walker 256 carcinosarcoma	49	42
Flexner-Jobling carcinoma	121	90
Jensen rat sarcoma	85	66
Primary hepatoma	95	60

[a] Micrograms per gram net weight.

TABLE 25

CITRIC ACID CONTENT OF NORMAL AND NEOPLASTIC TISSUES (118)

	Citric acid (mg./100 gm. tissue)	Tissue	Citric acid (mg./100 gm. tissue)
Normal		Neoplastic (animal)	
Liver (rabbit)	2.8	Brown-Pierce carcinoma (rabbit)	13.6
Skeletal muscle (rabbit)	2.5	Walker 256 carcinosarcoma (rat)	17.0
Kidney (rabbit)	6.0	Guerin tumor (rat)	16.8
Brain (rabbit)	4.6	Crocker sarcoma (mouse)	14.3
Testis (guinea pig)	11.5	Crocker sarcoma (non-necrotic)	12.3
Skeleton (mouse)	46.0	Crocker sarcoma (necrotic)	7.7
Skin (mouse)	12.2	Neoplastic (human)	
Fur (mouse)	13.3	Seminoma of testis	9.9
Seminal vesicles (mouse)	128	Carcinoma of stomach	2.7
Gastric mucosa (man)	2.0	Carcinoma of vulva	7.0
Skin (human)	5.3	Fibroadenoma of uterus	5.0
Fresh bone (ox)	272		

TABLE 26

ENZYMES OF THE CITRIC ACID CYCLE AND DEHYDROGENASES OF NORMAL AND TUMOR TISSUES (499)

Tissue	Condensing enzyme (μM citrate/ 10 min./100 mg. acetone powder)	Aconitase (units/mg. dry weight)	Fumarase (units/mg. dry weight)	Dehydrogenase (acetone powder)			Oxalo-acetic carboxy-lase (Q_{CO_2})	α-Keto glutaric oxidase (Q_{O_2})
				Lactic (units/ mg.)	Malic (units/ mg.)	Isocitric (units/ mg.)		
Liver (mouse)	1.53	33	132	—	—	—	3.21	—
Heart (rat)	—	72	96	320	383	56.0	—	—
Liver (rat)	—	25	66	200	256	10.8	2.40	7.7
Kidney (rat)	—	50	62	104	173	66.0	—	11.3
Muscle (rat)	—	12	—	520	330	15.6	—	—
Hepatoma (rat)	—	—	133	238	540	17.6	2.45	2.4
Hepatoma (mouse)	2.90	8.3	62	168	380	16.0	0.78	2.1
Rhabdomyosarcoma (mouse)	1.45	2.2–5.0	50	108	288	14.8	2.17	3.2
Mammary adeno-carcinoma (mouse)	3.3	3.1–5.9	50	190	178	6.7	1.24	3.2
Ascites (mouse)	—	—	—	165	200	4.8	3.48	—

most rat tissues. (Rat tumor accumulates 4.5–6.1 µmoles citrate per gram of wet tissue per 2 hours; liver, brain, diaphragm, and spleen, 6.3–7.7; and kidney, 26–28). Busch suggested that the failure to accumulate citrate after fluoroacetate injection *in vivo* may be due to a low tissue pH *in vivo,* to a lack of substrate, or to low tissue oxygen tension in the living animal.

C. The Levels of Krebs Cycle Enzymes

The best studies available on the levels of the enzymes of the citric acid cycle, those of Wenner *et al.* (499), are summarized in Table 26. Enzymes of the citric acid cycle, particularly condensing enzyme, malic, lactic, and isocitric dehydrogenase, fumarase, and oxaloacetic decarboxylase were present in tumors in amounts comparable to the levels in normal tissues. Aconitase and α-ketoglutarate oxidase were present, but the levels in tumors were lower than in the normal tissues studied.

D. Studies with Radioactive Carbohydrate Substrates

Isotope studies have played an important role in revealing the similarity in carbohydrate substrates and in metabolic pathways between normal and neoplastic tissues. Work with radioactive substrates has established beyond question that tumors oxidize glucose, lactate, pyruvate, and succinate. Olson (350) reported an extensive series of studies involving the measurement of $C^{14}O_2$ production from isotopic precursors in slices of hepatoma induced with butter yellow (aminoazobenzene) and in normal rat liver. Table 27 and Fig. 4 indicate that slices of hepatoma oxidize carboxyl- and carbonyl-labelled pyruvate to $C^{14}O_2$ at a rate comparable to that of

Table 27

Metabolism of Labelled Pyruvate in Slices and Homogenates of Hepatoma and Normal Liver (350)

		Values in terms of $Q_{pyruvate}$					
Tissue	Preparation	Q_{O_2} pyruvate	Total Δ	Lactate formed	Net Δ	$C^{14}O_2$ (from 1-C^{14})	$C^{14}O_2$ (from 2-C^{14})
Hepatoma	Slice	7.0	−4.1	+1.8	−2.3	+1.8	+1.2
	Homogenate	1.5	−2.5	+2.3	−0.2	+0.1	+2.0
Liver	Slice	7.4	−5.0	+1.0	−4.0	+2.1	+0.9
	Homogenate	6.8	−4.0	+0.7	−3.3	+3.0	+0.4

normal liver. The lack of appreciable oxidation of pyruvate by hepatoma homogenates in this experiment may be due to the failure to add external DPN, a requirement that was not recognized at the time this work was done. Labelled lactate was actively oxidized by both hepatoma and liver slices, accounting for about 30% of the oxygen consumption at all substrate levels. Hepatoma utilized labelled succinate (Fig. 5) at about 30% of the rate of normal liver but, in contrast to earlier studies (147), the uptake of oxygen was increased from 6.5 to 10.1 (compared with an increase from 8 to 30 in liver).

