

## Separation of Subcellular Particles with Rate and Isopycnic-zonal Centrifugation

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In order to improve the drawbacks of differential pelleting zonal centrifugation, a new method of combined zonal centrifugation was carried out to fractionate all subcellular particles in rat liver homogenate including nuclei and cell-debris. The polytron homogenizer was used to overcome the resistance of AH 7074 and other tumor cells to normal homogenization, such as Daunce, Potter-Elvehjem and Chaikoff. Minimum damage of nuclear fraction and favourable separation of other sub-cellular fractions were achieved with 0.25M sucrose, 10mM Tris buffer pH 7.2, 5mM MgCl<sub>2</sub>. The homogenization was carried out with Polytron at 31V for 2 min. Under these conditions, less than 10% of DNA was detected in supernatant measured by SDS Burton's method. A gradient of 25–35% sucrose, superimposed with the addition of 40, 45 and 50% sucrose and finally

CsCl, separated rat liver homogenate into nuclear, cell debris, mitochondria, peroxysomes, heavy microsomes, light microsomes and supernatant. Typical marker enzymes, such as catalase (peroxysomes), cytochrome oxidase (mitochondria), beta-glucuronidase (lysosomes), NADPH cytochrome c reductase (microsomes) and electron microscopy were used for proving each subcellular fractions. According to continuous zonal rotor method, Brown reported that lysosomes and a new granules have affinity to <sup>67</sup>Ga in normal as well as in tumor cells. However, we found by our method that the new component in problem is probably microsomes, as determined by w<sup>2</sup>t values and electron microscopy. The detailed study on intracellular turnover of tumor-radionuclides is in progress, and it will be reported elsewhere.

## Evaluation of <sup>201</sup>Tl Stress Myocardial Perfusion Scintigraphy in Patients with Ischemic Heart Disease

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1) In an attempt to visualize the localization and extent of myocardial ischemia produced by exercise testing in patients with effort angina, <sup>201</sup>Tl thallium stress myocardial perfusion scintigraphy was performed at rest and during exercise in 45 cases.

The paired <sup>201</sup>Tl images were collected in 5 projections of anterior, left anterior oblique (30°, 45° and 60°) and left lateral views, stored in the computer (TOSBAC 40-C) and processed subsequently. To delineate the changes of the distribution of local myocardial perfusion, these images were divided into 15 segments and analyzed.

Perfusion ratio of these segments were calcu-

lated as the count density ratio/normal area before and after exercise.

Using selected segments, no significant perfusion defects were observed on either the rest or exercise studies in any of the normal subjects. On the contrary, an appearance of local perfusion defects were frequent in patients with angina pectoris during anginal attack and ischemic ST changes produced by exercise testing.

The segmental perfusion ratio of well perfused areas showed  $1.01 \pm 0.10$ , but significantly lower value ( $0.76 \pm 0.10$ ) was obtained in under-perfused areas elicited by submaximal exercise in patients with effort angina.