

## 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.

Correlation of perfusion defects with arteriographically proven significant coronary stenosis was good for the left anterior descending and right coronary arteries, but not so good for circumflex artery disease.

Using 201 Tl stress myocardial scintigraphy, coronary artery disease could be detected non-invasively in sensitivity of 88% (21/24), with specificity of 95% (20/21).

2) To examine the ischemia-induced left ventricular dysfunction during exercise testing in pa-

tients with effort angina, 99m-technetium radio-nuclide angiocardigraphy was performed at rest and during submaximal exercise testing.

5 normal subjects showed no regional dysfunction and each slightly increased ejection fraction (average increase,  $9 \pm 5\%$ ) during exercise.

On the contrary, in 4 patients with effort angina who showed normal left ventricular ejection fraction at rest ( $54 \pm 7\%$ ), decreased ejection fraction ( $31 \pm 9\%$ ) was observed during anginal attack and ischemic ST changes.

### **Localization of Technetium-99m Pyrophosphate in Experimental Myocardial Infarcts—Its Relationship to Histological Findings, Regional Perfusion and Tissue Calcium Level**

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We have successfully estimated the size of anterior wall myocardial infarct using technetium-99m pyrophosphate ( $^{99m}\text{Tc-PYP}$ ) myocardial scintigram in eight dogs.  $^{99m}\text{Tc-PYP}$  is reported to accumulate in necrotic myocardium. In the present study, we tried to evaluate three possible determinates of  $^{99m}\text{Tc-PYP}$  accumulation: extent of tissue necrosis determined histologically, regional perfusion measured with thallium-201 ( $^{201}\text{Tl}$ ) and tissue calcium level (Ca) measured by atomic absorption spectrometry.

Methods: Experimental myocardial infarct was produced in four dogs by ligation of left anterior descending artery. Eight millicurie of  $^{99m}\text{Tc-PYP}$  was injected intravenously 48 hours after the ligation. 50 minutes after  $^{99m}\text{Tc-PYP}$  injection, 300 microcurie of thallium-201 ( $^{201}\text{Tl}$ ) was injected intravenously, and the dog was sacrificed 10 minutes thereafter. The left ventricle was cut into several transverse slices. One of the slices which contained grossly necrotic myocardium was divided into 10 sections, and each section was divided into two layers—endocardium and epicardium. Thus, the left ventricular slice was divided into 20 segments. These segments were weighed, placed in plastic tubes containing 10% formalin, and  $^{99m}\text{Tc}$  radioactivity was counted within 6–12 hours after injection of  $^{99m}\text{Tc-PYP}$  in well-type gamma

counter (Thyro-net, Aloka Inc.)  $^{201}\text{Tl}$  counts were obtained 5 days after dogs were sacrificed when samples were free of significant  $^{99m}\text{Tc}$  activity, and corrected for radioactive decay during 5 days. The raw  $^{99m}\text{Tc}$  counts were corrected for  $^{201}\text{Tl}$  activity. Each segment was divided into two parts, one for histological study and the other for the measurement of Ca.

Histological section was stained with hematoxylin-eosin, and the extent of infarction was expressed as percent of whole section.

For Ca study, myocardial samples were weighed, and then digested overnight in one milliliter concentrated nitric acid, and the digest was then diluted to 10 milliliter with deionized water. The Ca were measured by atomic absorption spectrometry (Type 308, Hitachi Inc.), and the results were expressed as parts per million per gram wet tissue.

Results: (1) Relationship between  $^{99m}\text{Tc-PYP}$  activity and the extent of myocardial infarct: All myocardial segments which showed elevated  $^{99m}\text{Tc-PYP}$  activity had histological evidence of infarct. However, there was no linear relationship between  $^{99m}\text{Tc-PYP}$  activity and the extent of myocardial infarct. (2) Relationship between  $^{99m}\text{Tc-PYP}$  activity and regional perfusion measured by  $^{201}\text{Tl}$ :  $^{201}\text{Tl}$  activity decreased in the segments