This sequence is similar to that proposed for the metabolism of itaconate by liver mitochondria[3] but differs from that suggested for itaconate utilization by another pseudomonad[4].

Investigations into the nature of enzyme *D*, which is believed to catalyse the direct formation of itaconyl-CoA from itaconate, ATP and CoA, and into the properties of the enzymes here reported, are continuing.

We thank Mr. R. ILEY for technical assistance. This work was supported in part by the Air Force Office of Scientific Research, OAR, through the European Office, Aerospace Research, U.S. Air Force, under Grant AF EOAR 61-12.

Department of Biochemistry,
University of Leicester, Leicester
(Great Britain)

R. A. COOPER
H. L. KORNBERG

[1] R. A. COOPER AND H. L. KORNBERG, *Biochim. Biophys. Acta*, 59 (1962) 480.
[2] A. M. GOTTO AND H. L. KORNBERG, *Biochem. J.*, 81 (1961) 273.
[3] S. F. WANG, J. ADLER AND H. A. LARDY, *J. Biol. Chem.*, 236 (1961) 26.
[4] V. BRIGHTMAN AND W. R. MARTIN, *J. Bacteriol.*, 82 (1961) 376.

Received June 22nd, 1962

Biochim. Biophys. Acta, 62 (1962) 438–440

PN 10003

Some observations on the lactate dehydrogenase of human neoplasic tissue

The electrophoretic heterogeniety of lactate dehydrogenase (L-lactate: NAD oxidoreductase, EC 1.1.1.27) has been well documented[1–5]. Although various workers use different methods for isolating the active components, it is generally agreed that five isoenzymes of lactate dehydrogenase can be demonstrated in mammalian tissues. The enzyme electrophoretic pattern obtained from a given tissue appears to be specific for that tissue with respect to the lactate dehydrogenase isoenzymes. The data of PFLEIDERER, AND WACHSMUTH[6] indicate that human fetal tissues demonstrate considerable similarity in their isoenzymic components. Tissue lactate dehydrogenase undergoes a differentiation process prenatally and during the early post-partum period, finally emerging as the adult-type pattern with multible fractions. This communication compares normal adult tissue lactate dehydrogenase with that obtained from neoplastic tissue and describes an apparent de-differentiation of theenzyme in malignant disease.

Specimens were obtained at autopsy no later than 4 h post-mortem and rapidly frozen. The tissue was washed once in ice water and then repeatedly in cold saline to remove the majority of contaminating erythrocytes, and subsequently homogenized in ice cold 0.02 *M* veronal buffer (pH 8.6). Supernatant fractions for analysis were obtained after centrifuging at 2000 × *g* for 0.5 h.

The isoenzymes of lactate dehydrogenase were obtained using a Beckman-Spinco continuous--flow electrophoresis cell, assembled in a cold room, thus maintaining temperatures of less than 10° across the paper curtain. 32 fractions were obtained for analysis. The reaction mixtures contained 2.5 ml of a fraction, 60 m*M* sodium L-lactate and 0.94 m*M* NAD in a total volume of 3 ml. The reaction was

followed at 340 mμ in a Beckman DU spectrophotometer at 1-min intervals for 5 min. Percent enzyme activity in the fractions were determined by planimetric analysis. The results obtained with normal and neoplastic tissue are described in Table I.

In contrast to the diverse isoenzymic pattern of lactate dehydrogenase observed in normal tissue, neoplastic tissue appears to have lost this differentiation to a high

TABLE I

RACTATE DEHYDROGENASE PATTERNS OBSERVED IN NORMAL AND NEOPLASTIC TISSUE

Specimen	*Number of samples*	*Percent activity in fractions*				
		V	*IV*	*III*	*II*	*I*
Cardiac muscle	3	0.2	3.0	11.9	27.8	56.8
Skeletal muscle	2	33.2	30.0	34.1	2.0	1.0
Liver	4	54.0	14.8	17.2	8.0	5.7
Lung	4	0.3	22.2	46.1	22.9	8.6
Kidney	3	0.7	12.4	30.1	27.9	28.6
Spleen	5	0.5	18.4	50.5	20.7	9.9
Testes	2	0.2	31.2	43.7	20.2	4.5
Erythrocytes	8	—	1.9	30.9	40.4	26.6
Leucocytes	8	—	11.8	76.1	6.1	—
Melanoblastoma (lung)	1	—	—	100	—	—
Adenocarcinoma (lung)	2	18.7	30.9	42.7	—	—
Squamous cell carcinoma (lung)	2	—	16.2	75.4	8.4	—
Seminoma	2	—	1.3	72.9	19.2	6.7
Plasmacytoma	4	—	—	100	—	—
Liver infiltrate (acute granulocytic leukemia)	1	1.6	21.7	44.3	18.6	14.0
Liver (metastases from lung, primary)	2	1.0	4.5	86.2	8.1	—
Erythrocytes (erythroleukemia)	3	—	12.6	82.8	4.6	—
Spleen (acute granulocytic leukemia)	2	—	10.3	85.2	4.5	—
Leucocytes (acute granulocytic leukemia)	4	—	—	100	—	—
Leucocytes (chronic lymphocytic leukemia)	4	—	—	100	—	—

