than that obtained by the thymol–sulphuric acid method of estimation. Furthermore Fraction X of which there is 20–25% present in the KEILIN–HARTREE beef-heart cytochrome *c* preparation[5] consists mainly of glycoprotein. Whether or not this material is present with cytochrome *c* in the mitochondrion is not yet known; an attempt is now being made to elucidate this point.

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PN 10071

Alterations of tissue lactate dehydrogenase in human neoplasms*

Conflicting views have recently appeared regarding the presence or absence of qualitative changes of lactate dehydrogenase (L-lactate:NAD oxidoreductase, EC 1.1.1.27) in tumor tissues. STARKWEATHER AND SCHOCH[1] have pointed to a shift towards Fraction III (H_2M_2) in neoplasms as well as a consistent alteration of the Michaelis constant in this fraction, suggesting that this lactate dehydrogenase moiety may represent "a structurally different protein characteristic of neoplastic tissue". NISSELBAUM AND BODANSKY[2], on the other hand, have purified the M_4 subunit from normal human liver and a hepatocellular carcinoma and report no significant differences between these two fractions either catalytically or immunologically. It is the purpose of this brief report to present evidence suggesting that the differences

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in lactate dehydrogenase between normal and neoplastic tissues are quantitative only and that a new type of lactate dehydrogenase is not a characteristic of tumors.

The preparation of tissue extracts, starch-gel electrophoresis, separation of hybrids on starch grain, enzyme assay method (utilizing pyruvate as substrate) and derivation of coenzyme analogue ratios were carried out as described by FINE *et al.*[3] with one exception: the concentration of extracts utilized in starch-grain separation was 1.0 g/ml. The separation of white cells followed the method devised by FREIREICH[4], one modification being the use of 6% fibrinogen for sedimentation of erythrocytes.

As indicated in Fig. 1, we have always been able to demonstrate all five major

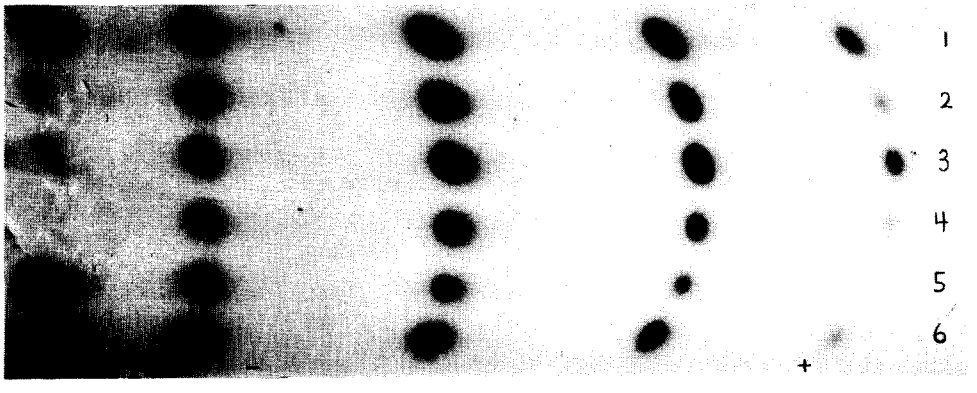

Fig. 1.

Lactate dehydrogenase hybrids (starch-gel electrophoresis) leukemic white cells.

Band	*Material*	*NH X DH_L*/ $NADH_H$***
1	Normal white cells	1.68***
2	Acute stem cell leukemia	1.95
3	Chronic lymphocytic leukemia	1.87
4	Acute monocytic leukemia	2.04
5	Leukemoid reaction (CA lung)	0.94
6	Chronic lymphocytic leukemia	1.38

* NH X DH_L, reduced nicotinamide hypoxanthine dinucliotide at low concentration (3×10^{-4} M pyrovate).

** $NADH_H$, reduced nicotinamide adenine dinucliotide as high concentration (1×10^{-2} M pyrovate).

*** For explanation of ratios, see refs. 3 and 6.

subtypes of lactate dehydrogenase in neoplastic tissues, this being consistent with the concept of the genetically determined formation of the intermediate forms from the two parent types (pure M_4 and H_4)[5]. Furthermore, it may be seen that the analogue ratios and electrophoretic patterns tend to support one another and to be mutually predictable. A binomial distribution of lactate dehydrogenase forms in both normal and tumor tissues is evident (a finding associated with the enzyme arising from a uniform cell population)[3] with the exception of samples 1 and 6 in the leukemic group. These two tissues, however, were found to contain a mixed cell population. In this regard, it should be mentioned that routine washing of the tissues for the

purpose of removing excess erythrocytes was not performed. A washing experiment was carried out, however, and the results indicated no appreciable change in either analogue ratio or electrophoretic pattern in the washed tissues as compared with unwashed controls. However, there was a lowering of lactate dehydrogenase activity in the washed tissues ranging from 29 to 82%, a finding consistent with the high solubility of lactate dehydrogenases.