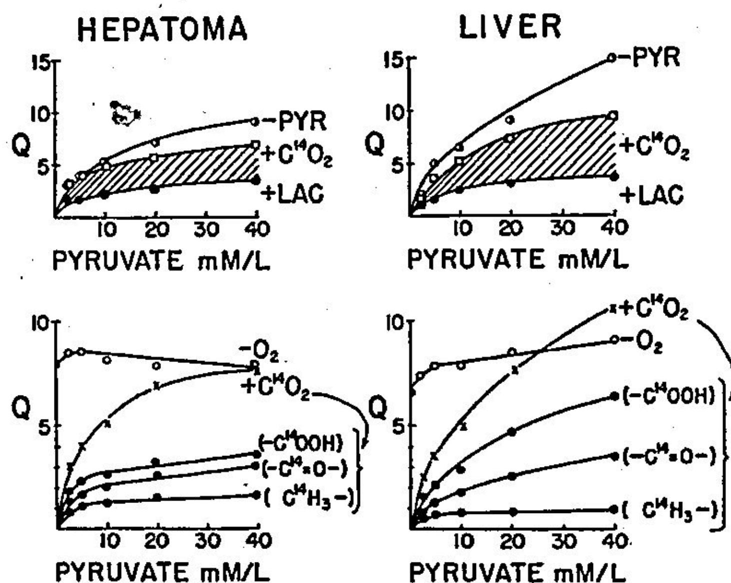

Fig. 4. The metabolism of pyruvate-1-C^{14}, -2-C^{14}, and -3-C^{14} in slices of hepatoma and rat liver. Metabolic quotient is plotted against substrate concentration. Disposition of substrate is presented in the top graphs and gas exchange in the bottom. The curve marked (x) is a summation of the $C^{14}O_2$ produced from all three carbons of labelled pyruvate. From Olson (350).

Studies by Olson (350) on the oxidation of uniformly labelled glucose by liver, hepatoma, heart, and brain are included in Table 28 and Fig. 6. It is well known that the liver slice displays a very small aerobic and anaerobic glycolysis unaffected by the presence or absence of carbohydrate, a respiratory rate which is not altered by the presence or absence of glucose, and a respiratory quotient which approaches that of fat oxidation. It is therefore necessary to compare the glucose oxidation of hepatoma with that of tissues that display a greater metabolic interest in the presence of this compound than does normal liver. Compared to the very low oxidation by liver (accounting for only 2% of oxygen uptake), hepatoma

displayed an active oxidation of labelled glucose (accounting for about 30% of its total oxygen consumption). The rate of oxidation in hepatoma is comparable to that in heart but less than that in

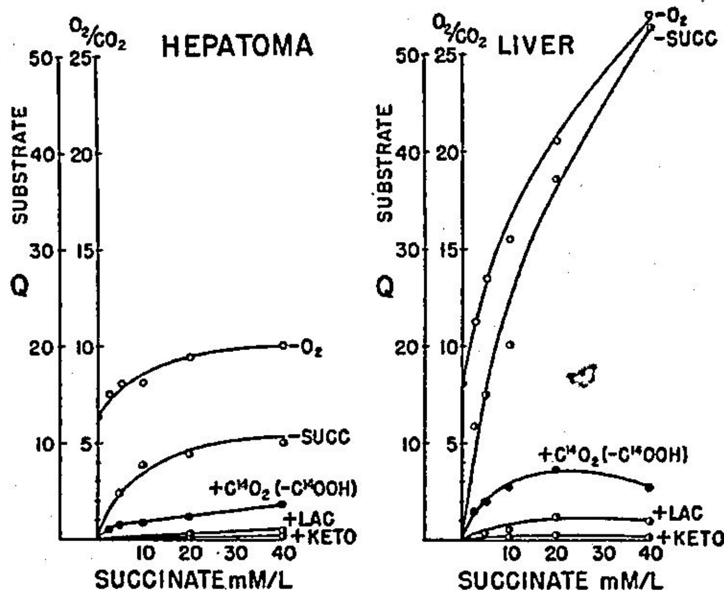

FIG. 5. The metabolism of carboxyl-labelled succinate-C^{14} in slices of hepatoma and rat liver. Substrate disposition and gas exchange are plotted on different scales on the ordinate so arranged that 1 µl. change in $O_2/C^{14}O_2$ exchange is equivalent to 2 µl. change in substrate utilization. From Olson (350).

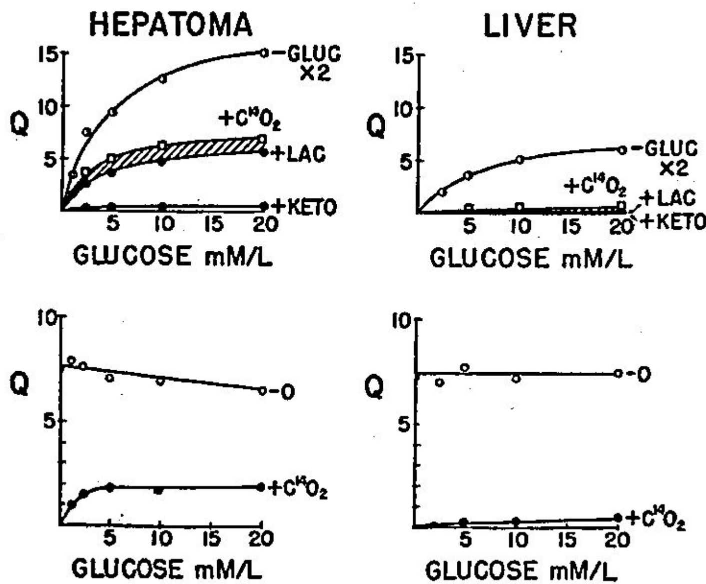

FIG. 6. The metabolism of uniformly labelled glucose-C^{14} in slices of hepatoma and liver. Metabolic quotient is plotted against substrate concentration. Disposition of substrate is presented in the top graphs and gas exchange in the bottom ones. From Olson (350).