degree, the majority of activity now appearing in Fraction III. Those tissue homogenates containing all of the lactate dehydrogenase in Fraction III were concentrated in an effort to detect other areas of activity. These experiments were uniformly negative indicating that the other 4 fractions were probably absent.

TABLE II

ACTIVITY PERCENTAGE PATTERN AND MICHAELIS CONSTANTS FOR LACTATE ACID OBSERVED IN A MALIGNANT NEOPLASM METASTATIC TO LIVER, ADJACENT AND NON-NEOPLASTIC TISSUE, AND DISTANT NORMAL LIVER

Specimen	*Percent activity*				
	V	*IV*	*III*	*II*	*I*
Malignant tissue	1.0	4.5	86.2	8.1	—
Adjacent non-neoplastic tissue	14.3	20.2	48.1	14.1	3.3
Distant normal liver	56.0	16.1	19.2	7.1	1.6
	K_m for lactate acid acid (mM)				
Malignant tissue	—	6.6	10	3.2	—
Adjacent non-neoplastic tissue	7.2	7.0	8.1	3.0	1.4
Distant normal liver	7.0	6.1	3.9	2.6	1.6

Histological sections of the malignancies that displayed multiple lactate dehydrogenase fractions demonstrated surrounding non-neoplastic tissue. The activity patterns in these instances could in no way be correlated with the patterns of normal tissue indicating a loss of specificity possibly due to the adjacent tumor. The data in Table II summarizes results obtained when a nodule, metastatic to liver, an area adjacent to this neoplastic tissue and a section of liver far removed from the tumor were analyzed for lactate dehydrogenase isoenzymes. These results indicate that non-neoplastic tissue immediately surrounding the tumor has lost the pattern specificity of the parent tissue while an area not involved has retained the normal pattern.

The Michaelis constants for lactate acid indicate subtle differences between each of the five isoenzymes in normal tissue (Table III). The tumor enzyme appears

TABLE III

MICHAELIS CONSTANTS FOR LACTATE ACID OBSERVED IN NORMAL AND NEOPLADTIC TISSUE

Specimen	K_m *(mM)*				
	V	*IV*	*III*	*II*	*I*
Skeletal muscle	7.3	5.6	3.0	1.9	—
Liver	8.4	6.0	3.8	2.3	
Heart	—	—	3.4	2.0	1.1
Spleen	—	5.7	3.3	2.2	1.3
Seminoma	—	7.1	11.2	4.1	2.0
Melanoblastoma (lung)	—	—	12.2	—	—
Leucocytes-(granulocytic leukemia)	—	—	10.5	—	—

similar to Fraction V in this respect but has the electrophoretic mobility of Fraction III.

In summary, tumor lactate dehydrogenase has lost the high degree of heterogeneity observed in normal human tissue, and the data suggests that Fraction III associated with neoplastic tissue may represent a structurally different protein characteristic of neoplastic tissue. Further investigations are currently being undertaken to elucidate more precisely the nature of tumor lactate dehydrogenase.

This work was aided by grants from the National Institutes of Health (Grant No. C-4843), the Horace H. Rackham School of Graduate Studies of the University of Michigan, and by an Institutional Research Grant to the University of Michigan from the American Cancer Society.

General Medical Research Laboratories,
U.S. Veterans Administration Hospital,
and the Department of Internal Medicine,
University of Michigan, Ann Arbor, Mich. (U.S.A.)

WILLIAM H. STARKWEATHER
HENRY K. SCHOCH

[1] E. S. VESELL AND A. G. BEARN, *J. Clin. Invest.*, 37 (1958) 672.
[2] T. H. WEILAND AND G. PFLEIDERER, *Biochem. Z.*, 329 (1957) 112.
[3] B. R. HILL, *Cancer Research.*, 21 (1961) 271.
[4] T. H. WEILAND, G. PFLEIDERER AND K. RAJEWSKY, *Z. Naturforsch.*, 15 (1960) 434.
[5] P. G. W. PLAGEMANN, K. F. GREGORY AND F. WROBLEWSKI, *J. Biol. Chem.*, 235 (1960) 2282.
[6] G. PFLEIDERER AND E. D. WACHSMUTH, *Biochem. Z.*, 334 (1961) 185.

Received May 15th-1962.