The one striking finding in most of our solid tumors has been a shift toward predominance of M-type on electrophoresis with an associated lowering of the analogue ratios. This is in marked contrast to the findings in leukemic cells where there is a tendency towards a shift in the opposite direction particularly in the "more malignant" forms. One problem in this regard relates to the erroneous use of a mixed cell population as a normal control and we are therefore attempting to devise a method of leukocyte separation which will provide pure cell populations in large enough quantities for enzyme analysis ($100 \cdot 10^6$ cells).

TABLE I

ANALOGUE RATIOS (NH X $DH_L/NADH_H$)* OF H_2M_2 (III) HYBRID IN NORMAL AND TUMOR TISSUES

For explanation of ratios, see refs. 3 and 6.

Normals		*Tumors*	
Lung	1.65	Liver metastases (bronchogenic primary)	1.40
Lung	1.55	Liver metastases (bronchogenic primary)	1.62
Lung	1.63	Colon primary	1.56
Kidney	1.61	Renal metastases (primary unknown)	1.41
Pancreas	1.51	Pancreas primary	1.45
Heart	1.40	Acute myelogenous leukemia white cells	1.68
Mean	1.56	Mean	1.52

* See legend Fig. 1.

The various hybrids of normal and neoplastic tissues were eluted from starch grain with cold bovine serum albumin and analogue ratios were obtained. Table I shows that this measure of catalytic behavior is essentially constant for the H_2M_2 hybrid (III) regardless of tissue source. In general, the ratios of each of the other four lactate dehydrogenase forms were also similar when determined on different tissues.

Inactivation of some of the lactate dehydrogenase forms has occurred in association either with heating during electrophoretic separation or with storage of the eluted forms at 4° prior to assay. The relative heat lability of M_4 (V) and the M_3H hybrid (IV) has been documented by KAPLAN AND CIOTTI[6] and it is of interest that these are usually involved when loss occurs (as determined by comparison with the original gel-electrophoretic pattern and the crude extract analogue ratio). In view of our finding of a shift towards M-type subunits in neoplasms, we wonder if the apparent localization of lactate dehydrogenase in one form is not merely artifactual

secondary to loss of M_4 and M_3H forms, this perhaps being magnified by the washing out of considerable enzyme activity during tissue extraction plus the utilization of lactate as substrate, an assay system which requires relatively large amounts of enzyme.

In summary, a comparison of lactate dehydrogenase patterns in normal and tumor tissues suggests only a quantitative difference in tumors represented by the appearance of greater relative amounts of M-type subunits. A more complete presentation of our data in this regard is planned shortly.

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Composition en amino acides et hydrolyse trypsique des anhydrases carboniques humaines X_1 et Y

Un ensemble de recherches poursuivies depuis 1956 nous a conduits (a) à individualiser un groupe de protéines érythrocytaires "lentes" désignées par Y, X_1, X_2 dans l'ordre de leur mobilité électrophorétique croissante à pH 8.6 (ref. 1); (b) à les isoler par chromatographie sur Amberlite CG-50 de l'"extrait éthanol–chloroforme" de l'hémolysat, dans lequel elles sont régulièrement présentes à raison d'environ 0.07, 0.6 et 0.05% de l'hémoglobine totale[2] et (c) à les identifier respectivement par un ensemble de leurs propriétés[3] aux trois formes moléculaires de l'anhydrase carbonique (EC 4.2.1.1) que NYMAN[4] a désignées par CA V, CA III et CA II.

Nous avons ainsi confirmé la multiplicité de ces enzymes et leur distinction en anhydrases carboniques de haute et de faible activité spécifique, Y étant environ 15 fois plus active que X_1 et X_2 (ref. 3). Ces données sont également en accord avec celles d'EDSALL *et al.*[5] qui ont isolé et désigné par CA II l'enzyme de haute activité et par CA I le plus abondant des deux enzymes de faible activité, seul retrouvé par ces auteurs.

Il importait, dès lors, de chercher à caractériser les diverses formes moléculaires de l'anhydrase carbonique par une étude comparée de leur composition en acides aminés et des peptides libérés par leur hydrolyse enzymatique. La présente note a pour objet de rapporter les premiers résultats des recherches entreprises dans cette