brain, a tissue which utilizes glucose almost exclusively. At comparable substrate concentrations, glucose oxidation by slices of hepatoma was 25% that of pyruvate, and 75% that of lactate. Other studies in the same report indicated that liver showed a more active rate of synthesis of glycogen from pyruvate and lactate than did hepatoma. Olson concludes that liver shows a rapid rate of glycogen formation from glucose with little conversion of glucose to lactate or CO_2, a metabolic pattern which, he suggests, results from a lack of phosphohexokinase activity. Hepatoma, on the other hand, displays little glycogen synthesis, but active conversion of glucose to lactate and CO_2. He attributes this to active hexokinase and phosphohexokinase with a rate of glycolysis in the tumor that exceeds its ability to oxidize small carbohydrate fragments.

TABLE 28

AEROBIC GLYCOLYSIS AND OXIDATION OF GLUCOSE-C^{14} IN TISSUE SLICES[a] (350)

Tissue	$+Q_{lactate}$	$+Q_{C^{14}O_2}$
Rat brain	4.5	10.5
Rat heart	1.1	2.8
Rat hepatoma	5.1	2.1
Rat liver	0.0	0.2
Duck heart	1.2	2.5
Duck liver	0.5	1.2

[a] Glucose concentration = 10 μM per milliliter.

Early studies on glycogen synthesis in the surviving slice (33) indicated that liver and kidney displayed net synthesis of carbohydrate from lactate and pyruvate, but brain, testis, Philadelphia 1 sarcoma, and Walker 256 carcinosarcoma did not. Consistent with this observation are the findings of several groups of investigators that the glycogen content of tumors is low (23, 184). This absence of tumor glycogen has been related by Nirenberg (338, 339) to a lack of glycogen phosphorylase activity. The glycogen phosphorylase activities of seven types of ascites tumors, a tumor grown in tissue culture, and a solid virus-induced neoplasm (as determined by chemical and histochemical techniques) was found to be at negligible levels when compared to normal liver or kidney. In three tumors in which it was studied the ATP-dependent phosphorylase activating enzymes were found to be present, but there were negligible amounts of the dephosphorylase.

Returning to a consideration of oxidation of radioactive carbo-

TABLE 29

$C^{14}O_2$ FORMATION FROM LABELLED LACTATE AND $C^{14}O_2$ FORMATION AND CITRATE FORMATION FROM LABELLED GLUCOSE IN SLICES OF NORMAL AND NEOPLASTIC TISSUE (494)

Tissue	Radioactive lactate	Radioactive glucose		
			Radioactive citrate formation	
	$C^{14}O_2$ formation (μatoms carbon, O.C.)[a]	$C^{14}O_2$ formation (μatoms carbon, O.C.)[a]	Activity (c.p.m.)	Quinidine citrate activity (c.p.m.)
Normal				
Rat liver	44	23	—	—
Rat kidney	152	111	—	—
Rat heart	148	87	—	—
Rat muscle	3	7.0[b]	—	—
Rat brain	112	93[b]	—	—
Rat spleen	130	—	—	—
Mouse liver	32	—	5.5×10^5	126
Mouse kidney	440	—	5.5×10^5	2.460
Mouse heart	108	—	5.5×10^5	150
Mouse muscle	5	—	—	—
Neoplastic				
Rat hepatoma	61	38.3	—	—
Mouse hepatoma	87	43.8	1.37×10^6	1.225
Mouse Andervont hepatoma	48	—	—	—
Mouse sarcoma 37	104	—	—	—
Mouse rhabdomyosarcoma	72	35	5.5×10^5	1.000
Mouse mammary adenocarcinoma	65	25.5	1.37×10^6	1.750
Mouse Ehrlich ascites	116	—	5.5×10^5	910

[a] O.C. refers to the microatoms of substrate carbon oxidized to CO_2 per gram dry weight of tissue per hour at 38° C. at a substrate concentration of 0.005 *M*.

[b] Homogenates.

hydrate substrates, the experiments of Weinhouse (494; Wenner, Dunn, and Weinhouse, 497) on a larger group of tumors are included in the data of Table 29. They are in agreement with Olson's results that tumor slices display an active oxidation of glucose and lactate. Further evidence that glucose was oxidized in tumors via the citric acid cycle was obtained when it was found that an appreciable part of the radioactivity of labelled glucose was transferred to the citric acid of the tumor (Table 29).

Busch and Nyhan have studied the operation of the citric acid cycle *in vivo* in tumor-bearing rats through analysis of the radioactivity of alanine, glutamic, aspartic, lactic, pyruvic, and succinic acids, and other Krebs cycle intermediates following the injection of labelled pyruvate (68), succinate (346), and glutamate (344, 345). Studies with pyruvate indicated that, whereas normal tissues (liver, brain, testis, muscle, intestine, heart, and kidney) converted the major part of the isotope present into the amino acids which are in equilibrium with the Krebs cycle compounds (alanine and aspartic and glutamic acids), tumor (Flexner-Jobling carcinoma, Walker 256 carcinosarcoma, and Jensen sarcoma) converted a very small amount into amino acid, converting the major part (over 50%) to lactate. This was interpreted by the authors as indicating a minor *in vivo* role for the Krebs cycle in tumors. However, other studies with labelled succinate and glutamate, in the presence and absence of malonate block, indicated that the citric acid cycle was active in tumors *in vivo* and that transfer of radioactivity from amino acids to lactate was via the cycle (345, 346). Freedman and Graff have found (171) that, in contrast to normal liver, the Murphy-Sturm lymphosarcoma converts a significant amount of pyruvate to acetate, and decarboxylates a considerable amount of oxaloacetate to a two-carbon fragment.

