Purpose

Two membrane fractions were prepared from a rat liver tissue: a microsomal enriched fraction and a mitochondrial enriched fraction. The lipids were extracted from the microsomal fraction, separated using 2D thin layer chromatography and were then compared with lipids extracted from the entire tissue. The 2D TLC plate is composed of mildly polar silica gel, which will bind polar compounds more tightly than non-polar ones. The first dimension of the plate was developed in a basic solvent and the second dimension was done in a basic solvent. The purpose of these 2 dimensions was to be able visualize the various polarities of the lipids and how they react on the silica gel in varying pH environments. In the second part of the experiment, the lipid/protein ratio in the mitochondrial fraction was analysed. Brilliant Blue Dye R was used to estimate the lipid concentration of the fraction and an absorbance assay was used to estimate the protein concentration.

Comment [S1]: 5/5

dilute samples are shown. The linear trendlline equation is y = -11.411x + 0.6306 with an R^2 value of 0.9926. The slope of the trendline shows the overall decrease in absorbance in the samples as the dilution fraction increases.

Comment [S9]: 5.6 / 6

-0.4 meaning of the slope [the slope corresponds to the decrease in absorbance corresponding to a dilution factor of one which is the undiluted sample]

Table 3: Proteins assay. 2 g of rat tissue liver was minced and placed in 10 mL of homogenization buffer (10mM Tris, 0.25M sucrose, 1mM EDTA, 1 mM DTT, pH 7.5) and then homogenized. The tissue was then centrifuged at 4°C for 10 min at 1000 xg after being paired and balanced. The supernatant was filtered and then centrifuged at 4°C for 10 min at 25000 xg after being paired and balanced. After completion, the supernatant (microsomal fraction) was transferred to a 15 ml Falcon tube and the pellet was suspended in 10 mL of homogenization buffer and then sonicated. 4 assay tubes were then prepared with 1 mL phosphate buffer (pH 11, 0.2M) and variable amounts of water and membrane suspension (mitochondrial membrane suspension) for a final volume of 2 mL per tube. The dilution fractions show the concentrations of the membrane suspension of each tube. All tubes were incubated for 15 mins at room temperature and then absorbance readings of each tube were taken with the spectrophotometer at 280 nm using a 1 mL plastic cuvette. The extinction coefficient of proteins is 2 $Lg^{-1}cm^{-1}$ at 280 nm.

Comment [S10]: 5/5

Sample	Dilution fraction	A_{280}
1) Blank	0	0.014
2) 1/100	0.01	0.035
3) 1/25	0.04	0.147
4) 1/5	0.2	0.643