to 2,500 units of streptokinase for 70 min of incubation. The clots of all seven cases of thromboangiitis obliterans lysed when exposed to 2,500 units of streptokinase in less than 90 min (Fig. 1).

That streptokinase has an optimal concentration for fibrinolysis has been known but not adequately explained. The possibility of an inhibitor preventing clot lysis at higher concentration has been suggested[4]. Investigations are being carried out to explain further this mechanism in the normal individual and the difference noted in cases of Buerger's disease. The unusual behaviour of blood clots obtained from cases of thromboangiitis obliterans suggests that an abnormality in clot formation or lysis may be the ætiological factor in Buerger's disease.

This work was supported by grant *H*–6567 from the U.S. Department of Health, Education and Welfare.

NORMAN ENDE

Clinical Laboratory, Veterans Administration Hospital,
and Department of Pathology,
Vanderbilt University School of Medicine,
Nashville, Tennessee.

[1] Buerger, L., *Amer. J. Med. Sci.*, **136**, 567 (1908).
[2] Buerger, L., *J. Med. Res.*, **31**, 181 (1914).
[3] Wessler, S., Ming, Si-Chun, Gurewich, V., and Freiman, D. G., *New England J. Med.*, **262**, 1149 (1960).
[4] Sherry, Sol, Tillett, W. S., and Read, C. T., *J. Thoracic Surg.*, **20**, 393 (1950).

Lactic Dehydrogenase in *L*1210 Cells

PROMPTED by the striking difference in lactic dehydrogenase activity observed by Woodliff[1] in sensitive *L*1210 leukæmic cells compared with those resistant to amethopterin and 6-mercaptopurine we have examined lactic dehydrogenase-levels in sensitive *L*1210*Z* and a strain (*L*1210*C*59) resistant to both amethopterin and 6-mercaptopurine and partially resistant to cytoxan.

Tumour-bearing mice were kindly supplied by Dr. A. Goldin and Mr. S. R. Humphreys and maintained as already described[2]. The mice used were $B6D2F_1$ ($C57BL/6J♀ \times DBA/2J♂$). Local tumours (average weight 250 mg) from the site of injection and infiltrated spleens (500 mg, 5 times normal weight) were removed 9–12 days after inoculation and stored at −15° two weeks prior to preparation of the enzyme extracts. These extracts were prepared by homogenizing the tissues in 3 volumes of chilled 0·1 M sodium phosphate buffer, *p*H 7·0, followed by centrifugation at 60,000*g* for 30 min. Protein content was measured spectrophotometrically[3].

Each 1-ml. incubation contained DPNH 0·00013 M, sodium pyruvate 0·002 M, sodium phosphate, *p*H 7·0, 0·1 M, and 14 μg protein; read in a Beckman *DU* spectrophotometer at 32°. The Δ absorbance for a control incubated without pyruvate was 0·004/min.

As shown in Table 1, we could reveal no difference in lactic dehydrogenase activity in extracts of sensitive and resistant cells.

Table 1. LACTIC DEHYDROGENASE IN TUMOUR CELL EXTRACTS

	Initial Δ absorbance/min (340 mμ)
Sensitive local tumour	0·062
Resistant local tumour	0·058
Sensitive spleen	0·052
Resistant spleen	0·052

Extracts of local tumours have been analysed by starch electrophoresis[4] by Dr. L. A. Costello of Brandeis University and shown to be predominantly the muscle (*M*) type. Heart (*H*) type was barely detectable. M_3H_1, M_2H_2, M_1H_3 were present in trace amounts which decreased in the order named. There was no difference in the pattern obtained from sensitive or resistant cells.

The difference between our results and those of Woodliff may be explained by: (1) different strains of tumour were used; (2) Woodliff used the ascites form of the tumour; (3) the cytochemical technique used by Woodliff to measure lactic dehydrogenase may reveal a difference in staining properties of the cells unrelated to the amount of enzyme present.

We conclude that lactic dehydrogenase in extracts of the drug-sensitive and drug-resistant *L*1210 cells examined is quantitatively and qualitatively the same.

R. L. KISLIUK *
G. ZEL
H. A. WALTER, JUN.
G. F. NORBERT
U. F. HUBLITZ
R. P. D'AMICO

Department of Pharmacology,
Tufts University School of Medicine,
Boston 11, Mass.

* Leukæmia Society Scholar.

[1] Woodliff, H. J., *Nature*, **191**, 1313 (1961).
[2] Goldin, A., Venditti, J. M., Humphreys, S. R., and Mantel, N., *J. National Cancer Inst.*, **21**, 495 (1958).
[3] Layne, E., *Methods in Enzymology*, **3**, 451 (Academic Press, New York, 1957).
[4] Kaplan, N. O., and Cahn, R. D., *Proc. U.S. Nat. Acad. Sci.*, **48**, 2123 (1962).

Distribution of Endogenous and Parenterally Administered Porphyrin in Viable and Necrotic Portions of a Transplantable Tumour

TETRAPHENYLPORPHINESULPHONATE (TPPS) is a synthetic, non-naturally occurring porphine derivative which accumulates to a greater extent in the tumours than in the other tissues of tumour-bearing rats after parenteral administration[1]. Because of its optical and solubility properties it can be differentiated from, and analysed in the presence of, endogenous porphyrin. These properties made possible quantitative study of two aspects of the tumour localization of porphyrin that had been seen grossly with other parenterally administered porphyrine: the greater apparent accumulation in the necrotic portions of the tumours[2,3], and the absence of apparent red fluorescence in some tissues despite high porphyrin concentrations[4].

Quantitative analyses of endogenous porphyrin and TPPS were done by an extraction procedure and spectrophotometric assay developed for this purpose and described previously[1]. This assay utilizes the complete separation of the major absorption peak of TPPS at 438 mμ from that of endogenous porphyrin at 407 mμ in dilute acid. Three zones of the Walker carcinosarcoma 256, grown 18 days, were separated by dissection: (1) sub-capsular tissue, comprising about 2 mm depth from the surface; (2) viable tissue of a homogeneous grey-white colour; (3) non-hæmorrhagic necrotic zone in which small discrete, pearly white areas, identified histologically as regions of necrosis, merge towards the centre of the tumour.

In Table 1 is shown the distribution of endogenous porphyrin in the three zones of control tumours, and of TPPS in tumours from rats that received 5·0 mg TPPS intravenously 24 h before killing. The concentration of TPPS was considerably higher than that of the endogenous porphyrin in all zones of the tumour. TPPS was most highly concentrated in the necrotic area, as was the endogenous porphyrin. TPPS concentration in the viable portion of tumour was also high, while that of the endogenous porphyrin was low, about that found in 12-day-old non-necrotic tumours[5]. The difference between

Table 1. ENDOGENOUS PORPHYRIN AND TPPS IN SUB-CAPSULAR VIABLE AND NECROTIC ZONES OF WALKER 256 CARCINOSARCOMA

	Control (6 animals) mg endogenous porphyrin*/ gm net weight	Experimental (6 animals) mg TPPS/g net weight
Sub-capsular mean ± *S.D.*	5·6 ± 3·3	22·5 ± 5·9
Viable mean ± *S.D.*	2·3 ± 1·7	34·5 ± 10·6
Necrotic mean ± *S.D.*	16·0 ± 6·3	52·1 ± 13·8

*Calculated on the basis of $\left(E_{1\ \text{cm.}}^{1\%}\right) = 4{\cdot}9 \times 10^3$ (ref. 6)