4. The Metabolism of Fatty Acids by Tumor Tissue

A. The Response of the Respiratory Rate to Added Fatty Acid

The understanding of the role of the oxidation of fatty acids by tumors has followed a pattern similar to the one outlined in the previous section for carbohydrate oxidation. Initial studies with unlabelled fatty acids suggested a deficiency in oxidation of these compounds by tumor, a deficiency that was later shown not to exist when the pathways were studied with isotopic materials.

Normal tissues such as liver, kidney, spleen, or testis show a marked increase in oxidative rate upon the addition of fatty acid (277, 386). In contrast to this finding, early studies by Ciaranfi with slices of Ehrlich adenocarcinoma, chicken sarcoma, and a variety of human sarcomas and carcinomas failed to show any increase in oxidative rate upon the addition of propionic, butyric, valeric, caproic, heptylic, caprylic, crotonic, and oleic acids (85, 87). Later investigations (88) by the same author showed that, in contrast to studies with free fatty acids, methyl esters of the fatty acids C_1 to C_8 increased the respiratory rate of tumor slices up to 240%, an effect which he attributed to omega oxidation of these latter compounds. (For comparison, the methyl esters increased the oxygen uptake of brain cortex up to 11 times, liver to 8 times, and spleen to 17 times.) More recently, Baker and Meister have shown (17) that the rate of oxygen uptake (per milligram of nitrogen) of particles derived from rat and mouse hepatoma and from rat fetal liver, in the presence of either octanoate or hexanoate, is less than 10% of the rate of oxidation of normal adult liver particles. (No attempt was made to increase the feeble fatty acid oxidation of the tumor or fetal mitochondria by addition of DPN or coenzyme A.) The influence of leukemic infiltration on the octanoate oxidation by homogenates of C-58 liver was studied by Vestling *et al.* (451). They found that 19 of 32 leukemia-infiltrated livers oxidized octanoate at a rate less than 50% of that of noninfiltrated control liver, with 13 of the 19 displaying a rate less than 20% of the control.

B. Studies with Radioactive Fatty Acid Substrates

Extensive studies on the oxidation of C^{14}-labelled fatty acids have been performed by Weinhouse and his group (496); their results are summarized in Tables 30 and 31. It was found that slices of transplanted mouse and rat tumors did oxidize C^{14}-labelled fatty acids, although at a lower rate than did liver slices. Differences between liver and tumor were greater for the short chain than for the long chain fatty acids. In part, this difference between long and short chain fatty acids could be attributed to the inhibitory effect of the shorter chain acids on fatty acid respiration (demonstrated in the case of octanoate). The lower rate of $C^{14}O_2$ production from labelled fatty acids in tumor tissues was due to both a lower overall oxidative rate and a lower percentage of $C^{14}O_2$ in the CO_2. This

last finding indicated a greater dependence of the tumor tissue on endogenous substrate. Hepatoma was found to resemble peripheral tissues rather than liver: careful trapping experiments failed to reveal significant ketogenesis in hepatoma, while there was active oxidation of ketone bodies by the tumor.

TABLE 30

OXIDATION[a] OF ACETATE-1-C^{14} BY SLICES OF LIVER NEOPLASTIC TISSUES (496)

Tissue	O_2 uptake (μmoles)	Respiratory CO_2: CO_2 (μ*M*)	Respiratory CO_2: R.S.A.[b] (%)	Respiratory CO_2: C.C.[c] (atoms carbon)
Normal liver	520	337	17.1	57.8
Host liver	486	350	14.0	49.0
Hepatoma 98/15	341	325	5.8	18.9
Host liver	617	463	17.7	82.0
Mammary adenocarcinoma	151	180	3.3	5.9
Host liver	473	317	12.5	39.7
Sarcoma 37	266	229	2.5	5.6
Host liver	615	448	15.0	67.4
Rhabdomyosarcoma	253	254	2.8	7.2

[a] Substrate concentration = 0.01 *M*.

[b] R.S.A. (relative specific activity) $= \frac{\text{Specific activity of product} \times 100}{\text{Specific activity of acid}}$.

[c] C.C. (conversion capacity) $= \frac{\text{R.S.A.}}{100} \cdot \frac{\mu\text{atoms carbon in product}}{\text{hr.} \times \text{gm. dry wt. tissue}}$.

A detailed study on incorporation of acetate-1-C^{14}, acetate-2-C^{14}, proprionate-1-C^{14}, octanoate-2-C^{14}, and pyruvate-2-C^{14} into the respiratory CO_2 and the oxidative intermediates of mouse liver and C-954 hepatoma slices has been done by Brown *et al.* (58). Table 32 contains their experiments with acetate and octanoate, the compounds which were examined most carefully. Both liver and hepatoma utilized octanoate in preference to acetate, as judged by conversion to $C^{14}O_2$, conversion to total or specific non-volatile radioactive compounds, and incorporation into acetoacetate. After incubation with labelled octanoate the major C^{14}-containing intermediates were the same in liver and hepatoma, although the quantitative partition differed to some extent. Thus, normal liver converted more radioactivity to β-hydroxybutyrate, glucose, and glutamine, while hepatoma converted more to glutamate, di- and tricarboxylic acids, aspartate, and alanine. There was no conversion

TABLE 31

OXIDATION[a] OF CARBOXYL-LABELLED FATTY ACID BY SLICES OF LIVER AND NEOPLASTIC TISSUES (496)

