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Letter to the Editor

A Procedure for the Application of Cell Kinetic Techniques to Human Tumor Samples*

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SEVERAL reports have suggested that an in-depth cytokinetic profile of malignant cell renewal systems would provide a rational basis for the design of oncotherapeutic strategies [1–3]. Indeed, in experimental systems where adequate data can be collected to define proper scheduling, marked improvements in the therapeutic responses are observed [4–6]. However, other authors have raised questions concerning the applicability of kinetically based therapy in humans [7–9]. A major reason underlying these objections is the difficulty in the application of existing cell kinetic techniques to human solid tumors, thereby limiting their widespread application and evaluation. Certain of these techniques require *in vivo* tritiated thymidine (^{3}H-TdR), multiple sampling and time consuming autoradiographic and microscopic analysis.

Recent studies have described the use of *in vitro* cell kinetic techniques based on a single tumor sample to estimate tumor growth fraction, via the primer available DNA polymerase (PDP) assay, the tritiated thymidine labeling index (^{3}H-TdR L.I.) and the DNA synthesis time (T_s) [10–11].

Potentially, these techniques could provide for the large scale accumulation of baseline human cell kinetic data as well as specific kinetic profiles on individual patients. Of practical importance to the widespread application and evaluation of these techniques is the development of storage conditions which do not alter the cytokinetic profiles of the sample.

Since tumor sampling would, in most cases be done in non-laboratory settings, sample preparation techniques should be rapid and based on a minimal use of equipment and manipulations. The storage techniques described herein are based on a modification of a tissue culture freeze preservation technique [12].

Both human and animal tumors samples were evaluated in this study. Approximately 1 cm^3 of tumor was minced in 3 ml of Eagles minimal essential media (EMEM, without Ca^{2+}, Mg^{2+}, $NaHCO_3$, Gibco) containing 20% fetal calf serum (FCS) (Gibco) and 10% dimethyl sufoxide (DMSO) (Sigma) and transferred to serum storage vials. Freezing was accomplished by placing serum vials in an ice bath (0°–4°C) for 30 min, transferring to a freezer (−20°–0°C) for 1 hr and finally placing on dry ice or in a −70°C freezer for final storage. In the present studies, a 7-day storage period was routinely used, as this would allow for the shipping and receiving of samples under most conditions from most locations. In some instances, samples were stored for up to 30 days.

For kinetic analysis, samples were rapidly thawed and diluted with 10 ml EMEM + FCS (without DMSO), triturated gently and allowed to stand at room temperature for 10 min. The samples were then filtered through a stainless steel grid and centrifuged at 400 *g* for 5 min. Viabilities were determined by trypan dye exclusion.

For the PDP assay, the pellet was re-

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suspended in FCS and slides prepared using a Cytocentrifuge® (Shandon Elliott, Cheshire, England). The PDP index was determined as described in detail elsewhere [10]. This *in vitro* assay has been shown to accurately reflect the growth fraction of experimental tumors as measured by the percent labeled mitosis technique [10].

The ^{3}H-TdR L.I. and T_s were determined as described in detail elsewhere [11]. *In vitro* labeling was terminated on ice, the viable cells isolated by a trypsinization procedure [13], and cytospin preparations made.

Control kinetic values were taken from tumor samples analyzed immediately prior to freezing.

All slides were subjected to gold activation autoradiographic techniques employing Kodak NTB-2 emulsion [11].

Text Figs. 1 and 2 show the relationship between the cell kinetic values obtained from samples immediately prior to freezing and after 7 days storage at −70°C. Cell viabilities in the freeze-stored samples showed little or no change from their pre-storage values. The

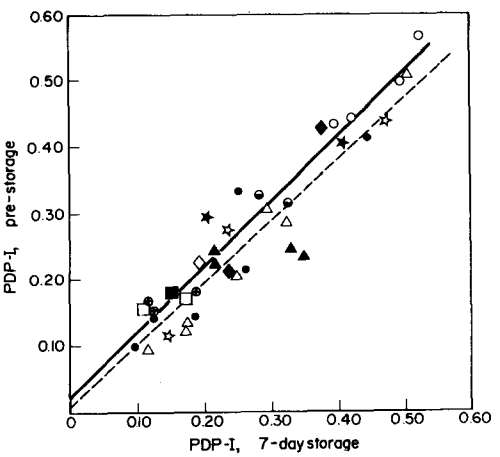

Fig. 1. Pre-storage PDP-I values expressed as a function of the values obtained after 7 days *of freeze storage. Human tumors: brain* (⊕), *breast* (▲), *colon* (△), *lung* (◇), *stomach* (☆). *Animal tumors: C_3H/HeJ mouse spontaneous mammary* (●), *transplantable fibrosarcoma* (□), *T*-1699 *mammary* (◆), *Lewis lung* (★), *P815X2 mastocytoma* (◒), 13762 *rat mammary* (○), *DMBA induced rat mammary* (■). *Lines fitted using a linear regression analysis. Human* (———), *animal* (– – – –).

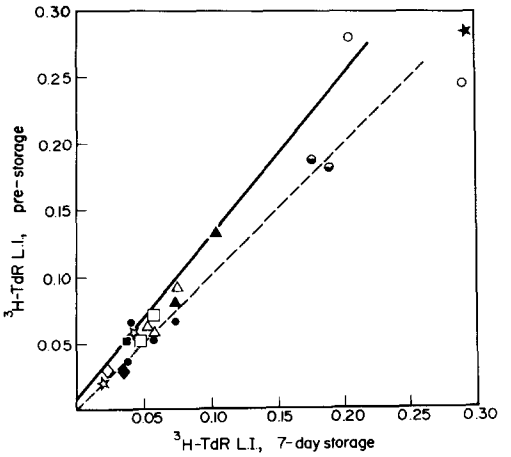

Fig. 2. Pre-storage ^{3}H-TdR L.I. values expressed as a function of the values obtained after 7 days *of freeze storage. Human tumors: breast* (▲), *colon* (△), *lung* (◇), *melanoma* (◆), *stomach* (☆). *Animal tumors: C_3H/HeJ mouse spontaneous mammary* (●), *transplantable fibrosarcoma* (□), *Lewis lung* (★), *P815X2 mastocytoma* (◒), 13762 *rat mammary* (○), *DMBA induced rat mammary* (■). *Lines fitted using a linear regression analysis. Human* (———), *animal* (– – – –).

data was subjected to a linear regression analysis and the lines fitted, employing the least squares technique. Correlation coefficients were 0.921 and 0.956 for human and animal PDP-Indices respectively. Correlations for human and animal ^{3}H-TdR labeling indices were 0.972 and 0.959 respectively. Excellent correlations (>0.90) were also observed in the T_s determinations prior to and after 7 days of freeze storage (not shown).

Human and animal samples analyzed at various times during the 7-day storage procedure showed no statistically significant fluctuations from their prestorage kinetic values nor did the sample kinetics vary after 30 days of storage at −70°C.

These results indicate that tumor specimens could be analyzed at locations other than the point of sampling, thereby eliminating the need to equip and staff individual kinetic facilities. Since this procedure is rapid and requires a minimum of equipment and technical manipulations it would be relevant to the surgical nurse or pathology technician.

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