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Density gradient centrifugation of tumor cells from needle biopsies and their respective source tumors: a comparison of density distributions[1]

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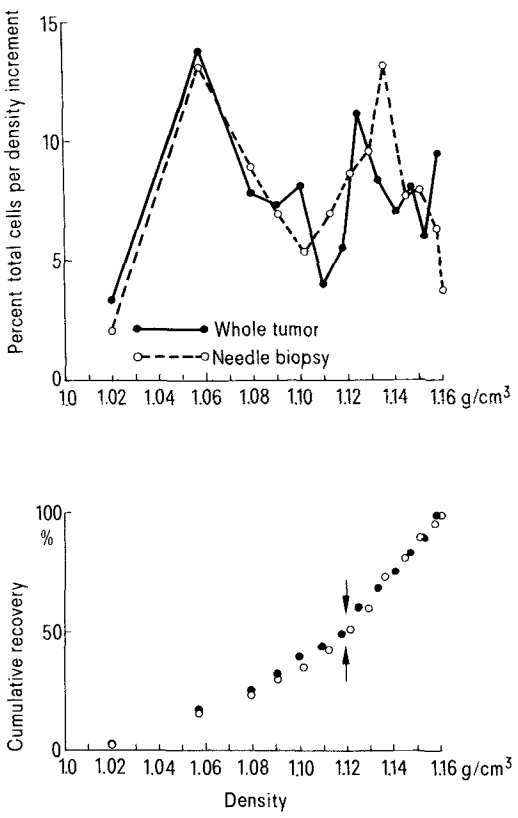
Summary. A needle biopsy technique was applied to a murine fibrosarcoma (FSa) tumor system. FSa tumors of 8 mm diameter in size were biopsied and then made into single cell suspensions. Resulting density profiles of cells from both sources were compared following centrifugation in Renografin gradients. In all cases, there was excellent agreement between the density profiles of the material from each of the biopsies and the corresponding solid tumors.

Following centrifugation of fibrosarcoma (FSa) cells in linear continuous density gradients of Renografin, at least 5 subpopulations of tumors cells are obtained, each of which differs in clonogenic ability[2], proliferative capacity[3,4], and radiation sensitivity[5,6]. The relative contribution of normal cells to each of these bands can be identified and quantitated by immunological and/or cytometric methods[7,8]. These data suggest that density distributions of tumor cells may reflect the relative composition in the tumor of relatively resistant vs sensitive, proliferating vs quiescent, and/or oxic vs hypoxic cells. Before such data can be utilized in a predictive manner to describe tumor response, it is required that a biopsy system be developed in which biopsy material obtained is sufficiently representative of the tumor and that any density profiles observed are reflective of those found when the entire tumor is dissociated and made into suspension.

Materials and methods. FSa tumors were grown to 8 mm in diameter in the hind legs of C_3Hf/Kam specific pathogen-free mice. Prior to obtaining a biopsy, each animal was anesthetized with Nembutal sodium (0.06 mg/g b.wt; Abbot Lab, Chicago, IL). Using a Tru Cut disposable biopsy needle (Travenol Laboratories, Inc., Deerfield, IL), tumor biopsy samples containing 2×10^6 cells were routinely obtained. These samples, as well as their respective solid tumors, were removed aseptically. Tumor tissue was finely minced and then digested for 20 min at room temperature with trypsin, made up to 0.025% in solution A (8.0 g NaCl, 0.4 g KCl, 1.0 g glucose, and 0.35 g $NaHCO_3$ in 1 l of water). The suspension was then passed through a stainless steel mesh (200 wires/2.54 cm) and then centrifuged at $225 \times g$ for 5 min. The resulting single cells were counted by hemocytometer and viability was determined by trypan dye exclusion and phase contrast microscopy. Viability was routinely greater than 95% and the yield of viable cells was about 10^8 per g of tumor tissue[5].



Comparison of density banding profiles obtained from an individual FSa solid tumor made into single cell suspension and a needle biopsy obtained from that tumor.