Tissue	Butyrate-1-C^{14}		Octanoate-1-C^{14}		Palmitate-1-C^{14}	
	R.S.A.[a] (%)	C.C.[a] (μatoms carbon)	R.S.A. (%)	C.C. (μatoms carbon)	R.S.A. (%)	C.C. (μatoms carbon)
Normal liver	33.8	59.5	31.6	43.3	4.9	11.1
Host liver	41.9	82.5	51.4	104.0	6.6	17.2
Hepatoma 98/15	33.3	51.3	24.7	45.0	5.5	11.4
Host liver	36.0	53.5	49.3	89.8	5.5	15.9
Mammary adenocarcinoma	9.4	12.0	1.0	0.6	3.1	5.4
Host liver	36.8	53.8	40.2	62.7	4.5	13.1
Sarcoma 37	7.4	9.8	0.2	0.2	5.0	8.7
Host liver	21.5	30.6	32.7	72.4	5.2	15.0
Rhabdomyosarcoma	13.0	15.8	0.5	0.6	4.4	10.7

[a] See Table 30.

of C-2 fragments to glucose in tumor. The pattern obtained with labelled acetate resembled that with octanoate. Studies by the same authors (57, 99) on the tumor distribution of radioactivity in acetoacetate and CO_2 after incubation of hepatoma slices with butyrate-1-C^{14}, octanoate-1-C^{14}, and octanoate-7-C^{14} were consistent with beta oxidation of the fatty acid to C-2 fragments and recombination of the C-2 units to acetoacetate or oxidation to CO_2 in the same manner as in normal liver and kidney.

TABLE 32
INCORPORATION[a] OF ISOTOPE FROM ACETATE AND OCTANOATE INTO MOUSE LIVER AND HEPATOMA (58)

	Normal liver		Hepatoma	
Intermediate	Acetate-1-C^{14}	Octanoate-1-C^{14}	Acetate-1-C^{14}	Octanoate-1-C^{14}
Glutamine	23.4	12.0	10.2	5.0
β-Hydroxybutyrate	22.2	7.9	9.1	12.4
Glucose	18.7	7.7	1.2	—
Glutamate	10.2	7.0	40.0	33.7
Pyrrolidone-COOH acid	8.6	1.7	4.7	4.2
Lactate	4.7	2.3	12.2	11.9
Di- and Tri-COOH acids	3.2	5.7	12.5	19.0
Alanine	2.7	1.4	3.0	4.9
Urea	1.4	1.4	—	—
Aspartate	0.7	Trace	6.5	6.5
Glutathione	0.9	0.4	2.9	3.2
Origin	0.3	0.3	1.3	3.4

[a] Expressed as percentage of total nonvolatile radioactivity.

Experiments by Pardee *et al.* (355) have stressed the relatively low level of $C^{14}O_2$ formation by tumor homogenates from acetate-1-C^{14} as contrasted with homogenates of normal tissue. However, it has since been found (6) that the simultaneous addition of fluoride and CoA to tumor homogenates results in a 40-fold increase in $C^{14}O_2$ formation from labelled acetate by Walker 256 carcinosarcoma, and a 20-fold increase by Flexner-Jobling carcinoma. The resulting activation of acetate by the Walker tumor (in the presence of CoA and fluoride) is 23% that of normal liver and 7% that of normal kidney, while for the Flexner-Jobling the figures are 15% and 4% respectively, which are results consistent with the slice studies.

Busch and co-workers (67, 69, 73) have studied the metabolism of acetate-1-C^{14} in malonate- and nonmalonate-treated tumor-bear-

ing rats. In the rat tumors (Jensen sarcoma, Walker 256 carcinosarcoma, Flexner-Jobling carcinoma, and a transplantable lymphphoma) both in the presence and absence of malonate there was a very slow rate of conversion of acetate-1-C^{14} to $C^{14}O_2$ and non-volatile radioactive compounds, suggesting a slow rate of acetate utilization *in vivo* (the half time for acetate-1-C^{14} disappearance was 6–15 seconds for normal tissue, 48 seconds for liver, and 4½ minutes for tumor). In malonate-treated animals the specific activity of the succinate of the tumor was only 1/20 that of normal tissues. Glutamate, aspartate, and succinate accounted for 33–75% of the radioactivity of non-tumor tissues, but only 4–6% of the radioactivity of the tumor in the absence of malonate.

C. Lipid Synthesis

With regard to lipid content, neoplasms contain in general a greater concentration of phospholipids and cholesterol and a lower concentration of neutral fat than do the tissues of origin (203). In 1931 a study by Yasuda demonstrated (531) that the iodine number of the tumor phospholipid fatty acid was dependent on the food fat. These experiments involved feeding rats and mice bearing transplanted tumors diets of fats of varying degrees of unsaturation (cod liver oil, linseed oil, and coconut oil). Haven *et al.* found (202, 204) that after feeding elaidin to rats with the Walker 256 carcinosarcoma, elaidic acid could be demonstrated in the tumor, again suggesting the dietary origin of the tumor lipid.

A number of studies of tumor lipogenesis have been done with radioactive precursors. Medes *et al.* (307) have studied the lipid synthesis of slices of neoplastic tissue (rat and mouse hepatoma, mouse mammary tumor, and sarcoma 37) by measuring the incorporation of radioactive acetate and glucose into fatty acids. A slow rate of fatty acid synthesis was found, which the authors felt was too slow to supply the lipid needs of the tumor, and led them to conclude that the tumor must obtain preformed lipid from the host. (Rough calculation suggested that only 5% of the fat content of the tumor was derived from endogenous synthesis.) To study further the source of tumor lipids the same authors in a later investigation (310) fed palmitic acid-1-C^{14} to mice bearing transplanted tumors (Lettré-Ehrlich ascites tumor, a rhabdomyosarcoma, TA-3 carcinoma in solid and ascites form, hepatoma 98/18, and sarcoma 37). Normal liver was most active in taking up the labelled fatty

acid, with a peak at 6 hours, the relative specific activity of which was 5–20 times that of the tumors (Table 33). The uptake of the neoplastic tissues resembles that of the internal organs and muscle in both the time of the peaks (24–48 hours) and their lower specific activity. Slices of tumor, which had been labeled with C^{14}-palmitic

TABLE 33

In Vivo INCORPORATION[a] OF RADIOACTIVE PALMITATE INTO THE LIPIDS OF NORMAL AND NEOPLASTIC TISSUES (310)

	Hours after administration						
	6	12	24	36	48	72	96
Liver	6.49	2.95	3.12	0.37	—	0.40	0.06
Internal organs	3.40	6.83	3.86	4.05	—	2.33	2.04
Muscle	2.84	3.44	4.18	3.46	—	2.19	1.81
Hepatoma 98/15	1.45	—	5.14	1.52	0.37	0.91	—
Mammary adenocarcinoma TA3	0.02	0.55	0.24	0.09	0.10	0.56	0.04
Rhabdomyosarcoma	0.01	1.27	0.82	1.78	0.00	0.00	0.01
Sarcoma 37	0.05	—	0.34	—	0.95	0.12	0.53
Ehrlich-Lettré ascites cells	0.00	0.00	0.53	—	—	0.11	—

[a] As per cent of administered radioactivity.

acid *in vivo*, were incubated *in vitro*, with the finding that the $C^{14}O_2$ produced had the same specific activity as that of the fatty acids of the tumor. These authors thought the studies indicated that the host's fatty acids formed important respiratory substrate for the tumor (310). Several other laboratories have obtained evidence of more active lipogenesis in tumors than is indicated by the results of Medes *et al.* Thus, the radioactivity of the lipids of the Novikoff hepatoma following radioactive glucose administration exceeded that of the liver or carcass (230), and the capacity of four transplanted tumors to incorporate radioactive acetate into lipid was equal to that of the most active normal tissues (32).

Medes *et al.* (306) have also studied the over-all hepatic metabolism of fat during carcinogenesis with butter yellow (*p*-dimethylaminoazobenzene) employing acetate-2-C^{14}. They found (Table 34) that conversion of C^{14}-acetate to acetoacetate and fatty acid was much decreased in the liver tumor from the levels of the normal and preneoplastic liver. Glycogen content was also decreased, but conversion of acetate to $C^{14}O_2$ and cholesterol was unchanged. In regard to fatty acid metabolism, the authors found there was no

TABLE 34

ACETATE METABOLISM *in Vitro* DURING HEPATOCARCINOGENESIS BY *p*-DIMETHYLAMINOAZOBENZENE (AAB) (306)

Tissue	Analytical			Acetate-2-C^{14} C.C.[a] (μatoms carbon)			
	Glycogen (mg./5 gm. tissue)	Fatty acid (mg./5 gm. tissue)	Cholesterol (mg./5 gm. tissue)	CO_2	Aceto-acetate	Fatty acid	Cholesterol
Normal liver (normal diet)	405	102	7.4	70.4	130	19.8	1.40
Normal liver (AAB-fed)	390	99	7.3	55.2	90	21.0	1.84
Pathological, no tumor (AAB-fed)	260	96	10.4	57.2	63.2	20.4	3.04
Nontumor lobe of tumor liver (AAB-fed)	216	90	10.4	96.8	87.0	24.0	2.05
Hepatoma (AAB-fed)	11	127	15.0	54.8	13.7	2.32	1.99

[a] See Table 30.

evidence of there being a preneoplastic state, but felt there was an abrupt change in the pattern of fat metabolism associated with frank tumor formation. An abrupt fall in acetoacetate formation associated with the neoplastic state was also reported by Dickens (132). Another group of investigators has reported that the lipogenesis of the azo dye hepatoma (studied in the slice with radioactive glucose) is equal to that of normal liver (230).

A number of workers have reported that tumors are able to synthesize cholesterol from radioactive acetate or glucose (16, 158, 307, 351). In the Yoshida ascites tumor, after incubation with radioactive acetate, there was no radioactivity in the purified dibromocholesterol, although there was radioactivity in the less pure digitonin precipitate (415).

With regard to lipid synthesis, investigators disagree on whether the activity of tumor is equal to or less than the more active lipid synthesizing normal tissues. This difference may reflect differences in the particular tumors studied or in the experimental conditions employed. It appears well established, however, that the tumor utilizes to a considerable extent the fatty acids of the host. This nutritional parasitism of the tumor, involving proteins (167), fatty acids, purines, and glucose, is discussed in greater detail in a recent review by Henderson and LePage (206).

5. The Hexose Monophosphate Shunt in Tumors

A. Introduction

There can be little doubt that the principal pathway of carbohydrate oxidation in mammalian systems is via the Embden-Meyerhof scheme and the citric acid cycle. However, the discovery of a portion of the total respiratory activity that persisted despite blocking this major pathway with iodoacetate, fluoride, or arsenite suggested the existence of an alternate route of carbohydrate oxidation. This other pathway, known as the hexose monophosphate shunt or pentose phosphate pathway, was worked out through the studies of Warburg, Dickens, Lipmann, Horecker, Racker, and others. The initial steps involve triphosphopyridine nucleotide (TPN)–coenzyme oxidations of glucose-6-phosphate and of the resulting 6-phosphogluconic acid. The oxidation of the latter compound involves (probably via an intermediate) a decarboxylation at C-1, resulting in the formation of ribulose-5-phosphate, which in

turn is isomerized to ribose-5-phosphate. A series of complex transfer reactions (transaldolase and transketolase) follow, with the result that for every six molecules of hexose that enter the cycle one is completely oxidized to CO_2 and water. The evidence available at the present time suggests that the oxidation of the TPNH generated in the hexose monophosphate shunt is not associated with the production of high energy phosphate (236). There can be little doubt that a major function of the shunt in many tissues is the synthesis of pentose (222). One group of investigators (498) has obtained evidence that in ascites tumor cell the main role of the TPNH generated in the monophosphate shunt is in reductive syntheses. The quantitative importance of the alternate pathway is most readily studied by comparing the rate of $C^{14}O_2$ formation from glucose labelled in the 1 position with glucose labelled in the 6 position. Excess $C^{14}O_2$ from C-1 over C-6-labelled glucose indicates operation of the monophosphate shunt, since this pathway involves a C-1 decarboxylation.