Density banding experiments were performed using Renografin gradients[5]. Approximately 2×10^6 cells from biopsy samples or their respective source tumors were loaded onto each 14 ml preformed gradient (10-35% Renografin in Ringer's solution), centrifuged at $13{,}000 \times g$ for 30 min in a swinging bucket rotor (Beckman L5-50, SW 27.1 rotor) at 4 °C. Each gradient was fractionated into 1 ml samples. The refractive index (N_{24}) was determined for each fraction and the density calculated using the formula $\rho = (g/cm^3) = 3.4683 \times N_{24}$ 3.6267. The number and viability of cells recovered in each fraction was determined using a hemocytometer and a phase contrast microscope. The resulting data were presented as percent total and cumulative percent total of cells recovered per density increment.

Results and discussion. Thus far, 24 tumors have been biopsied using this procedure and there has been good agreement between the density profiles of the material from each of the biopsies and their corresponding solid tumors. Representative data are presented in the figure.

There is currently considerable interest in developing suitable biopsy procedures for application as prognostic indicators. It is unclear, however, as to how well biopsy material reflects the source tumor. In particular, it is of interest to know the minimum requirement of biopsy material required for a given size of tumor to accurately reflect both the relative composition and proportion of classes of tumor cells present. In this study, the Tru Cut needle was used because it allowed for the largest and least fragmented tissue samples[9]. The adequacy of a single biopsy sample as being suitably reflective of a 8 mm in diameter tumor is demonstrated by the close correlation of density distributions presented in the figure. It is most probable, however, that larger size tumor masses will require proportionally larger sized biopsy samples. The applicability of this procedure to other tumor systems requires the development of adequate single cell suspension methods. Currently this approach has been applied successfully to an L-P59 sarcoma and a fibrosarcoma of spontaneous origin. Its applicability to carcinomas, however, has not as yet been tested.

While the density of each FSa tumor subpopulation remains relatively constant from tumor to tumor, the relative number of cells comprising each subpopulation will vary in each individual tumor. In addition, normal cell populations present in the tumor can be assayed. By combining density gradient centrifugation with centrifugal elutriation, nontumor cells can be selectively isolated from the tumor and enriched to over 80%[2]. Earlier studies have indicated that relatively radiation resistant FSa cells are collected at densities greater than 1.12 g/cm^3[3,5,6]. With this as a reference density the relative proportion of sensitive vs resistant FSa cells in each tumor can be approximated using the Renografin density gradient system. In conclusion, the good correlation between the density profiles of biopsies and their corresponding disaggregated tumors thus allows the consideration of the biopsy technique together with density centrifugation as a prognostic indicator system for FSa tumor response.

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Electrophysiological evidence of nervous involvement in the control of the prothoracic gland in *Periplaneta americana*[1]

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Summary. The nervous activity of the prothoracic gland nerve in the last larval instar of *Periplaneta americana* was investigated by means of suction electrodes. Nervous activity is low immediately before and after the last larval molt, and increases in the middle of the last intermolt phase. The level of nervous activity is in remarkable coincidence with the course of the ecdysteroid titer in the hemolymph. An involvement of nervous control mechanisms in the regulation of the prothoracic gland function should be taken into account.

It is well known that molting in insects is induced by the prothoracicotropic hormone of the neurosecretory cells of the brain, stimulating the prothoracic gland. However, some physiological indications of nervous influences on prothoracic gland activity[2-5] as well as anatomical evidence of prothoracic gland innervation from the suboesophageal, prothoracic, and mesothoracic ganglia in different species[6-14] have remained mostly disregarded. Recently the prothoracic gland of *Periplaneta americana* was demonstrated by cobalt chloride-iontophoresis to be innervated by 4 neurons of the prothoracic ganglion via a side branch of nerve 4 rlb[15,16], emerging from the 4th segmental nerve of the prothoracic ganglion (fig. 1). It enters the prothoracic gland on each side at the gland's posterior distal end. These findings raise the question of the involvement of nervous control in prothoracic gland function.

Material and methods. Registration of the activity of the prothoracic gland nerve of *Periplaneta americana* in the last larval instars and, in some cases, in penultimate instars just before molt was performed after opening the prothoracic region of the animals dorsally. The prothoracic gland was dissected by removing the oesophagus and the main tracheae. After cutting the prothoracic gland nerve near the prothoracic gland the nerve was drawn into a suction