B. Levels of Hexose Monophosphate Shunt Enzymes

The alternate pathway of glucose oxidation, the hexose monophosphate shunt, has been studied with several methods in a number of tissues, and its existence in neoplastic tissue has been clearly established. In an early study, Dickens and Glock (121) demonstrated that extracts of rat liver carcinoma actively oxidized glucose-6-phosphate, 6-phosphogluconate and ribose-5-phosphate, resembling liver, kidney, and brain in this respect. A more detailed study was later done by Glock and McLean (179) on the activity of the hexose monophosphate shunt enzymes (glucose-6-phosphate dehydrogenase, 6-phosphogluconic dehydrogenase, and the enzymes that catalyze ribose-5-phosphate breakdown) in extracts of a large group of normal and tumor tissues (Table 35). These enzymes, like the glycolytic enzymes, are localized in the soluble cytoplasmic fraction of the cell. The shunt dehydrogenase activities were found to be the highest in adrenal cortex, in lactating mammary gland, in lymphatic tissues (spleen, thymus, and lymph nodes), and in whole rat embryos in early stages of development. The enzyme levels in tumors were within the range of those of normal tissues, being relatively high in tumors of lymphatic origin. Studies from another laboratory have confirmed the presence of the enzymes for ribose-5-phosphate metabolism in extracts of the Gardner mouse lympho-

TABLE 35

LEVELS OF ENZYMES OF THE DIRECT PATHWAY (HEXOSEMONOPHOSPHATE SHUNT) OF CARBOHYDRATE METABOLISM IN NORMAL MAMMALIAN TISSUES AND TUMORS (179)

Tissue	Enzyme activity (units/gm. tissue at 20°)			
	G-6-P dehydrogenase	6-Phosphogluconic dehydrogenase		Ribose-5-phosphate breakdown
		pH 9.0	pH 7.6	
Adrenal cortex (ox)	730	315	232	286
Adrenal gland (rat)	163	350	185	425
Spleen (rat)	305	96	64	287
Thymus (rat)	98	87	49	359
Lymph node (rat)	76	76	44	227
Liver (male rat)	46	147	59	345
Liver (female rat)	104	290	130	371
Lung (rat)	88	106	59	282
Kidney (rat)	69	62	56	259
Ovary (cow)	30	52	35	108
Testis (rat)	30	44	22	224
Placenta (rat)	37	54	32	222
Brain (rat)	32	22	11	75
Skeletal muscle (rat)	8	15	8	91
10-Day embryo (rat)	186	140	71	430
20-Day embryo (rat)	50	45	30	198
Mammary gland (21 days' lactation, rat)	5452	1734	883	1300
Mammary gland (2 days' involution, rat)	50	93	45	147
Mouse sarcoma 37	58	60	54	—
Benzpyrene mouse sarcoma	63	98	60	128

TABLE 35 (*Continued*)

Tissue	Enzyme activity (units/gm. tissue at 20°)			
	G-6-P dehydrogenase	6-Phosphogluconic dehydrogenase pH 9.0	6-Phosphogluconic dehydrogenase pH 7.6	Ribose-5-phosphate breakdown
Benzpyrene mouse epithelioma	126	137	84	289
Mouse squamous cell carcinoma	72	71	37	121
Spontaneous mouse adenocarcinoma	103	109	55	480
Mouse-lung carcinoma	19	69	42	202
Spontaneous lymphoma (C-57 mice)				
Lymph nodes	290	140	97	378
Liver	222	153	129	344
Spleen	266	172	110	378
Walker rat carcinoma	65	140	87	254
Spontaneous rat carcinoma	127	193	148	306
Fowl tumor	33	63	50	281

sarcoma (459), while glucose-6-phosphate and 6-phosphogluconate dehydrogenase activity have been shown to be present in acetone powder preparations of the Ehrlich ascites tumor (508).

In using cell-free extracts, homogenates, and slices of transplanted mouse tumors (mammary carcinoma, a hepatoma, ovarian tumors, and a sarcoma), enzymes, intermediates, and reactions of the hexose monophosphate shunt were demonstrated to be present by Bosch *et al.* (49). Oxidation of glucose-6-phosphate and 6-phosphogluconate, ribolysis and formation of sedoheptulose, hexose, and triose were all studied and found to occur in these tumors.

C. Isotope Studies with Specifically Labelled Glucose

Although the above-described enzymatic studies indicate the presence of shunt enzymes in tumor extracts, a better quantitative estimate of the importance of the shunt pathway is through isotopic studies with specifically labelled glucose. Studies of $C^{14}O_2$ formation from slices of mammalian liver incubated with C-1 and C-6 glucose are presented in Table 36 (4). The $C^{14}O_2$ ratio of C-6:C-1

TABLE 36

RATIO OF $C^{14}O_2$ FORMATION FROM GLUCOSE-6-C^{14} TO $C^{14}O_2$ FORMATION FROM GLUCOSE-1-C^{14} IN SLICES OF NORMAL AND NEOPLASTIC LIVER (4)

Tissue	R_6/R_1
Normal rat liver	
Chow diet	0.33
High glucose	0.29
Fasted 48 hr.	0.36
Fasted 72 hr.	0.70
Maternal rat liver	0.20
Fetal rat liver	0.42
Regenerating rat liver	
Third day	0.48
Fourth day	0.74
Butter yellow hepatoma (rat)	0.60
Normal mouse liver	0.65
Transplanted hepatoma (mouse)	0.33

for normal rat liver was about 0.3 and for normal mouse liver 0.65, indicating a selective removal of C-1 in liver via the monophosphate shunt. The ratio was 1.0 for kidney, evidence that all the CO_2 was being formed via the Embden-Meyerhof scheme in this tissue. The normal liver ratio was elevated (suggesting less par-

ticipation of the shunt) in regenerating rat liver, fetal liver, fasting and induced rat liver tumor, and was decreased (suggesting greater participation of the shunt) during pregnancy and in transplanted mouse hepatoma. In similar experiments, Abraham *et al.* (1) found a ratio of 1.0 for mouse liver, while with mouse hepatoma the ratio was 0.3, indicating that the alternate pathway for glucose was operating. Studying a group of mouse tumors including induced and spontaneous mammary tumors, hepatomas, ovarian tumors, and a sarcoma, Emmelot *et al.* (157, 159, 449) found ratios of C-6:C-1 of the $C^{14}O_2$ varied from 0.40 to 0.70. These authors found that in a number of tissues the shunt was only revealed in the presence of malonate.

A study of the hexose monophosphate shunt in normal lymphatic tissues and lymphatic tumors, with specifically labelled glucose, was made by Kit (255). He found that the following percentages of CO_2 were not derived from the Embden-Meyerhof pathway (presumably this CO_2 was formed via the shunt): appendix, 11%; thymus, 13%; spleen, 37%; Gardner lymphosarcoma, 32%; and Ehrlich ascites tumor, 23%. This author also found that intact tumor cells formed from 2 to 5 times as much pentose from glucose as did normal lymphatic cells.

Wenner *et al.* (503) studied the fate of labelled glucose in a number of normal and tumor tissues, in terms of conversion to lactate and to $C^{14}O_2$ via the Embden-Meyerhof pathway and the alternate pathway (hexose monophosphate shunt). From 2 to 16% of the total glucose disappearing in normal and tumor tissue was oxidized via non-Embden-Meyerhof pathways. In tumor, 77–94% of the total glucose undergoing catabolism was first converted to lactate, but the lactate was a transient intermediate rapidly being converted to CO_2 and other substances.

Thus studies of enzyme and coenzyme levels, as well as studies with specifically labelled glucose, indicate the presence of the hexose monophosphate shunt in tumors. Quantitatively, the pathway in tumors appears to be at least as important as in the homologous normal tissue.

D. The Metabolism of Glucose-6-Phosphate

Weber and Cantero have studied the metabolism of tumors from the particular point of view of the fate of glucose-6-phosphate (15, 487, 490, 491). This compound has four metabolic pathways open

to it: isomerization to fructose-6-phosphate followed by glycolysis via the Embden-Meyerhof scheme; oxidation to 6-phosphogluconate with subsequent oxidation via the monophosphate shunt; isomerization to glucose-1-phosphate prior to conversion to glycogen; and, finally, cleavage by glucose-6-phosphatase to glucose. It is therefore a critical intermediate in carbohydrate metabolism. Whereas there was considerable glucose-6-phosphatase activity (487) in many normal tissues (liver, 213 units; kidney, 203 units; spleen, 16 units; brain, 11 units), there was no demonstrable enzyme in a group of rat and mouse tumors (sarcoma 37, adenocarcinoma EO771, Gardner lymphosarcoma, Novikoff hepatoma, and spontaneous and aminoazo dye hepatomas of the rat). It should be noted that some normal tissues including lung and muscle also showed no activity. During the feeding of butter yellow there was a decrease in the glucose-6-phosphatase of the liver (436), while the enzyme was absent from the hepatoma (488). The enzyme was also absent from fetal liver, but was present in regenerating and newborn liver.

Ashmore *et al.* (15) have found that phosphohexoisomerase, the enzyme which directs glucose-6-phosphate into the Embden-Meyerhof pathway, is markedly increased in the Novikoff hepatoma, while it is relatively unchanged in embryonic and regenerating liver. Phosphoglucomutase, the enzyme which shunts glucose-6-phosphate toward glycogen synthesis, is markedly decreased in the Novikoff hepatoma but not in any of the non-malignant hepatic states studied. These results are summarized in Table 37. Glucose-6-phosphate dehydrogenase was found to be elevated in the Novikoff hepatoma, as other authors have also reported. The result of the enzyme pattern of the tumor is to favor the Embden-Meyerhof pathway and the monophosphate shunt in tumor at the expense of glycogen synthesis.

Glucose-6-phosphate metabolism was also studied with labelled glucose (15) in work from the same laboratory. On a per cent basis, it was found that the Novikoff hepatoma took up 58% as much glucose as did the normal liver, incorporated 6% as much into glycogen and 11% as much into fatty acid, oxidized 58% as much, and converted 400% as much to lactate. Fructose-1,6-diphosphatase, the enzyme that removes the phosphate in the 6 position from hexose diphosphate, was found to be absent in Novikoff hepatoma (491). In embryonic liver the enzyme was 30–40% of the normal

TABLE 37

METABOLISM OF GLUCOSE-6-PHOSPHATE IN SLICES OF NORMAL AND NEOPLASTIC RAT LIVER (488)

Substance	Novikoff hepatoma	Embryonic liver	Newborn liver	Normal liver	Regenerating liver
Glucose-6-phosphatase	Absent	Absent or minimal	Increased	Normal	Normal
Phosphohexose isomerase	Increased on N basis but normal on a cell basis	Increased	Increased	Normal	Normal
Phosphoglucomutase	10% of normal	Normal	Normal	Normal	Normal
Glucose-6-phosphate dehydrogenase	Increased 500%	Decreased	Normal	Normal	Normal